Evaluation of antimicrobial activities of medicinal plants used against microbial infections in Pregnant women in Delta State, Nigeria

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 because they believe it is safe and effective for curing ailment and maintaining good health. Microbial infections that infect pregnant women are numerous, there is 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 investigate the effects and phytochemical activities of medicinal plants used in pregnancy care in Warri South local government, Delta State.Medicinal plants used were identified by oral interview. Microorganisms were identified by standard methods from urine. Result from oral interviewers showed that some medicinal plants and substances including kaolin clay were used by pregnant women, which tallied with former study. Bacteria identified from pregant women´s urine were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and others including *Candida albicans*. *Ficus exasperata* and *Slenostemon monostachyus* were active againgst *Candida albicans* at 12.5mg/ml. However not active on any bacteria. *Peperomia pellucida* had no inhibitory effect on any of the microorganisms. *Ficus exasperata Slenostemon monostachyus* could be used to treat microbial infections caused by *Candida ablicans* during pregnancy.

Key words: *Ficus exasperate*, *Slenostemon monostachyus*, *Peperomia pellucida*, *Candida albicans*, antimicrobial activity, pregnant women, local midwives.

Introduction

In the last two decades, the usage of medical plants, which translates to herbal medicines such as medicinal herbs as botanical pharmaceuticals, teas, nutritional supplements, or indigenous formulations including herbs, has expanded dramatically (Enioutina *et al.*, 2017). Due to 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%. (Dugoua, 2010). 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 (Seema, 2020). Herbal products are commonly seen by pregnant women as a safe, natural alternative to pharmaceuticals (Frawley *et al.*, 2015) and often

use them to improve their well-being or for the treatment of non-life threatening conditions like nausea, constipation (Louik *et al.*, 2010).
Pregnant women are likely to get a range of illnesses, according to epidemiological research. These illnesses include influenza, varicella, measles, severe acute respiratory syndrome, tuberculosis, vaginal yeast infection, urinary tract infection, group B streptococcus, bacterial vaginosis, listeriosis, pneumocystis, toxoplasmosis, and malaria (Sappenfield *et al.*, 2013). Urinary tract infection is one of the most common infections reported in pregnant women and it is mostly caused by *Escherichia coli, Pseudomonas aeruginosa, Staphylococuss aureus, Enterococcus faecalis, and Candida albicans.* The severity of infection has been suggested to vary at different stages of pregnancy, likely from the unique immunological alterations that occur during different stages of pregnancy (Faucette *et al.*, 2015). Pregnant women are susceptible to some of these infections as a result of immunological changes, changes in hormones and stress. If infections are left untreated it 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 (Seema, 2020). Early detection, prevention, and treatment are important to help reduce and eradicate these complications (Jamieson *et al.*, 2018).

A number of medicinal plants used during pregnancy , including *Ficus exasperata* (Vahl), have been used in traditional medicine for many years because of their antimicrobial properties (Sofowora, 2013). *Ficus exasperata* belongs to the family Moraceae, with 800 species occurring in the tropical part of the globe, mainly in and Polynesia and Indomalaya (Odunbaku *et al.*, 2008). *Ficus* species are plants well known all over the world as 'fig plant'. *Ficus exasperata*, is a medicinal plant locally known as 'sandpaper plant' and used for treating eyesores, stomach pains, ring worm infection, leprosy, controlling bleeding and easing childbirth (Burkill, 2017). In Nigeria, the young leaves of *Ficus exasperata* are prescribed as a common antiulcer remedy in addition to various pharmacological actions, such as antidiabetic, antihypertensive, lipid-lowering and antimicrobial activities (Buniyamin *et al.*, 2016).

