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Synergistic Effect of oils extracted from *Bryophyllum pinnatum* Leaf and *Prunus dulcis* in Formulated Herbal Soap

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Abstract

Natural remedy have been in use by homeopathic and herbalists in handling skin problems, in this present study, synergistic effects of essential oils extracted from local plants *Bryophyllum pinnatum* (odaa opue) leaves and *Prunus dulcis* (almond) seeds in formulated herbal soap was investigated. These bio materials were dried at temperature of 80 ^{0}C for three days and extracted using solvent extraction method with acetone via soxhlet extractor. Oil yields were 22.58/287g and 16.88/281 g for B. *pinnatum* and *P. dulcis, respectively*. The oils (*B. pinnatum* and *P. dulcis*) were used in single and combined **doses** in the ratios of 2:8, 4:6, 6:4, 8:2, 0:0 (blank),0:5, 5:0 and 5:5, respectively to formulate the soaps of varying concentrations. Ciprofloxacin and ketaconazole were positive controls whereas *pseudomonas aureginosa, staphylococcus aureus, Escherichia coli* and *candida albicans* were clinical isolates of microbes used. Sensitivity test was conducted and an *in vitro* evaluation of the anti-microbial activity was conducted using ditch-plate techniques. Strains of reference microbes namely *Candida albicans*, *Staphylococcus aureus* , *Pseudomonas aureginosa* and *Escherichia coli* were tested at four different soaps' concentrations (0.0, 2.0, 5.0 and 8.0 mg/ml). ANOVA mean results of zones of inhibition also revealed variability of antimicrobial activity among the natural formulated soaps with positive correlation **($P<0.01$)** between zones of inhibition and soaps' concentrations was evidenced. Synergistic effects of (P. *dulcis*: B. *piñata*) ratios 2:8 soap exhibited the largest zone of inhibition (36 mm) on *S. aureus,* followed by *Candida albicans* with (30 mm) zone of inhibition whereas single formulations gave the least zone of inhibition (9mm and 13mm) respectively for P. *dulcis* and B.*pinatum* ,respectively. Therefore with local plant materials in synergy, an effective, cheap and environmentally friendly herbal soap was explored hence, we recommend the product to homes, pharmaceutical companies, health organizations and government in handling topical issues related to skin infections caused by micro organisms.

Keyworks: *Bryophyllum pinnatum* leaf; *Prunus dulcis*; herbal soap.

INTRODUCTION

Soap is a mixture of sodium and potassium of various naturally occurring fatty acids and substance of ancient origin (Gunstone *et al.*,1986). Several millennia ago, mixture of animal fats and alkaline potash were found to generate crude soap which lathered and cleaned effectively (Phansteil *et al.*, 1998). Generally, antimicrobial soap could be any cleaning soaps to which Antibacterial Active Ingredients (AAIs) have been added (Chemother, 2001). These chemicals kill bacteria and other microorganisms, though they are not effective at deactivating viruses just like any other kind of soaps. Soaps are intended for reduction of the inoculums sizes of both pathogenic and non-pathogenic microorganisms; the latter include the normal flora.

Of these, two types are well known viz. resident flora that are the normal flora of the skin and other human body parts, and transient flora that are usually picked up from objects or other human beings (Chemother, 2001). The reaction for making soap (saponification) is a base hydrolysis of triglycerides to make salts soap and glycerol. Several factors affect the soap making process and the quality of the soap produced such as the quality of oil and the amount of water and soap used to make it. Using medicated soaps in washing of hands helps in promoting defense mechanisms against bacteria and other pathogens that can cause colds, flu, skin related infections and deadly communicable diseases (Kimel, 1996); (Poole,2002). According to (Levy, 2001), antimicrobial portion of soap is effective at preventing communicable diseases, however a good soap should be free from harmful chemical that will affect the skin and environment. Nature has bestowed our planet with an enormous wealth of medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention and cure of diseases and ailments (Young *et al.*, 2004). Numerous cleaning agents are available in the market, which are presented in various forms with distinct formulations. Triclosan, trichlorocarbamide and p-chloro-m-xylenol (PCMX/chloroxylenol) are the commonly used antibacterials in medicated soaps. These are generally, only contained at preservative level unless the product is clearly marked as antibacterial, antiseptic, or germicidal (Larson, 1988). In making soap, triglyceride in fat or oil are heated in the presence of a strong alkali base such as sodium hydroxide, producing three molecules of soap for every molecule of glycerol. The process is called saponification. Like synthetic detergents, soap are "surface active substances (surfactant) and as much make water better at cleaning surfaces. In studying how soap works, it is useful to consider the general rule of nature, like the non polar hydropholic tail of soap are lipopholic (oil-loving) so will embed into the greese and oil to help dirt and stain adhere to surfaces hydropholic heads.

