Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/10428
DOI URL: http://dx.doi.org/10.21474/IJAR01/10428

RESEARCH ARTICLE

EVALUATION OF ANTISEPTIC PROPERTIES OF SOAPS PRODUCED WITH *PHYSCIA GRISEA* EXTRACT

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Manuscript Info

Manuscript History
Received: 01 December 2019
Final Accepted: 03 January 2020
Published: February 2020

Key words:-
Evaluation, Antiseptic Soap, Properties, Physcia grisea, E. coli

Abstract

Evaluation of soaps produced with ethanol extract of Physcia grisea was carried out to determine the antiseptic properties of the ethanol extract when used in soap production. The soap production was carried out by saponification process using ethanol extract as an antiseptic agent instead of synthetic chemicals. The quality of the soap produced from antiseptic agent and the extract were assayed for antimicrobial activity using Agar Cup Diffusion Technique. The result showed that the soap had a significant activity on Escherichia coli (E. coli), which is an indicator organism for fecal contamination. The minimum inhibitory concentration (MIC) of P. grisea extract was 10 mg/ml while that of the P. grisea antiseptic soap was 12.5 mg/ml. The extract also gave a natural brownish colour to the soap. This showed that antiseptic soap produced using P. grisea extract has the advantage of possessing attractive brown colour in addition to the novel antiseptic properties.

Introduction:-

In recent times, production of antiseptic soaps with so many synthetic chemicals has been on increase. Some of these synthetic chemicals used in antiseptic soap production have harmful effects on both human skin and environment. For instance, while some synthetic chemicals used in antiseptic soap production attack human skin (Okumura and Nishikawa, 1996), others can corrode bathroom wall paint (Sooknah *et al*., 2007).

Generally, antiseptic soaps are known for their antimicrobial actions on human skin. They are soaps, which contain chemical ingredients that assist in killing of microbes on human skin (FDA, 2016). In most cases, producers of these antiseptic soaps use common synthetic chemicals such as triclosan, trichlorocarbanilide and chloroxylenol that have both carcinogenic and mutagenic effect on human. In some cases, these synthetic antiseptics cause allergic reactions to human skin, dry the skin and even poison the nervous system (Okumura and Nishikawa, 1996). Besides, certain bacteria easily develop resistance to some synthetic chemicals used in antiseptic soap production such as triclosan and this could cause change in both microbial morphology and physiology (Aiello *et al*., 2007).

Since triclosan and other related synthetic antiseptics work principally by blocking the enzyme in the bacterial fatty acid-biosynthesis pathway, their constant use in antiseptic soap production have proliferated resistant bacterial strains (Yazdankhah, 2006). These disadvantages of antiseptic soaps produced from synthetic chemicals amongst

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others call for the need to research for a more user and environmental friendly antiseptic soaps from safe sources such as plant extracts (Ameh *et al*., 2013). However, research in that direction is still limited. This could be because of limited knowledge on plant extracts with safe and powerful antiseptic properties that could be used for such soap production. One of the plants with such safe and broad-spectrum antiseptic attributes that can be harnessed is *P. grisea* (Eze and Ogonnaya, 2010).

P. grisea is lichen found on walls, rocks and trees attached by short threads that grow from the underside and are white with black tips (Eze and Onumah, 2010). The plant is light grey or slightly brownish and is almost covered near the tip of the lobes with a very fine powder. The plant has been found to possess both antibacterial and antifungal properties, which can be, harnessed (Eze, 2007).

Production of antiseptic soaps with *P. grisea* may therefore result in an environmental friendly soap which will be safe to human body and hence this study.

The Aim of the Study:
The aim of the study was to produce and evaluate the antiseptic activity of soap produced with safe and affordable *P. grisea* extract.

Materials and Methods:-

Collection and identification of *P. grisea*:
The *P. grisea* plants were obtained from Amogu Ezimo-Uno Community, Udenu Local Government Area of Enugu State and were identified in the department of Crop Science, University of Nigeria, Nsukka.