Solenostemon monostachyus P. Beauv of the Lamiaceae family commonly called Monkey Potato. This plant is an important herb that is widespread in West and Central Africa. It occurs as an annual weed in anthropogenic habitats and rocky savannahs. It is slightly fleshy, aromatic and grows up to 1m tall (Jude *et al.*, 2015). 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 (Adebayo and Krettli, 2011). The decoction of the plant is also used as a diuretic as well as to treat hypertension

(Jude *et al.*, 2015). 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 (Jude *et al.*, 2015). Reported biological activities of the plant include; antioxidant and antihypertensive, antimicrobial activities and antiulcer (Amazu *et al.*, 2015). Peperomia pellucida (L.), known commonly as silver bush or shiny bush is of the family Piperaceae. The plant is and annual weed native to Africa, tropical North and South America and Asia .Nigeria, Ghana, Sierra Leone, and Democratic Republic of Congo (DRC) Regions are African countries where the plant has been reported to be common. The infusion of plant with milk boost the immune system of sick people as reported in ethnomedicine (Idris and Olatunji,2016) The leaves of the plant is used to treat excited mental disorder in Bangladesh (Khan et al., 2008). Topical and warm poultice of pounded whole plant is used for skin diseases like pustules, boils, acnes, pimples. Also the plant is used for impotence headaches, rheumatic pains (Idris and Olatunji, 2016). In Ayurvedic medicine, the plant is crushed and mixed with water to form a mixture, heated and administered orally to cure hemorrhage and also against kidney and prostate problems and against high blood pressure (Idris and Olatunji, 2016). *Peperomia pellucida* contain secondary metabolites including, carotol, saponins, cardenolides, essential oils, flavonoids, and tannins (Khan and Omotoso, 2002).

In this study, the ethanolic, methanolic and aqueous extracts of *Ficus exasperata Slenostemon monostachyus* and *Peperomia pellucida* leaves were evaluated for their antimicrobial effects on Microorganisms isolate from the urine of pregnant women. Microbial infections have been documented to inflict pregnant women. Common infections that occur during pregnancy include influenza, vaginal yeast infections, eczema, bacterial vaginosis, group B streptococcus (GBS) and uterine infections. Other infections are listeria, herpes simplex virus (HSV), cytomegalovirus, rubella; human immunodeficiency virus (HIV), varicella zoster virus, hepatitis, influenza and Ebola. Changes in immune function, such as reduction in T- and B-cell activity and natural killer–cell activity and increases in dendritic-cell activity, may cause increased risk of infection. Low birth weight, birth defects, learning problems preterm birth and pregnancy loss are complications from severe infections that may occur during pregnancy (Seema, 2020).

Materials and Methods

After due consultation with Local midwives at Warri, from the list of local herbs Table 1) frequently administered to pregnant women, three plants *Ficus exasperata*

(Vahl) and *Solenostemon monostachyus* were selected for this study. Fresh plant leaves of *Ficus exasperata* (Vahl), *Solenostemon monostachyus* and *Peperomia pellucoia* 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 Botany Department, Delta State University, Abraka then to Microbiology laboratory for further analysis.They were then air dried at room temperature for 21days and pounded to powdered.

Preparation Of Extracts

100g of the powdered plant leaves of *Ficus exasperata* (Vahl) and *Solenostemon monostachyus* and *Peperomia pellucia* were weighed into a conical flask and soaked with 125ml of each solvent (aqueous, ethanol and methanol). For the aqueous extraction the duration was 24hours while the ethanol and methanol extraction were 7 days. The filtrate obtained was evaporated to dryness in water bath at 70^{o}C.

Preparation Of Culture Media

Nutrient agar, Potato Dextrose Agar (PDA), Blood agar, MacConkey Agar, Mueller Hilton Agar were prepared following manufacturer's specification. All media were sterilized in the autoclave at 121^{0}C for 15 mins at 15psi.

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.

