ANTIMICROBIAL EFFECT OF YOYO CLEANSER BITTERS AGAINST SELECTED MICROBIAL ISOLATES

BY

OLUSEYI OMOWUMI ROSEMARY

16/MHS07/028

Department of Pharmacology, Medicine and Health Science, Afebabalola University, Ado-Ekiti, Ekiti State.

Supervised by

Pastor Oni and Mr. Olatunji Kazeem

An IT report submitted to the Department of pharmacology and therapeutics, Afebabalola university, Ado-Ekiti, Ekiti-state And submitted to the Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, Idu, Abuja.

**CERTIFICATION**

This is to certify that this report is a detailed account of the student industrial work experience scheme (SIWES) undertaken by OLUSEYI OMOWUMI ROSEMARY at NATIONAL INSTITUTE FOR PHARMACEUTICAL RESEARCH AND DEVELOPMENT, IDU, for a period of 3months and has been prepared in accordance to regulation guiding the proportion of reports in the Department of Pharmacology and Therapeutics, Afebabalola University, Ado-ekiti, EkitiState

DR. MOHAMMED B.S (HOD) **……………………**

Signature and date

MR OLATUNJI KAZEEM **......................................**

Supervisor signature and date

PASTOR ONI **....................................**

School supervivor Signature and date

**ACKNOWLEDGEMENT**

My profound gratitude to the almighty God for enabling me to carry out this study. I want to thank my parent for their love and support throughout this work. I want to thank the Director General/Chief Executive officer and the Director of Administration, National Institute for Pharmaceutical Research and Development (NIPRD) for granting me the opportunity to undergo my SIWES program. To the HOD, Dr. Mohammed B. S.and staff of the Department of Microbiology and Biotechnology. To my supervisor Mr. Olatunji kazeem for his support, contribution, and supervision during this study. I also want to thank the research fellows and Technologist in Microbiology and Biotechnology department for their help and commitment in impacting me positively. Finally, I want to thank my IT colleagues and friends in the department of Microbiology and Biotechnology for their supports and contributions throughout my stay. Thank you all I really appreciate. I also want to thank my lecturers, the HOD of Pharmacology And Therapeutics, My school supervisor Pastor Oni who come all the way from Ekiti to come and supervise me,I am very grateful.I want to thank all the lecturers in general and the school of letting us learn more and to experience Industrial training. I’m really grateful. Thank you all.

**TABLE OF CONTENTS**

1. Introduction 5-7

1.1Aim and objectives 7

2.0 Materials and methods 8

2.1 Materials used 8

2.2 Microorganism used 8

2.3 Media preparation 8

2.4 Preparation of inoculums 9

2.5 Sample preparation 9

2.6 Procedure 9,10

3.0 Results 11

4.0 Discussion 12

5.0 Conclusion 13

Recommendation 13

References 14

1. **INTRODUCTION**

A major source of active compounds is medicinal plants that are thought to be effective with minimal side effects than conventional drugs (Shinwari, 2010). In some countries, particularly developing ones, individuals that play an active role in their health care have chosen herbal medicine as a common choice in self-therapy against oxidative stress and inflammationmediated diseases (Ernst, 2011,Leornardo and Maes, 2012).More than three-quarter of the world’s population is increasingly diverting to herbal medicine due to its capacity to reduce ailments at an economical rate, and the trend is increasing globally (Oreagba *et al.,*2011). Herbal medicines which are alternatively called botanical medicine or phytomedicine refer to herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients (Pfserschy-Wenzig and Bauer, 2015). According to the World Health Organization, herbal medicines among the general population are medications prepared from one or more herbs or plant parts including roots, stem, bark, seeds, and leaves (Zhang *et al.,*2012). In Nigeria, herbal preparation of yoyo bitters have become a slight medical option in many Nigerian homes due to its easy access and affordability.

Yoyo bitters is a plant based medicine in the class of herbal bitters that was launched into the market in 2003 by Abllat Company Nigeria Limited. Abllat Nigeria limited is an indigenous manufacturer nature green medicine. After it was introduced into Nigeria drug market, yoyo bitters tonic has received wide acceptance and usage by general populace. The drug is certified by National Agency for Food, Drugs and Control (NAFDAC) as the first real bitters without alcohol, colouring or artificial preservatives produced in Nigeria (Ganong, 2003). Yoyo cleanser bitters is a powerful blend of some premium quality herbs well formulated to reduce free radical damage and removal of harmful toxins in the body, thereby supporting the immune system and the body’s ability to resist disease. Yoyo bittershelp the following systems in the body;Urinary and Excretory system, Circulatory System, through arterial dilation, Nervous system, Hardening of tissues, Weight control and It enhances bodies’ immunity (Ganong, 2003).