Bryophyllum pinnatum, known as the air plant, cathedral bells, life plant, miracle leaf, and Goethe plant is a succulent plant native to Madagascar, which is a popular house plant and has become naturalized in tropical and subtropical areas. It is distinctive for the profusion of miniature plantlets that form on the margins of its phylloclades, a trait it has in common with some other members of its genus. It is a succulent, perennial plant, about 1m tall, with a fleshy cylindrical stem and a reddish color for the youngest and it flowers most of the year (Ali, 2013)

It has been used in the Indian medicine for treatment of headaches (National light, 2019). For the people of the Amazon, kalanchoe has multiple uses: the Creoles use it roasted against inflammations and cancer and as an infusion, and as a popular remedy for fevers. Against headaches, Palikur rubs their forehead with a mixture of kalanchoe leaf juice with coconut oil (Hermann ,1983).

Prunus dulcis also known as Almond is a fruit of the deciduous tree native to Iran and surrounding countries but widely cultivated elsewhere (Harvard, 2020). It also penetrates deep into the skin to kill bacteria and remove dirt. Almond oil keeps the skin moisturized for a long time. It is also helpful in treating acne. Fatty acids and vitamins A and E in almond oil are especially beneficial in repairing dry skin. When almond oil is heated to body temperature and used in skin massage, the skin will become moistened. Almond oil protects your skin from developing rashes. The oil is edible and hence, it can be applied to chapped or cracked lips to make them soft and pink again. Almond oil is extracted from raw almond seeds. Using almond oil to nourish your skin can clear it up, make it glow and give you a clear complexion too. Almond oil provides a lot of skin benefits like treating acne scars, moisturization, de-pigmentation and it helps in lightening the skin as well. Almonds are tasty and healthy snacks that are capable of increasing one's intellectual ability. Almonds are a healthy treat and possess several health benefits. It is one of the most beloved nuts that are consumed in various ways. Almonds are very good for skin as they help in nourishing the skin. Almonds are rich in vitamin E that helps in moisturizing your skin and also prevents the occurrence of various skin diseases, such as acne, pimple etc. Its vitamin E content gives it good blend in soap formation and hence stable emulsion. Almond oil is light and hence won't clog the pores. Consumption of almonds on daily basis helps humans to maintain a clear glowing skin.

Chemical constituents of *Bryophyllum pinnatum*

Bufadienolide compounds they are isolated from *Bryophyllum pinnatum* and include bryophillin A which showed strong anti-tumor promoting activity *in vitro*, and bersaldegenin-3-acetate and bryophillin C which were less active (Supratman *et al.*,2001). Bryophillin C also showed insecticidal properties (Supratman *et al.,* 2009).

Statement of the Problem

There have been problems as a result of bacteria, fungal and viral infections. According to World Health Organization drugs, micro-organisms like *Escherichia coli* and *staphylococcus aureus* are developing resistance to several drugs which is posing severe threat like inflammatory and other infections to the general public health. The importance of plants in medicine remains even of greater relevance with the current global shift to obtain drugs from plants sources as a result of which attention has been given to the medicinal value of

herbal remedies for safety, efficiency and economy (Akindehinci and Salawu, 2003). According to (Levy, 2001) and (White, 2001), over exposure to medicated soaps might result in antimicrobial resistance and even rendering an individual more vulnerable to microbial attacks such as opportunistic skin infections (Russell, 1998).

On the other hand, regardless of a wide-spread availability of the so-called medicated soaps; a number of communicable infectious and food-borne diseases as well as poor-hygienic conditions-related health problems are rampant. This can partially be explained by the fact that, occasionally some of these antimicrobial consumer products could have insufficient quantities of antimicrobials. It seems to be more of a marketing phenomenon. Unfortunately, in the long-run may adversely affect the consumers, because overuse of these agents can ascribe to the emergence of drug-resistant microorganisms (Chauncher, 2001). However, serious need for a non-synthetic approach to fight these microorganism, ,hence this investigation on synergistic effect of oils extracted from *Bryophyllum pinnatum* leaf and *Prunus dulcis* seed in formulation of herbal soap.

Aim and Objectives of the Study

The aim of this research is synergistic effect of oils extracted from *bryophyllum pinnatum* leaf and almond seed in formulated herbal soap: The objectives of the study are:

1. Drying of the plant material..
2. Extraction and distillation of *Bryophyllum pinnatum* leaves and *Prunus dulcis* seeds to obtain oils.
3. Produce of the herbal soaps using different blends of oils, blank and single doses.
4. Characterize the bio oils and soap, the product.
5. To estimate anti microbial profiles of the herbal soap blends(synergistic effects).