Isolation of Microorganisms

Isolation of microorganisms was carried out according to the streak plate method previously outlined by Cheesebrough, (2006). The urine samples were centrifuged and the pellet was streaked on different agar plates and incubation was carried out at 37°C for 24hours for bacteria while fungi incubated at 25±2°C for 7 days. Thereafter, distinct colonies were picked with the aid of a sterile wire loop for subculture and stock in refrigerator for further identification and characterization.

Identification of Isolates

Morphological Characteristics

Colonies of bacterial isolates that were cultured on the different agar mentioned above and was described according to their shapes, color, diameter and appearance

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 pigments.

Antibacterial sensitivity and phytochemical tests

The antimicrobial activity of the three plant extracts (nine extracts) against microbial isolates from the urine of pregnant women were 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 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 different extracts. The plates were incubated at 37^0 C for 24 hours and were observed for zone of inhibitions. Phytochemical experiment was determined by standard methods (Harborne,1998).

Results

The plants used to cure and maintain pregnancy by traditional Midwives in Okere community 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 was 6.60% (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 microorganism from the urine 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 on *F.exaperata*, *S monostachyus* and *P. pellucida* extracts were not potent against any bacterium however, *Candida albicans* was sensitive to methanol and ethanol of F *exasperate* and S. *monostchyus* respectively.

The phytochemical test results showed the presence of various compound such as tannin, alkaloids, saponins, and cardiac glycosidefor P. pellucida, and S. monchyus while *ficus exasperate* contanined tannin, alkaloids, steroids, phobatanin and flavonoid while anthraquinone, is not present for F. exasperate and S. *monostachyus* while steriods and phobatanins are absent for S *monostachyus* and *P. pellucida* (Table 6).

Discussion

The study was designed to investigated the antimicrobial activity of medicinal plants used against microorganisms from urine of infected pregnant women. Oral interview revealed plants (table 1) used for treating and maintaining health of feotus 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).*

Solanostemon monostachyus leaves are used to treat infections. The plant is boiled and taken orally. *Ocimum gratissimum* stops bleeding during pregnancy, *Ficus exacerparta* is used for easy delivery, when foetus 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 (Dafam et.al., 2021). Which are quite different from those mentioned by midwives in Okere and Ugbuwangue communities. One similarity, however, was detected among pregnant women is the consumption of clay (geophagy). 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 (Madziva and Chinonay 2020). However, in this study the midwives reported that clay consumption build baby's body. Madziva and Chinonay, 2020) also reported that macro and micro elements were present in the clay they and elements content 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 mention earlier, Microrganisms 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 exaperata* leaf extract was reported to have antimicrobial activity. In another report, the plant was active against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia, Aspergillus niger, Candida albicans, Rhizopus stolonifera, Penicillium notatum* (Ajala *et al*., 2020). *Peperomia pellucida* was activie against some pathogenic disease causing organisms. Plant was active against *S. aureus*, ATCCA35218, *Klebsiella pneumonia*e ATTC34089, *Salmonella typhi* ATCC22648 and *Pseudomonas aeruginosa* has indicated the potential usefulness of *P. pellucida* in the treatment of various pathogenic disease. However, was not potent against any of the organism isolated from urine in this study. Nevertheless, *F. exacerpata and S. monostachyus* were potent against *Candida albicans* in this study. Also phytochemical compounds included alkaloids, saponins, tannins and glycocides and absence of steroids for *P. pellucida* which concurs with previous study (idris et al., 2019).

Conclusion

Plants used in Okere community in Warri South local Government area, Delta State were investigated by oral interview of which three plants were randomly selected and investigated against microorganisms from pregnant women's urine. *F. exacerpata and S. monostachyus* had inhibitory effect on *Candida albicans*. *Peperomia pellucida* had no inhibitory effect on microorganisms though phytochemical compounds were present.

Table 1: Plants used by pregnant women in Okere community in Warri South Local Government council Delta State.

