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Comparative Survey of Antibacterial Potency of Formulated Black Soap with Aloe Vera Extracts on Some Clinical Pathogens from the Skin

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Abstract

A comparative survey to determine the antibacterial potency of black soap formulated with *Aloe vera* and the Aloe vera extracts against some clinical pathogens (*Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*) of skin origin was carried out using agar "well" diffusion method. The *Aloe vera* formulated black soap and the *Aloe vera* extracts shows appreciable antibacterial potentials at all the concentrations (10%, 30%, 50% and 70%) used against the test organisms. The highest activity of 20.0 mm was recorded against *S. aureus* at the highest concentration of 70% used for the Aloe vera formulated soap and no activity was observed at the lower concentration against *Ps. aeruginosa*. Both the *Aloe vera* Gel and ethanolic leaf extracts recorded the highest activity of 18.0 mm against *Ps. aeruginosa* at the highest concentration of 70% used; no activity was recorded against *S. aureus* at lower concentrations used for both extracts and also no activity was observed against *S. pyogenes* for the *Aloe vera* ethanolic leaf extract. The MIC and MBC survey of the Aloe vera extracts and the formulated soap from *Aloe vera* indicates high antibacterial potentials against the test organisms except for the ethanolic leaf extract that fails to show any activity against *S. pyogenes*. The phytochemical survey of the extracts also revealed the presence of many important bioactive compounds including saponins, tannins and anthraquinones. These surveys have given a pointer to the possibility of using *Aloe vera* extracts for the formulation of skin care and skin remedies.

Key words: comparative, survey, antibacterial, potential, *Aloe vera*, extracts formulated, soap

Introduction

The use of natural plant remedies has become increasingly popular around the world, which has increased the demand for knowledge about the properties and applications of medicinal plants [1]. For centuries, people have used the intricate properties of plants to treat a wide range of illnesses, diseases and disorders [2]. Humans are not sufficiently aware that natural products drug discovery is important for new generations as a tool for their health care. We know that for the major lethal diseases, there are no truly effective drug

treatments and another area that have been neglected over the years, is skin hygiene practice that have not been widely put into consideration [3], [4]. In addition, drug resistance to existing chemotherapeutic agents for fungal and bacterial infections, AIDS, cancer, and malaria is increasing on a daily basis. This has pose serious challenges for health care both now and in the future and thus, calls for decision-makers, governments, international agencies, and pharmaceutical companies to commit to the sustainable development of natural products as medicinal and hygiene agents particularly in developing countries [5], [6].

Aloe vera (*Aloe barbadensis miller*) is a plant, which belongs to the family of Liliaceae and is mostly succulent with a whorl of elongated, pointed leaves [7], [8]. The name is derived from the Arabic word 'alloeh' which means 'bitter', referring to the taste of the liquid contained in the leaves. The term Aloe refers to a solid residue obtained by evaporating the latex derived from the outer layers of the plant leaf. The central bulk of the leaf contains colour-less mucilaginous pulp that is made up of large, thin-walled mesophyll cells containing the *Aloe vera* gel itself. Despite its wide use as a folk remedy over a long period of time, the biochemical details of its action on physiological or pathophysiological functions have not been systematically documented [2]. The plant has a long history as a multipurpose folk remedy, and has been associated with myth, magic, and medicine since pre-biblical times. Historical evidence indicates that *Aloe vera* originated in the warm, dry climate of Southern and Eastern Africa, and was subsequently transported into Northern Africa, the Arabian Peninsula, China, Gibraltar, the Mediterranean countries, and the West Indies [9][1].

Aloe vera is described as one of the most talked about, yet most misunderstood plants in history. Modern clinical use of Aloe vera began in the 1920s and claims now abound, in numerous research and commercial literature, in journals and on the Internet, regarding its numerous therapeutic potentials when used both topically and parentally. It is averred to cure ailments ranging from mild fever, wounds and burns, gastrointestinal disorders, diabetes, sexual vitality and fertility problems to cancer, immune modulation, and even AIDS [10], [11]. Among more than 360 *Aloe vera* species, *Aloe vera* (*A. barbadensis miller*) has been the most popular in both folk and modern medicine [12], [4], [13]. *Aloe vera* extracts are widely used in a variety of over-the-counter and dermatological products.