Materials and methods

Collection and Preparation of Plant Materials

The fresh leaves of Bryophyllum pinnatum and *Prunus dulcis* seed were collected from farms around Oko, Anambra State, Nigeria in March 2019. The plants were identified by a plant Taxonomist/Botanist Dr S.I Okeke of Science Laboratory Technology Department Federal Polytechnic Oko, Anambra State.

Materials/ Apparatus

Instrument and glassware's used for this work were the facilities of the Science Laboratory Federal Polytechnic Oko, while the chemicals and reagents were of analytical grade and standard.

Methods

The collected plant materials (leaves and seeds) were oven dried at 80°C for 72 hours and ground to powder. Each powdered sample was kept in a clean specimen bottle or container and stored in a freezer till used for analysis.

Extraction of Plant Material

The powdered plant materials were extracted by solvent extraction method with acetone for 3 hours. Oil was extracted from each sample. The oils (*P. dulcis* and *B. pinnatum*) were used in single and combined doses in the ratios of 2:8, 4:6, 6:4, 8:2, 0:0 (blank),0:5 and 5:0 and 5:5, respectively.

Formulation of Herbal Soap

Cold process soap making was used.

This process requires large stainless bowl, rubber gloves, molds, cardboard, plastic bag,B. *pinnatum* and P. *dulcis* oils ,natural dye, distilled water and fragrances. The cold process gets its name from the general low temperature that is used to mill this type of soap.

Eight samples of blends, single and blank doses of the active ingredients from *B. pinnatum* and *P. dulcis* were used in the formulation of the soap. The soap samples were prepared separately using each dose of the oil samples. The caustic soda solution and oils were mixed in the

ratio of (1:2) the hydrometer gauge was used to check the density of the solution. the oil phase solutions were gradually transferred into the water phase with constant stirring of the solution in one direction to form a homogeneous mixture, hence stable emulsion of the products were achieved (Mabrouk, 2005).

Determination of Percentage Yields of the Product.

The products in emulsion form were transferred to moulds to solidify at room temperature. The yields of the products were obtained by measuring the mass in grams using top loading balance.

Determination of pH the Products.

This was achieved using pH meter by making 2% solution of the product and the result were recorded.

Antimicrobial Analysis of the Product.

Antimicrobial susceptibility profile of the herbal soap blends on microbes was carried out. This was achieved using different concentrations of soap suspensions (0.0, 2.0, 5.0 and 8.0) mg/ml, respectively. Ciprofloxacin and ketaconazole were positive controls whereas *pseudomonas aureginosa, staphylococcus aureus, Escherichia coli* and *candida albicans* were clinical isolates of microbes used. The results recorded as (--) which denotes no inhibition; (ND) which denotes not done and (ZI values) denotes zone of inhibitions.

Sensitivity Test

Sensitivity test was an *in vitro* evaluation of the anti-microbial activity using ditch-plate techniques. Ciprofloxacin and ketaconazole positive controls where used whereas *staphylococcus aureus, Escherichia coli* and *candida albicans* were clinical isolates of microbes used. Strains of reference microbes namely *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aureginosa* and *Escherichia coli* were tested at four different soaps' concentrations (0.0, 2.0, 5.0 and 8.0 mg/ml). ANOVA mean results of zones of inhibition obtained and recorded.

Results and Discussion

The result of characterization of the oils showed that *P. dulcis* yielded 22.58 % while *B. pinnatum* yielded 16.88%; *P. dulcis* gave pH 5.0 as against *B. pinnattum* of 5.80; acid value were 2.24 mg/KOH/g and 5.6mgKOH/g respectively for Almond and *B. pinnatum*, (Table 1). The result of characterization of the oil shows high yield of the oil as source of income generation and can be used in the production of other things. The extracted oils were used in the formulation of soap in varying ratios of both oils. Low acid values of the oil is an indication of low susceptible to oxidative rancidity.

