ANTIMICROBIAL ACTIVITY OF FORMULATED LOCAL HERBAL SOAP MADE FROM PAW-PAW (*CARICA PAPAYA)* LEAF EXTRACT.

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ABSTRACT
Herbal soap has been found to be effective against most microorganisms because it is mostly formulated from plants. This study aimed at formulating herbal soap from paw paw (*Carica papaya*) leaf extract (*Caricapapaya*) tested against 3 microbial isolates: *Staphylococcusaureus,Pseudomonasaeroginosa* and *Candidaalbicans* using agar well diffusion method. The herbal soap showed significant activity at ($P<0.05$), demonstrating its activity against the tested isolates with the highest activity (24.00 mm zone of inhibition) demonstrated against *Candida albicans.Pseudomonas aeruginosa* had its zone of inhibition at (18.00 mm) remove brackets for meaningful statement while *Staphylococcus aureus* was at (13.00 mm) remove brackets respectively. The Minimum Inhibitory Concentration (MIC), was most effective at 80 and 100 mg/ml of the extracts except for *C. albicans* with the MIC at 100mg/ml. The Minimum Bactericidal Concentration (MBC) of the extract was at 100 mg/ml.
Keywords: Leaf Extract, Antimicrobial quality, Local Herbal Soap, Zones of Inhibition, Natural products

INTRODUCTION

Soaps aid in general body hygiene by physical removal of microorganisms from the skin. The act of washing or scrubbing the body with the soap is expected to lead to a reduction in the microbial load on the skin and this contributes to a reduction in the incidence of skin infections. Apart from this physical removal of organisms on the skin, the achievement of therapeutic effect of an herbal soap can be due to direct antimicrobial activity on microorganisms present on the skin. (Igenegbu, 2013).

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Traditional healers have long used plants to prevent or cure infectious conditions; Western medicine is trying to duplicate their successes (Trautmann and Halder, 2008). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties (Georges *et al*., 1949).Ethnobotany is define as a multi-disciplinary science that deals with the interaction between plants and people. The relationship between plants and human cultures is not limited to the use of plants for food, clothing and shelter but for orientation and health care (Choudhary, 2008). According to Clark (1996), plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population over 50% of all modern clinical drugs are of natural product origin (Kirbag*et al*., 2009). Phytochemical such as vitamins (A,C,E and K), Carotenoids, terpenoids, faronoids, alkaloids, tannins, saponins, pigments, enzymes and minerals that have antimicrobial and antioxidant activity (Madhuri and Pandey, 2009). Antimicrobial screening of plant extracts and phytochemicals, represents a starting point for antimicrobial drug discovery. Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques (Adebayo *et al*., 1989). These techniques played significant role in the search for additional resources of raw material for pharmaceutical industries (Shakeri*et al.,* 2012), medicinal plants possessingimmunomoulatory and antioxidant properties, leading to versatile immunomodulatory activity by stimulating both non-specific and specific immunity (Pandey and Chowdhry, 2006). Papaya is a small, sparsely branched tree usually with a single stem growing from 5 to10m (16 to 33ft) tall, spirally arranged leaves confined to the top of the trunk; the lower trunk is conspicuously scarred where leaves and fruit were borne (Rivera-Pastrana *et al*., 2010). The leaves are large, 50-70cm (20- 28ln) in diameter, deeply palmately lobed, with seven lobes. Papaya is native to Mexico and extends to South Africa and has become naturalized throughout the Caribbean islands, Floridaand several countries of Africa. From laboratory tests, extracts from skin, flesh, and seeds of both ripe and unripe papaya showed invitro antibacterial activity against several microorganisms, including *Bacillus cereus, Bacillus subtilis, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonellatyphi, Shigella flexneri*, and *Staphylococcus aureus, Candida albican* and *Streptococcus pyogenes.* In addition, Clinical isolated bacteria in the study are *Staphylococcus aureus* (gram positive), *Pseudomonas aeruginosa* and *Candida albican*(Fernadaz*et al*., 1996). The aim of this study was to determine the antimicrobial activity of local herbal soap made from paw paw (*Carica papaya*) leaf extract.

MATERIALS AND METHOD

The leaves of*Carica papaya* (Pawpaw) were collected, identified and confirmed in the Microbiology Laboratory by Laboratory attendant Biotechnology Department, Godfrey Okoye University, Enugu. Two (2) bacteria and one fungiwere obtained from stock cultures in the Microbiology Laboratory of the University of Nigeria Teaching Hospital, Enugu, Nigeria. *Staphylococcus aureus, Pseudomonas aeroginosa, Candida albicans.* The media used were Sabouroud Dextrose Agar, Blood agar, MacConkey agar, Mueller Hinton Agar and Nutrient agar. The Paw paw leaves were washed properly with clean running tap water. The washed leaves were placed on a cleaned plastic sieve to drain. The washed leaves were allowed to dry at room temperature. The extract was obtained using a dry extraction method. The dried leaves were blended into a powder form using a blender. Two hundred grams (200g) of the powdered *Carica papaya* sample (leaf) was weighed using a weighing balance into a five (5) litre capacity plastic can, with a lid. 25mls of the solvent,was added to the sample. The plastic container containing the sample was allowed to stand for 24 hours, with constant agitation of the container to ensure proper mixture of the content. After 24 hours of shaking and mixing, it was filtered using a sieve with tiny pores. The filtrates were then filtered again using Whatman's filter paper. The filtered extracts were concentrated by allowing to evaporate at room temperature to a semi-solid form. A sticky semi-solid greenish substance was obtained from the sample. The extract was stored in a well corked universal bottle at room temperature (Ewansiha*et al.*, 2012).