Peperomia pellucida leaves	Use for early pregnancy-when the pregnant woman is still experiencing her monthly period during the early stage of the preganancy	Pound and use to prepare plantain porridge
Solenostemon monostachyus leaves	Use to treat infection, can be used injury/sore.	For pregnant woman boil and drink
Ocimum gratissimum leave	Stops bleeding during pregnancy.	Pound and insert inside the pelvic, leave for a day, then remove, helps to close the pelvix
Ficus exasperate	It is used when the foetus is too big and ceaserian birth is suggested	
Eyibesesi/ertbotor leave	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 I shot every morning.
Bryophyllum pinnatum leave	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(l**%)W/V	**Methanol(**%)W/V
Ficus exasperate	9.30	5.20	11.00
Solenostemon monostachyus	10 .1	5.60	8.50
Peperomia pellucida	6.9	7.90	10.10

Table 3: Cultural, Morphological, and Biochemical Characteristics of Bacterial Isolates

	Staphylococcus aureus	*Escherichia coli*	*Streptococcus spp*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Candida albicans*
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_2S	-	-	-	-	-	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**
Candida albicans	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	*S. aureus*	*E. coli*	*Pseudomonas aeruginosa*	*Staphylococcus sp*	*Klebsiella pnuemoniae*	*Candida albicans*
Ficus exasperate						
Water	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0
Methanol	0	0	0	0	0	17
Solenostemon monostachyus	0	0	0	0	0	0
Water	0	0	0	0	0	0
Ethanol	0	0	0	0	0	18
Methanol	0	0	0	0	0	0
Peperomia pellucia						
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	Tannin	Saponin	Alkaloids	Steroids	Phobatanins	Anthraquinones	Falvonoids	Cardiac glycosides
Ficus exasperate	++	-	+	+	+	-	+	-
Solenostemon monostachyus	+++	++	++	-	-	-	+	+
Peperomia pellucida	+++	+++	+++	-	-	+	-	+

REFERENCES

Adebayo, J.O. and Krettli, A.U. (2011). Potential antimalarials from Nigerian plants: A review. *Journal of Ethnopharmacology,* 133: 289 –302.

Ajala, T.O., Olusola, A.J and Odeku, O.A. (2020) Antimicrobial activity of Ficus exasperate (Vahl) leaf extract on clinical isolates and its development into herbal tablet dosage from Journal of Medicinal Plant for economic development 4(1)a95.https:doiorg/10.4102/jompet.Vuil.95

Amazu, L.U., Antia, B. and Okokon, J.E. (2015). Antiulcer activity of S. monostachyus. *The Journal of Phytopharmacology,* 4: 97 – 101

Bauer AW, Kirby WWM, Sherris JC, TurkM. Antibiotic susceptibility testing by Standardized single disc method. Am J Clin Pathol. 1966;45:493-496

Blumenthal, M., Brusse, W.R., Goldberg, A. and Gruenwald, J. (1998). The complete German Commission E monographs', in M. Nathan and R. Scholten (eds.), *Therapeutic guide to herbal medicines*. The American Botanical Council, Austin, TX. pp. 16–25.

Buniyamin, A.A., Eric, K.I. and Fabian, C.A., (2007). Pharmacognosy and hypotensive evaluation of *Ficus exasperata* Vahl (Moraceae)', *Acta Poloniae Pharmaceutica Drug Research* 64(6):543–546.

Burkill, H.M. (2017). *The Useful Plants of West Tropical Africa*.Vol. 4. Richmond: Kew, Royal Botanic Gardens.

Cheesebrough, M. (2006). *District Laboratory Practice in Tropical Countries.* Cambridge University Press.

Cite as ALP conference proceedings: 1844. 020006(2017): htt63/1.4989417 hastuti, U.S.Irsadummondi, Y.B and Ichasanah, H.N

Dafam, D.G., Denou, A., Idoko, A. Jimam, N.S, Okwon, V.A., Ohemu, T. U. Akubu, T.P and David, S (2021) Use of herbal medicine during pregnancy and attitude of pregnant women in Jos, Nigeria. Journal of Pharmacy and Biosciences (18):1

Dugoua, J. J. (2010). Herbal medicines and pregnancy. Journal of Population Therapy and Clinical Pharmacology, 3(17):370–378.