Preparation of *P. grisea* Extract:
The extraction of *P. grisea* was carried out as described by Eze *et al*. (2010). About 56.0 g of pulverized *P. grisea* plants were weighed out using Mettler sensitive balance and poured into 500 ml flat bottom flask. This was added 1000 ml of absolute methanol to get 56 g/L. The mixture of the pulverized *P. grisea* and the absolute methanol in the flask was stirred with a magnetic stirrer for 18 h before it was allowed to stand for 24 h. This was filtered using a clean muslin cloth and the filtrate concentrated in the oven (Gallen Kamp, England) at 60 ^{o}C**.**

Sterilization of Materials:
The glassware used for this work was sterilized using hot air oven at the temperature of 160 ^{0}C for 1 hour. The Nutrient Agar medium used for the study was sterilized with autoclave at temperature of 121 ^{0}C for 15 min as described by Adibe and Eze (2004).

Preparation of Medium:
The preparation of Nutrient Agar medium was prepared according to manufacturer's guide as described by Eze and Onumah (2010). About 28 g of the nutrient agar was weighed 1 litre of distilled water in a sterile conical flask and was dissolved in 100 ml of distilled water. This was homogenized and sterilized with autoclave at temperature of 121 ^{0}C for 15 min. Thereafter, 20 ml sterilized medium was dispersed into Petri dishes and allowed to cool so as to solidify.

Preparation of Sodium Hydroxide:
About 500 g of NaOH was weighed out into 1,500 ml of water and put into a plastic bucket. This was stirred continuously until the solution was completely dissolved and was allowed to cool. This was tested for sterility within 24 h**.**

Preparation of the Test Organism:
Preparation of the test organism, *E. coli*, was done by transferring a colony the organism into a sterile test tube containing 2 ml of normal saline. It was then corked with sterile cotton wool and kept at room temperature to be used shortly**.**

Preparation of Antiseptic Soap using the Ethanol Extract:
The preparation of *P. grisea* antiseptic soap was carried out by saponification process using 200 ml of the palm kernel oil, 200 ml of soaked mixture of sodium hydroxide, 5 ml of analytical ethanol, 20 ml of *P. grisea* extract, 10

ml fuming agent, 10 ml silicate was added to the mixture, then fragrance, hardener with a continuous stirring and a solution of sodium chloride (NaCl) for salt out**.**

Molding of the antiseptic soap:
The molding of *P. grisea* antiseptic soap was done using a soap molder constructed in the Department of Science Laboratory Technology, University of Nigeria, Nsukka. The soap solution was poured into a soap molder greased with paraffin oil for easy of removal after solidifying. This was allowed to stay for 24 h for solidification**.**

Sensitivity testing:
The sensitivity testing of *P. grisea* antiseptic soap and *P. grisea* extract were evaluated by the agar diffusion method as described by Agboke *et al*. (2005) to determine the inhibition zone diameter (IZD) of the agents as well as sensitivity pattern of the clinical isolates of *E. coli*.

Determination of the Inhibition Zone Diameter of the Soap:
Sterile Petri dishes were aseptically seeded with 0.1 ml of freshly prepared suspension of *E. coli* using a sterile pipette. A 20 ml aliquot of a sterile molten nutrient agar at 45 ^{0}C in McCartney bottle was poured into each plate and swirled clockwise and anti-clockwise for even distribution of the organism. After solidifying, the agar plates were marked into four sections after solidifying. The four sections represented the four two-fold dilutions of the extract (1600 mg/ml, 800 mg/ml, 400 mg/ml and 200 mg/ml) and labelled 1 – 4 with an indelible marker. Using a sterile 6 mm cork-borer, cups were made in each of the four divisions. The two fold dilutions of the *P. grisea* extracts were aseptically added in the cups using standard sterile dropper starting with the highest concentration of the *P. grisea* extract to that with lowest concentration of the extract. The plates were incubated at 37 ^{0}C for 24 h and the zones of inhibition were measured. The graph of the inhibition zone diameter square against the logarithm of the concentrations of the dilutions used was plotted to extrapolate the minimum inhibitory concentration (MIC) of the extract (Eze and Onumah, 2010).