Table 1: Result of the Characterization of the Oils

Bio oils	% yield	pH	colour	mass of sample	acid value	sapon value
.ALMOND	22.58	5.0	colourless	287g	2.24mgKOH/g	27.28 mgKOH/g
B. Pinnata	16.88	5.80	greenish	281g	5.61mgKOH/g	11.76 mgKOH/g

The result of the anti microbial susceptibility profiles of herbal formulated soap blends on tested microbes shows strains of reference microbes namely *Candida albicans*, *Staphylococcus aureus* , *Pseudomonas aureginosa* and *Escherichia coli* at four different soaps concentrations (0.0, 2.0, 5.0 and 8.0 mg/ml). ANOVA mean results of zones of inhibition also revealed variability of antimicrobial activity among the natural formulated soaps with positive correlation **($P<0.01$)** between zones of inhibition and soaps' concentrations (Table 2). There are evidence of synergistic effects of *(P. dulcis: B. piñata)* ratios 2:8 soap. This is indicated by exhibition of largest zone of inhibition (36 mm) on *S. aureus,* followed by *Candida albicans* with (30 mm) zone of inhibition whereas single formulations gave the least zone of inhibition (9mm and 13mm) respectively for *P. dulcis and B.pinatum*, respectively. The result of the sensitivity test shows determination of minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC). All the media was prepared according to manufacturer's instructions and sterilized by autoclaving at 121^{0}C for 15 mins. Results from the incubation of herbal soap suspensions (30 µl) with the strains of reference microorganism-inoculated-agar plates depict a variability of antimicrobial efficacy (Table 2). The ANOVA mean results of zone of inhibition revealed variability of antimicrobial activity among the natural formulated soaps with positive correlation ($P<0.01$) between zone of inhibition and the tested blend soap formulations. Synergistic effects of (***P.dulcis : B pinattum)*** ratios **2:8** soap exhibited the largest zone of inhibition(36) on S.aureus followed by *candida albicans* with (30mm) zone of inhibition. Blends 4:6, 6:4, 5:5 and 8:2 were equally effective ($P<0.05$) against *S. aureus* (Table 2). Whereas blend 5:0 and 0:5 exhibited the

least zone of inhibition on the tested bacteria. *C. albicans* was the least susceptible showing very small zone of inhibition (3.3mm) at the highest assayed concentration (8 mg/ml) as depicted on (Table 2).This shows that majority of the assayed synergist soaps have satisfactory antibacterial activity, though its antifungal activities were not really highly effective with exception of blend (**2:8)** for *P.dulcis :B. pinattum* soap.

Table 2: Antimicrobial Susceptibility Profiles of Herbal Soap Blends (Synergistic Effects)

s/n	Blends	Conc.(mg/Ml)	Mean Diameter Zone Of Inhibition(mm)			
			P.aureginosa	*E.coli*	*S.aureus*	*C.albicans*
1	2:8	2.0	10.4		14.0	20.0 14.2
		5.0	15.4	21.4		24.9 18.2
		8.0	19.2	27.0	36.0	30.0
2	4:6	2.0	8.0	12.6	16.0	---
		5.0	13.3	19.0	20.1	---
		8.0	17.2	25.4	30.3	12.8
3	6:4	2.0	7.3	7.2		8.1 ----
		5.0	12.0	9.7	12.0	-----
		8.0	15.8	14.0	15.9	6.2
4	8:2	2.0	---		3.0	4.5 ----
		5.0	5.3		6.8	7.0 ----
		8.0	7.5		8.6	10.5 ----
5	0:5	2.0	6.0		11.6	4.2 ----
		5.0	10.4		13.0	7.0 ----
		8.0	13.8		18.2	13.0 12.0
6	5:0	2.0	---		0.5	---- ---
		5.0	4.2		5.9	2.3 ---
		8.0	6.8		7.5	4.0 3.3
7	5:5	2.0	7.8		12.4	15.0 ----
		5.0	12.3		18.7	18.5 ----
		8.0	15.9		22.4	25.4 6.8
8	Ketaconazole 15µ/disc		ND	ND		ND 12.5

9	**Ciprofloxacin 15µ/disc**	**26.4**	**33.0**		**28.8**	**ND**
10		**0.0**	**0:0**	**0:0**	**0:0**	**0:0**

Blend = ***P.dulcis : B pinattum*** (-) denotes no inhibition; ND denotes Not Done

The result of the characterization of the product shows that the pH of the products are slightly alkaline with the selected blend value of pH 7.5. This is an indication of the product in a stable form. This value is in line with the pH of standard tethmosol soap (8.0) being slightly alkaline too (Table 3). Also, the antimicrobial susceptibility profiles of herbal soap blends were carried out and result proved less effective for *P. dulcis* only blend(5:0); slightly effective for *B. pinattum* only blend(0:5) ; and highly effective for *P. dulcis : B. pinattum* blend (2:8) (Table 3).This is in line with work by(Nwambete and Lyombe, 2001) when they studied antimicrobial analysis of medicated soaps. It is an indication of the synergistic effects of the essential oils in the formulation of the herbal soap. They possesses antimicrobial and moisturizing properties.