Many studies report the effective use of this plant when applied topically for the treatment of burns, sunburns, inflammatory skin disorders and wound. *Aloe vera* is a plant that can produce latex and gel. The gel is extracted from the leaf, and it is this substance that is most used as a treatment. *Aloe vera* has been evaluated in several different clinical contexts and some promising results have been found for its use in controlling cardiovascular risk factors and diabetes, besides being beneficial in

areas of dermatology. One explanatory factor for this is the anti-inflammatory properties of the plant [14]. It contains over 70-75 biologically active compounds and is claimed to have anti-inflammatory, antioxidant, immune boosting, and anticancer, healing, anti-ageing and anti-diabetic properties. Aloes, by contrast, is an anthraquinone derivative of the sap of the Aloe leaf which has been used for centuries as a purgative [15], [5].

Aloe vera gel has been widely promoted and used by patients for the treatment of a range of inflammatory digestive and skin diseases. The antibacterial activities of *Aloe vera* were dependent on the dose of anthraquinone. It is reported that *Aloe vera* possesses antifungal, antiviral, and antibacterial activity against skin infections such as acne, herpes and scabies [16]. It contains a compound that neutralizes and binds with FGF-2 receptor, or otherwise alters signaling pathways for FGF-2 by affecting both GJIC and proliferation of diabetic fibroblast [17]. Several reports suggest that beneficial effects of Aloe gel are due to its high molecular weight components such as polysaccharide, lectin like proteins and prostaglandins.

Soap may be defined as a chemical compound or mixture of chemical compound resulting from the interaction of fatty acids or fatty glycerides with a metal radical (organic base) [18]. It is any water soluble salt of whose fatty acid which contains eight or more carbon atoms. Soap is class of product that serves for cleaning of human, property and surrounding. Hence, soap in its various forms, is in high demand. In all society, for generation its used has increased until its manufacture has become an industry essential to the comfort and health of civilized man. Soap is known for its laundry and cleaning purpose, though calcium soap has been used in animal feed formulation [18]. Soap depends for its washing action on the fact that its molecules possess one ionic (polar, water-attracting or hydrophilic) end and one covalent (non-polar, water repelling or hydrophobic) end which attracts oils and greases [19]. Thus, soap molecules can make water and oils come into an emulsion which can be washed away. Soaps are essentially the sodium or potassium salts of various fatty acids. Soaps also contain additives for some desired qualities. These include salt; soda ash, colour, citric acid, sodium silicate, sodium bicarbonate, perfume, borax trisodium phosphate and magnesium sulphate. A combination of inexpensive builders, example soda ash with more effective (an expensive) tetrasodium pyrophosphate or sodium triphosphate, is sometimes superior to the phosphate used alone [19].

Soap is one of the cleaning materials needed by every family. Soap is so important that there is hardly any family that does not use it in their daily activities either in the solid bars, liquid and detergent forms. Soaps are salts of fatty acids and it may be hard or soft depending on the type of ingredients used [19]. Soaps are generally made by the hydrolysis of fats with caustic soda (Sodium hydroxide), thus converting the glycosides of

stearic, oleic and palmitic acids into sodium salts and glycerol [18]. Soaps have a cleaning action because they contain negative ions composed of a long hydrocarbon chains attached to a carboxyl group. The hydrocarbon chain has an affinity for grease and oil and the carboxyl group has an affinity for water. That is why soaps are mostly used with water for bathing, washing and cleaning. They are also used in textile industries for textile spinning. There are many agricultural waste materials generated in homes and littered all over the environment. These materials include palm bunch, cocoa pod, plantain peels, banana peels, maize cobs, cassava peels and others. Some of these agricultural wastes like cocoa pod adversely affects soil fertility and so constitute environmental nuisance to man. However, they are a potential viable source which needs to be harnessed for other uses and to save the environment. According to [20].

The taxonomical classification of *Aloe vera* as per Ayurveda, Aloe is known as Kumari or "Young Girl". It is because; *Aloe vera* is believed to bring back youthful energy and femininity. Aloe is used as a tonic for the female reproductive system [21]. According to Ayurveda, Aloe is said to have alliterative, tonic, rejuvenating, purgative, and vulnerary actions. Aloe is also believed to give good solution to all the three Ayurveda constitutions [22]. It is mainly used as a remedy for constipation, colic, skin diseases, worm infestations and infections in traditional Indian medicine. It is also used as a laxative, anti-helminthic, for haemorrhoid treatment, and as a uterine stimulant (menstrual regulator). Aloe extract is also topically used to treat eczema or psoriasis, in combination with liquorice root. Aloe is also used as food. People in Tamil Nadu, India often prepare a curry using *Aloe vera* which is taken along with Indian bread (nan bread) or rice [23], [24], [25], [21].