Enioutina, E. Y., Salis, E. R., Job, K. M., Gubarev, M. I., Krepkova, L. V., and Sherwin, C. M. (2017). Herbal Medicines: challenges in the modern world. Part 5. status and current directions of complementary and alternative herbal medicine worldwide. *Expert Review on Clinical Pharmacology*, 10(3):327–338.

Faucette, A. N., Unger, B. L., Gonik, B. and Chen, K. (2015). Maternal vaccination: moving the science forward. *Human Reproduction Update*, 21:119–135.

Frawley, J., Adams, J., Steel, A., Broom, A., Gallois, C., and Sibbritt, D. (2015). Women's use and self-prescription of herbal medicine during pregnancy: an examination of 1,835 Pregnant Women. Women's Health Issues, 25(4):396–402.

Harborne JB. Phytochemical methods. Harborne JB, ed. Chapman & Hall,London; 1998.

Hastuti, U.S., Irsdil Ummah, Y. P. and Khasanah, H.N. (2017). Antifungal activity of piper odoncum and peperomia pellucida leaf ethanol extract against Candida albicans ALP Conference proceedings

Idris, O.O., Olatunji, B.P. and Madfur, P. (2016). Invitro antimicrobial activity of the extract of Peperomia pellucida L. British Microbiology Research Journal, 11(4):1

Jamieson, D.J., Theiler, R.N. and Rasmussen, S.A. (2018). Emerging infections and pregnancy. *Emerging Infections Disease*, 12(11):1638-1643

Jude, F.O., Koofreh, D., Lucky, L. and Nwidu, F. (2015). Anti-inflammatory and antinociceptive activities of *Solenostemon monostachyus* aerial part extract in mice. *Avicenna Journal of Phytomedicine*, 6(3):284-294.

Khan A, Rahman M, Islam MS.Neuropharmacological effects of Peperomia pellucida leaves in mice. DARU. 2008;16(1) 35-40.

Khan MR, Omotoso AD. Antibacterialactivity of Hygrophila stricta and Peperomia pellucida'. Fitoterapia. 2002;73(3):251-254.

Kortei, N.K., Annor, I.A. Aboagye, G, Manaphram, N.Y.B, Koryo-Dabrah, A., Awude, E, Essuman, E.K., Alidu, H.W. Tettey, C.O. and Anadzi, B. (2019). Elemental minerals and Microbial compositions as well as Knowledge and perception regarding Kaolin (clay) consumption by pregnant women in the HO MUNICIPALITY OF Ghana. Pan Africa medicinal Journal 113.doi.10.11604/pmj.2019.34.113.17394

Louik, C., Gardiner, P., Kelley, K. and Mitchell, A. (2010). Use of herbal treatments in pregnancy. *American Journal of Obstetric Gynecology*, 5(202):431-439.

Madziva, C. and Chinonya, M.J (2020) Clay ingestion during pregnancy among black African women in a north London Borough. Understanding cultural meanings, integrated indegenious and Biomedical knowledge systems Frontiers in Sociology 6:20 doi:103389/isoc.so20.00020

Pharmacy and bioresources. 18(1):64-73.

Sappenfield, E., Jamieson, D. J., and Kourtis, A. P. (2013). Pregnancy and susceptibility to infectious diseases. *Infectious Diseases in Obstetrics and Gynecology*, 9:752-759.

Seema, L. (2020). Infectious Diseases in Pregnancy. *Pharmacy Resource for Clinical Excellence*, 67:4-7

Sofowora, A., (2013). *Medicinal plants and traditional medicine in Africa*, Spectrum Books Ltd., Ibadan. , pp. 191–289.