Determination of Inhibition Zone Diameter of *P. grisea*:
This was carried out as described above except that the extract was replaced with 200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml of *P. grisea* extract only**.**

Results and Discussion:-

The photograph of the *P. grisea* antiseptic soap and the inhibition zone diameters on *E.coli* were showed in Figure 1.

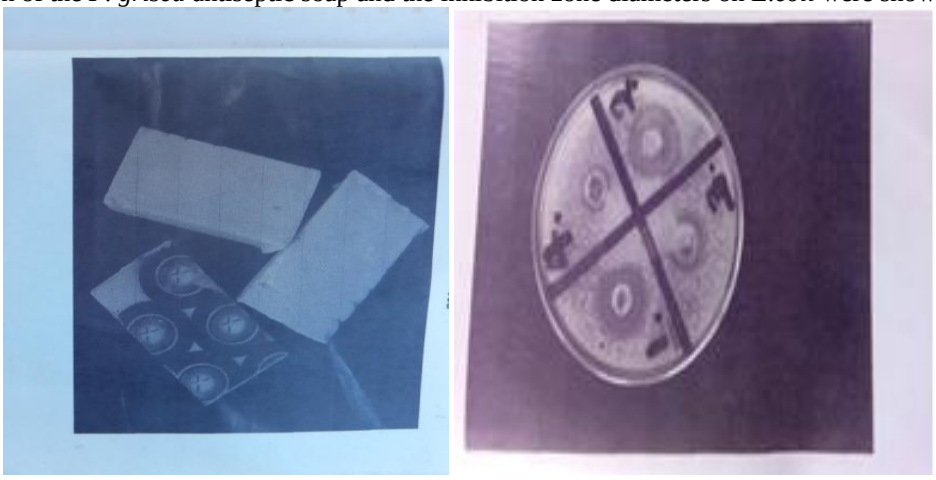

Figure: 1.A:- Bars of *P. grisea* antiseptic soap.

Culture plate showing the inhibition zone diameters of the *P. grisea* antiseptic soap on *E. coli*
The choice of *E. coli* used in this study was informed by the fact that *E. coli* is an indicator organism for fecal contaminant and had been reported *E. coli* as a relevant microorganism used for testing the efficacy of antiseptic soaps and hand washes (Mwambete and Lyombe, 2011). The brownish colour of the antiseptic soap looked very attractive and may have been impacted by the *P. grisea* extract. Eze *et al*. (2010) had reported *P. grisea* plant to be brownish and this could be responsible for the attractive brownish colour of the *P. grisea* antiseptic soap. Thus, the

P. grisea plant, in addition to possessing antimicrobial or antiseptic properties, has the capacity to impact natural brownish colour to the soap. This has a great advantage since the colour could be readily available as well as cheap for thousands of soap industries, which spend heavily in procuring synthetic colours used as soap raw materials.

The preliminary sensitivity test of *E. coli* to *P. grisea* and *P. grisea* antiseptic soap indicated that *E. coli* was moderately sensitive to *P. grisea* antiseptic soap and highly sensitive to *P. grisea* extract (Table 1).

Table 1:- Sensitivity of *E. coli* to *P. grisea* antiseptic soap.

Antimicrobial Agent	***E. coli***
P. grisea antiseptic soap	++
P. grisea extract	+++

++ = *E. coli* was moderately sensitive to *P. grisea* antiseptic soap; +++ = *E. coli* was highly sensitive to *P. grisea* extract.