The ingredients used for production of yoyo bitters as published by the manufacturers are: *Aloe vera, Acinosavensis*, *Chenopodiummurale*, *Citrusaurantifolia* and *Cinamomumaromaticum*. Each of these components has several medicinal properties. Yoyo cleanser bitters is reported to be formulated in such a way that the ingredients have a synergistic effect which has been listed above.

*Aloe vera:Aloe vera* is a succulent plant species of the genus Aloe. An evergreen perennial, it originates from the Arabian Peninsula but grows wild in tropical climates around the world and is cultivated for agricultural and medicinal uses.

*Acinosavensis*: It serves as antiseptic, stimulant tonic. It is also used for shortness of breath, improving digestion, treating bruises, toothache, Sciatica and neuralgia.

*Chenopodium mural*: It is commonly known as nettle leaf goosefoot and is an important annual weed. It is distributed throughout the temperate and tropical regions and found in Pakistan in almost every field in winter season.

*Citrus aurantifolia*: Citrus species are among the native plants of Iran and the history of their cultivation dates back to 4000 years ago from which time, they have been widely used in the ethno medicine. These species with the wide range of bioactive ingredients have been found to exert anti-infection and anti-inflammatory properties. In addition, Citrus fruits have been found to be beneficial for cancer prevention in an epidemiological survey. These fruits contain several classes of phytochemicals and micronutrients such as limonoids and flavonoids, which have been reported to have antitumor effects in vitro and in vivo.In addition, Citrus fruits have been found to be beneficial for cancer prevention in an epidemiological survey (Wattenburg, 1985). These fruits contain several classes of phytochemicals and micronutrients such as limonoids and flavonoids, which have been reported to have antitumor effects in vitro and in vivo (Das and Pereira, 1990, Guthrie and Carroll, 1998, Kawaii *et al.*,1999).

*Cinamomumaromaticum*: Aqueous Cinnamon Extract from the bark of *Cinamomumcassia* (*Cinamomumaromaticum*) causes apoptosis in human cervical cancer cell line through loss of mitochondrial membrane potential.

* 1. **AIM AND OBJECTIVE**

The aim and objective is to determine the antimicrobial effect of yoyo cleanser bitters against microbial isolates using Agar well diffusion technique.

1. **MATERIALS AND METHOD**

**2.1 Materials Used**

* Media(Mueller Hinton Agar and Broth)
* Foil paper
* Analytical weighing balance
* Conical flask
* Measuring cylinder
* Autoclave
* Petri dishes
* Oven
* Incubator
* Biosafety cabinet/laminar flow
* Inoculating chamber
* Inoculating wire loop
* Cork borer (6mm)
* Gas, bursen burner and lighter
* Micropipette/multichannel micropipette
* Micropipette tips
* Cotton wool and alcohol
* Hot plate
* Spatula
* Sterile water
* Yoyo cleanser bitters
* Sterile syringes

**2.2 Microorganisms Used**

Pure culture of *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Candida albicans*and *Salmonella paratyphi*were obtained from the Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development.

**2.3 MediaPreparation**

All media used were prepared according to the manufacturer’s instructions.

**2.4 Preparation of Inoculums**

A wire loop of the overnight culture of each test organisms was transferred into 5mL of sterile Mueller Hinton broth, and then incubated for 1 hour.

**2.5Sample preparation**

The registered yoyo cleanser bitters was given by my supervisor. Different concentrations of 100%, 80%, 60%, 40%, and 20% were prepared using sterile water into sterile container using sterile syringes.

**2.6Procedure**

The zone of inhibition were determined using the agar well diffusion technique. One hundred microlitre(100µL) of each organism was added to the media using a micropipette and dispensed in the various sterile petri dishes labelled with marker in duplicates for each microbial isolates. Then it was swirl and left to solidify, in each of the plate, holes of 6mm in diameter was being bored aseptically, using a sterile cork borer. A drop of molten MuellerHinton agar was use to seal the base of the holes been bored. The different concentrations of the samples was being dispensed in the holes respectively and the control drug (chloramphenicol) was also dispensed in the control hole of each petri dishes and was allowed to stand for 30mins. The plates were then incubated at 37ºC for 24hrs. The procedure was repeated for other test organisms. After incubation, the zone of inhibition were measured and recorded.

1. **RESULT**

After incubation for 24 hours, the plates were being brought out and observe their zones of inhibition. There was no inhibitory zones in all the holes at different concentrations except for the control drug (chloramphenicol), which had inhibitory zone against the test organisms (Table 1).

**Table 1: Antimicrobial activities of different concentrations of the Yoyo cleanser bittersagainst microbial isolates.**

 **ZONE OF INHIBITION**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  **Microbial isolates** | **100%** | **80%** | **60%** | **40%** | **20%** | **Chloramphenicol (1.25mg/mL)** |
| *Pseudomonas aeruginosa* | --- | --- | --- | --- | --- | 18.0 |
| *Staphylococcus aureus* | --- | --- | --- | --- | --- | 22.0 |
| *Escherichia coli* | --- | --- | --- | --- | --- | 28.0 |
| *Candida albicans* | --- | --- | --- | --- | --- |  |
| *Salmonella paratyphi* | --- | --- | --- | --- | --- | 22.0 |

Key: (---) Represent No inhibitory zone.