One gram of NaOH was dissolved in 5 ml of distilled water, into which one gram (1g) of sodium silicate was added and the mixture cooled to room temperature. Varying amounts of the *Carica papaya* leaf extract were sequentially added ranging from 0.0 to 15.0 % (w/w) into five separate batches of the latter mixture. Stirring was immediate until a thick paste of homogenous soap resulted. The soap samples were left to solidify at room temperature (Kareru, 2010).0.5ml of 1.175%w/v Barium Chloride Dehydrate ($BaCl.2H_2O$) solution was added to 99.5 ml of 15%w/v Sulphuric acid (H_2SO_4). The mixture was dispensed into tubes identical to the ones used in preparing inoculums suspension of the test organisms. The test tubes containing the McFarland standard were stored in well-sealed container in dark room at room temperature until when needed (Cheesebrough, 2006). The test inoculum was standardized using the method described by Vandepitte*etal.* (2003).Saline solution was prepared by dissolving 8.5g of NaCl into 100 ml of water, sterilized by autoclaving at 121^0C for 20 minutes. The pH was adjusted to approximately 7.0 and 4ml was dispensed into the test tubes. Sterile wire loop was used to pick a loopful of inoculum from the pure culture of the test organisms. The turbidity was compared with the turbidity standard and adjusted by adding more test organism or more normal saline until it gets to 0.5 McFarland which is approximately $1x10^6$CFU/ml. the inoculum suspension will then be used within 15 minutes to avoid further growth.

The agar well diffusion method recommended by CLSI (2006) was employed for antimicrobial testing. Two distinct colonies of each test organism were taken from a 24-hours agar culture and were suspended in 10 ml sterile distilled water in test tubes using a sterile loop. The suspension was thoroughly mixed with a spin mixer and then adjusted to 0.5 McFarland Standard. The suspension was applied in duplicates to the surface of dried Mueller Hinton agar (Oxoid) using sterile swab sticks. The soap discs were then applied in duplicates equidistant to one another on the inoculated plates. The plates were refrigerated at 4°C for 30 minutes to ensure adequate diffusion of the soap and all test plates were incubated at 37°C for 18–24 hours. At the end of incubation period the diameter of zones of inhibition around each disc was measured in millimeter and the mean of duplicate experiments were recorded. Similar procedure was carried out for the antifungal test but test fungi were picked from Sarbouraud Dextrose Agar (Oxoid) plates, suspended in water and swabbed on dried Sarbouraud Dextrose Agar plates. An antiseptic soap containing triclosan 0.6%w/w was used as a reference antiseptic soap(Igbeneghu, 2013).

The MIC helps to measure more exactly the concentration of an antibiotic necessary to inhibit growth of a standardized inoculums under defined conditions (Geo *et al.*, 2001). Serial dilutions of the soap in liquid medium were prepared. These were then challenged with small inoculums of an overnight broth culture of the test organisms. The culture was then challenged with small inoculums of an overnight broth culture of the test organisms. The culture was then incubated at 37°C for 48hours. The smallest concentration that inhibits the growth was taken as the MIC. The determination of the value of MBC follows the determination of MIC by the broth dilution technique. The MBC is the lowest concentration of the antibacterial agent that kills at least 99.9% of the test organism (Geo *et al*., 2001). To determination this value, about) 0.5ml of the sample was removed from the test tubes used in the determination of MIC in which there was no desirable growth spread over the surface of the over dried nutrient agar plates. The lowest concentration of the agent that prevent the growth of less than 0.1% of the test organism on the recovery plate was taken to be the MBC value for the extract.

RESULTS AND DISCUSSION

Table 1 showed the antimicrobial activities of the Herbal soap against the tested organisms. The herbal

soap was more effective on *Candida albicans* than *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The highest zones of inhibition (24mm) was produced from the 2g of the herbal soap sample. At concentration of 0.5g, the tested soap produced no zone of inhibition on all the tested organisms.The herbal soap at the quantity of 2g produced higher zone of inhibition of (24.00mm) compared to the control soap which produced a zone of 15.00mml at the same quantity.The result of this study showed that the herbal soap were more effective on the tested organisms than the control soap. This may be due to the active components of the *Carica papaya* contained in the soap. Table 2 showed the Minimum inhibitory concentration of the herbal soap on the tested organisms. 100ml of the herbal soap inhibited all the organisms while 40ml were not able to produce any inhibition. At concentration of 100ml, the herbal soap were bactericidal to all the tested organisms as shown in table 3.