The MIC of *P. grisea* extract was 10 mg/ml while that of the *P. grisea* antiseptic soap was 12.5 mg/ml (Tables 2 and 3). The results of the analysis of variance showed that the difference in the IZDs of *P. grisea* extracts alone and that of *P. grisea* antiseptic soap was statistically not significant ($p < 0.05$). Although that of *P. grisea* extract was lower, the MIC value was within the recommended value for antiseptic soaps (Mwambete and Lyombe, 2011). However, the increase in the MIC value of *P. grisea* antiseptic soap showed that the antibacterial activities of the *P. grisea* extracts were higher. Eze and Onumah (2010) had reported that decrease in MIC is always associated with increased antimicrobial properties**.**

At present, several research findings have shown that the values of MICs of antiseptic agents are increasing (Kaliyadan *et al*., 2014). This shows a global increase in the incidence of resistance strains of some species of organisms such as *E. coli* and related organisms associated with skin infection to different antiseptic agents used in many homes (Eze *et al*., 2009). The resistance developed by these organisms in this case, may have resulted from prolonged use and adaptability of the microbes to the antiseptic agents. Eze and Ogonnaya (2010) reported lengthened use of a particular antimicrobial agent to be associated with the organism's adaptability to the agent. The challenge therefore, has been to develop a low cost antiseptic soap with high level of efficacy and stability against these organisms that cause skin infections. One of the ways of approaching this challenge could be by evaluating antiseptic properties of a stable and broad-spectrum medicinal plant such as *P. grisea*. The use of such plant extract may broaden both the antibacterial and antifungal spectrum, with lower risk of resistance (Eze *et al*., 2009). Besides, biologics from such medicinal plants have opportunities for other health benefits because of their chemical diversity (Eze *et al*., 2007). In this case, harnessing *P. grisea* extract used in this study for production of antiseptic soap could be a noble invention.

Table 2:- The concentration, inhibition zone diameter and log concentration of *P. grisea* activities on *E. coli*.

S/N	**Conc mg/ml**	**IZD (mm)**	**IZD^2 (mm^2)**	**Log conc.**
1	1600	12.00	144	3.204
2	800	10.00	100	2.903
3	400	8.00	64	2.602
4	200	6.00	36	2.301

The inhibition zone diameter (IZD) and minimum inhibitory concentration (MIC) of the antiseptic soap were statistically the same with that of *P. grisea* extract when compared using Students-t test. These values of both IZDs and MIC indicate that there was a beneficial interaction between *P. grisea* extract and other components of soap such as caustic soda and acids. Eze (2007) had observed that combined interactions of agents can enhance IZDs positively. The ethanol extract of *P. grisea* has therefore numerous advantages over many other antiseptic agents used in antiseptic soap production.

Table 3:- The concentration, inhibition zone diameter and log concentration of soap on *E. coli*

Conc. mg/ml	**IZD (mm)**	**IZD^2(mm^2)**	**Log conc.**
200	23	529	2.30
100	21	441	2.00
50	15	225	1.70

25	11	121	1.40

Conclusion:-

The results of the study showed that soaps produced with *P. grisea* extract have good antiseptic attributes. The high level of inhibition zone diameters showed on *E. coli* suggested that *P. grisea* could be of great importance in alleviating harmful effects of synthetic/inorganic chemicals used for antiseptic soap production on both human body and environment. The safe natural brown colour of *P. grisea* impacted on the soaps was cheap and could be harnessed globally for production of antiseptic and other soaps. Utilizing these attributes of *P. grisea* in soap production will not only lower the cost of soap production, but could go a long way to encourage thousands of unemployed youths who could start soap production with limited resources to move into soap business.

Acknowledgements:-

This research was sponsored by the authors; however, fragrance and soap hardener were provided by Ever Prince Market Nigerian Ltd. The authors are highly indebted to the Department of Crop Science, University of Nigeria, Nsukka for allowing them use the Laboratory for the research.

Conflict of Interests:

The authors declare that there is no conflict of interests regarding the publication of this paper.

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