1. **DISCUSSION**

Result from the study shows that yoyo bitter cleanser has no antimicrobial activity against the selected micro-organism used which are *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Candida albicansand Salmonella paratyphi.* The only well which shows the zone of inhibition contains the control drugchloramphenicol.A study conducted by Godswill*et al*., (2017), showed that yoyo bitters had appreciably high antioxidant and anti-inflammatory properties. Also a work reported by Ali*et al*., (2014), revealed that all the biochemically active compounds (flavonoids, alkaloids, tannins, saponins, steroids and balsam) tested for were all negative and also reported that high consumption of yoyo cleanser bitters for long period of time will have a significant effect on the histology of the cerebellum of wistar rats which may lead to cerebellar dysfunction.

The medicinal properties of yoyo cleanser bitters preparations could be attributed to their antioxidant and anti-inflammatory activities that could mitigate against oxidative stress and inflammation mediated diseases

**5.0 CONCLUSION**

The yoyo cleanser bitters is used in this study, showed no activity against the test microorganisms. This does not necessarily imply they possess no substances that can exert some antimicrobial activity. The method of preparation as well as the age and time of the harvest of the plants used for the herbal mixtures can influence their potency.

**RECOMMENDATION**

Antimicrobial activity should be carried out using different microbial isolates to determine if the drug is active against them.

This is the work I did at NIPRD, I did it personally we were told that we should do this work after the end of our IT training. We were being taught other things I will list them below :

1.about how to know the difference between selective medium and differential medium

2. Preparation of broth and agar

3. My supervisor, Mr Kazeem taught us the procedure to gram stain, different steps used in gram staining and also the procedure to streak plate method.

4. We were also being taught types of agar mostly used in the microbiology lab

5. We were taught about serial dilution.

6. We were also taught three ways to test for antimicrobial growth which are commonly used in microbiology laboratory at NIPRD

i) Agar well diffusion

ii) Agar dilusion and

iii) Disc diffusion method

We were being taught a lot of things.I’m told to carry out this work at the end of my IT training to see how we understand what they taught us. I carried the practical in the lab. I was supervised by my supervisor Mr kazeem.

**REFERENCES**

Abllat Nigeria Company Limited (2009). New Manuscript of Yoyo bitters.

Ali I. S., Musa B. T. U., ChiomaU., Gana J. U., Muhammed B. M., Francis S., Ahmed M. R and Rene M. (2014). The effect of Yoyo cleanser bitters on the cerebellum of adult male wistar rat. Sky Journal of Medicine and Medical Sciences Vol. 2(5), pp. 021 – 030.

Das N. P and Pereira T. A. (1990). Effect of Flavonoids on Thermal Autooxidation of Palm oil; Structure Activity Relationship, J. Am. Oil chem. Soc., ,255-258.

Ernst E. (2015). Herbal medicine in the treatment of rheumatic diseases. Rheum Dis Clin North Am ;37:95-102.

Ganong W. F. (2003). Review of Medical Physiology, International Edition. Published by Lange. pp. 223-224.

Godswill N. A., Babafemi O and Ifeoluwani A. A. (2017). Antioxidant and anti-inflammatory properties of selected polyherbal preparations: Oroki herbal, Swedish bitters and Yoyo bitters. Oxidants and Antioxidants in Medical Science. 6(2): 25-29.

Guthrie N and Carroll K. K. (1998). Inhibition of mammary cancer byCitrus flavonoids, Adv. Exp. Med. Biol., 439, 227-236.

Kawaii S, TomonoY, Katase E, Ogawa K and Yano M. (1999). Antiproliferative effects of the readily extractable fractions prepared from various Citrus juices on several cancer cell lines, J. Agric. Food Chem., 47, 2509-2512.

Leonard B and Maes M (2012). Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. NeurosciBiobehav Rev;36:764-85.

Oreagba I. A., Oshikoya K. A., Amachree M. (2011). Herbal medicine use among urban residents in Lagos, Nigeria. BMC Complement AlternMed ;11:117.

Pferschy-Wenzig E. M and Bauer R. (2015). The relevance of pharmacognosy in pharmacological research on herbal medicinal products. Epilepsy Behav;52:344-62.

Shinwari Z.K. (2010). Medicinal plants research in Pakistan. J Med Plants Res 2010;4:161-76. 2.

Wattenburg L. W. (2012). Chemoprevention of cancer, Cancer Res., 45, 1985, 1- 8.

Zhang J, Wider B, Shang H, Li X and Ernst E. Quality of herbal medicines: Challenges and solutions. Complement TherMed ;20:100-6.