Table 3: Result of Characterization of the Product (Herbal Soap)

S/N	Soaps	pH	Foamability	Skin Reaction	Antimicrobial Conclusion
1.	*P. dulcis* only	7.2	good	none	less effective
2.	*B. pinattum* only	7.8	good	none	mildly effective
3.	2:8 blend ***P. dulcis :B. pinattum***	7.5	very good	none	highly effective
4.	Standard. Tethmosol	8.0	very good	none	highly effective

From the results obtained they proved that with local plant material in synergy gave an effective, cheap and environmentally friendly herbal soap . This can be a source of income generation for economy recovery. The soap oils produced from all the blends of the oils exhibited varying levels of zone of inhibition against fungi and bacteria; however soap of blend *P. dulci* and *B. pinnatum* of ratio 2:8 (synergy) gave

highest zone of inhibition. The antimicrobial effect exhibited by the soap in this study signifies the potential of the soap as a topical therapeutic and protective agent.

Recommendations

Therefore we recommend this product to homes, pharmaceutical companies, health organizations and government in handling topical issues related to skin infections since it is sustainability. It is friendly to the body with no adverse effects to man and environment due to absence of common synthetic antimicrobial active ingredients triclosan, trichloroxylenol and trichlorocarbanilide.

References

Akindahonsi A.A and S.O. Salawu (2005) Phyochemical screening and nutrient-anti-nutrient anti-nutrient composition of isolated green leafy vegetables.

Ali, E .A. (2013). The Chemical Constituents and Pharmacological Effects of Bryophyllum Calycinum. A review, International Journal of Pharma Sciences and Research (IJPSR) , vol. 4, n o 12.

Chemother ,A. K. and Schweizer, H. P.(2001),. Cross-resistance between triclosan and antibiotics is mediated by multidrug efflux pumps: Exposure of a susceptible mutant strain to triclosan selects *nfxb* mutants over-expressing MexCD-OprJ. *Antimicrob Agents.* ;45:

Hermann, J. (1983). The Succulent Encyclopedia . 3. Edition. Fischer, Jena 1983, p. 275.

Gunstone, F.D., Harwod, J.L., and Padley, F.B. (1986): The lipidHandbook. Chapman and Hall Limited London, pp236-261.

Kimel, L.S. (1996). Hand Washing Education Can Decrease Illness Absenteeism. *J School Nurs*.12:14–8. [PubMed] [Google Scholar].

Larson, E. (1988). A Causal Link Between Hand Washing and Risk of Infection? Examine the evidence. *Infect Control Hosp Epedemiol*. 1988;9:28–36. [PubMed] [Google Scholar]

Levy, S. B. (2001). Antibacterial household products: Cause for concern. *Emerg Infect* ;7:512– [PMC free article] [PubMed] [Google Scholar

Mabrouk, S.T. (2005). Making Useable, Quality Opaque or Transparent Soap. Journal of Chemical Education 82(10): 1534- 1537

Nwambete, K. D. and Lyombe, F. (2001).Antimicrobial Activity of Medicated Soaps Commonly Used By Dar es Salaam Residents in Tanzania

Phansteil, O.N., Dueno, E., and Xianghong Wang. (1998): Synthesis of Exotic Soaps in the Chemistry Laboratory. Journal of chemical Education, 75(5):612-614

Poole, K. (2002). Mechanisms of Bacterial Biocide and Antibiotic Resistance. *J Appl Microbiol*. ;92:S55–64. [PubMed] [Google Scholar]

Russell, A.D(1998). Mechanisms of Bacterial Resistance to Antibiotics and Biocides. *Prog Med Chem*.;35:134–97. [PubMed] [Google Scholar].

Supratman, U; Fujita, T and Akiyama K. (April 2001). Anti-tumor promoting activity of bufadienolidesfrom Kalanchoepinnata and K. daigremontiana x tubiflora" (PDF). Biosci. Biotechnol. Biochem. **65** (4): 947–9. doi:10.1271/bbb.65.947. PMID 11388478.

Supratman, U; Fujita T; Akiyama K; Hayashi H.(2000). New insecticidal bufadienolide, bryophyllin C, from Kalanchoe pinnata" (PDF). Biosci. Biotechnol. Biochem. **64** (6): 1310–2. doi:10.1271/bbb.64.1310. PMID.

White, D.G, and McDermott P. F(2001). Biocides, drug resistance and microbial evolution. *Curr Opin Microbiol*.;4:313–7. [PubMed] [Google Scholar]