Table 1: Zones of inhibitions for organismsisolated in mm

Microbial isolate	**Control (Antifungal soap) (2g)**	**Test Sample (0.5g)**	**Test sample(1g)**	**Test sample(1.5g)**	**Test sample(2g)**
Candida	**15.00**	**-**	**15.00**	**14.00**	**24.00**
Pseudomonas	**15.00**	**-**	**13.00**	**14.00**	**18.00**
Staphylococcus	**15.00**	**-**	**-**	**11.00**	

Table 2: Minimum inhibitory concentration of *Carica papaya* on different concentration.

Isolates	100	80	60	40
Staphylococcus	-	-	+	++
C. albicans	-	+	++	+++
Pseudomonas	-	-	+	++

+= growth, -= no growth

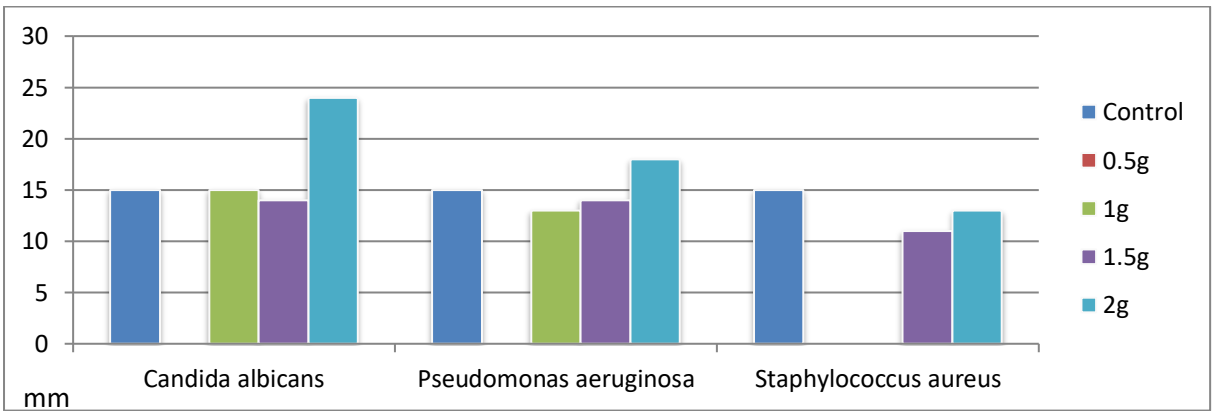

Figure 1: Comparison of zone of inhibition found in *Candida albicans*, *Pseudomonas aeruginosa*and *Staphylococcus auerus.*

Table 3: Minimum bactericidal concentration of*Carica papaya*.

Isolates	Concentration
Staphylococcus aureus	100
Candida albicans	100
Pseudomonas aeruginosa	100

The results of this study showed that the herbal soaps were more effective depending on the varying concentrations. This may be due to the better solubility of the active component of the paw paw leaves extract used in the production of the soap. (De Boer *et al*., 2005). Among the gram-positive and gram-negative bacteria tested against the herbal soap, Gram-negative bacteria was more susceptible which is *Pseudomonas aeruginosa* to the soap extracts. The soap was very efficacious against *Candida albicans,* indicating that fungi was very susceptible to the herbal soap as against findings from (Jigna and

Sumitra, 2006). Although, several factors may predispose bacteria or fungi to antimicrobial agents such as previous encounters with the agents or the nature of medium used, the demonstration of activity against the test bacteria and fungi provides scientific bases for the local usage of these plants in the treatment of various ailments. The fact that the extracts were active against both gram-negative, gram-positive bacteria and fungi that were tested may indicate a broad spectrum of activity. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multidrug-resistant organisms. The Minimum inhibitory concentration (MIC) in (Table 2) value observed is a good indication of high efficacy against this bacterium and fungi. This outcome is remarkable considering that boil, breast abscess and surgical wound infection etc (caused by *Staphylococcus aureus*) is on the rise and also becoming recalcitrant to first-line antibiotics for its treatment in developing countries, including Nigeria. High MIC may be an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds .Temperature stability of plant extracts has been reported earlier (Dougharie*t al.,* 2006).This may be an indication that the bioactive compounds form synergic relationship with inorganic solvents and explains the ethno-botanical application process of the plants where boiling at very high temperatures for extended time periods are often practiced without the concoctions losing their efficacy as reported in Borris (1996).

CONCLUSION

The findings of this study showed that the soap had its MBC at the concentration of 100mg/ml and is a considerable fact for the extract. In concluding, the demonstration of antimicrobial activity against both gram-negative and gram-positive bacteria and also fungi is an indication that the plant is a potential source for production of drugs with a broad spectrum of activity. Therefore, the formulated local herbal soap from *Carica papaya* leaf extract was found to be very effective on both bacteria and fungi. The results of the study also supports the traditional application of the plant and suggests that the plant extracts possess compounds with antimicrobial properties that can be used as antibacterial agents and antifungal agents in novel drugs for the treatment of skin infection, boil and surgical wound infections. Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antimicrobial from this plant are recommended for future plan and it is recommended that industries can make further contact for production of local herbal soap that handle problems peculiar to our environment.

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