The kingdom is Plantae, Clade: Angiosperm, *Clade*: Monocots, Order: Asparagales, Family: Asphodelaceae, Subfamily: Asphodeloideae, Genus: *Aloe*, Species: *Aloe vera*[3].

Figure 1: Aloe vera plant [26]

Microorganisms are ubiquitous and can invade and multiply most habitats, thereby displacing the normal bacteria flora of that environment. The human skin performs numerous functions to the human body and it is also colonized by a number of bacteria (Salt tolerant bacteria) that gain passage or penetrate the skin through the hair follicles to reach deeper tissues [6]. The secretion of sweat and sebaceous glands are also essential for normal microbial population of the skin because they supply water, amino acid and liquids which serve as nutrients for microbial growth while some are from the dust etc, some of these salt tolerant pathogenic microorganisms have the tendency of displacing the normal flora microbial population of the skin [11]. This invading salt- tolerant pathogenic skin bacterium can be taken care of by suitable skin preparation, or body formula, like antiseptics and medicated remedies prepared from plant origin since the conventional ones can be resisted by microbes, cause toxicity and allergy reactions, and may also not be available and affordable [8], [6]. This research work therefore, tries to explore one the potentials of Aloe vera leaf extracts to formulate black soap preparation or remedy and to survey the antibacterial potency of the formulated black soap and that of the extracts against some clinical pathogens of skin origin.

Materials and Methods

Sample collection

The *Aloe vera* plant used in this research was collected within Birnin Kebbi, Kebbi State, Nigeria. The sample was packed in polythene bags, to avoid contamination and was taken to the department of Science technology for identification and processing. The *Aloe Vera* leaf obtained was then washed and kept in a safe and clean container [27].

Extraction and formulation of the Aloe vera soap

The *Aloe vera* leaves were cut using a knife, and the gels from the leave were obtained with a clean bowl and the Aloe vera gels from the leaves were obtained in a clean bowl and were then stored to use to produce the soap [27].

Bedding of Soap with Aloe vera Gel

Chemicals and reagents

Distilled water	6 Litres
Natrosol	66.6g
Lauril Rice	58.5g
Texapon	97.1g
STPP	114.6g
Pine oil	10ml
Phenol	10ml
Glycerin	10ml
Vitamin E	10ml
Colour	1.1g
Aloe vera gel extracts	180ml
Perfume	10ml

Procedure

Exactly six (6) litres of distilled water were added into a bucket and then the following chemicals were added step by step. 66.6g of natrosol was first added and stirred for 5

minutes then 58.5 g of Lauril rice was also added to the mixture and they were mixed. After that 97.1 g of Texapon was added and continued the stirring then 180 ml of Aloe vera gel was poured into the mixture and 114.6 g of STPP was added and were, mixed. After putting all the listed chemicals above, the following chemicals were poured one after another [22], [19].

10cm^3 of pine oil

10 cm^3 of Phenol

10 cm^{3}of Glycerin

10 cm^3 of Vitamin E

1.1g of colour

10 cm^3 of perfume

The chemicals were mixed all together to ensure that a liquid soap was formed [19].

Media preparation

All the media used in this research work were prepared according to the instructions of the manufacturers as contained in the label of the container of each medium [27].

Confirmation of Pathogens

The organisms used in this research work are clinical isolates obtained from a hospital in Birnin Kebbi, Kebbi State. The isolates are *Staphylococcus aureus, Streptococcus pyogenes and Pseudomonas aeruginosa.* The isolates were transported to the microbiology laboratory of Waziri Umaru Federal Polytechnic, Birnin Kebbi, Kebbi State, and were sub cultured into appropriate media and then gram stained and subjected to different biochemical tests, to confirm the claim the Hospital.

Formulation of varying concentration of the Aloe vera formulated soap and that of the Aloe vera extracts

About 1 cm^3 of the formulated black soap was placed in 10 cm^3 of distilled water and was done for 3 cm^3, 5 cm^3 and 7 cm^3 in other to obtain various concentrations of 10%, 30%, 50% and 70% respectively. The same procedure was repeated for the *Aloe vera* gel extract while 1 g of the ethanol extract was weighed and place in 10 cm^3 of distilled water and was also done for 3 g, 5 g and 7 g to obtain 10mg/ml, 30 mg/ml, 50 mg/ml and 70 mg/ml of the concentrations of the *Aloe vera* ethanol leaf extract [27], [12], [2].

Antibacterial testing

The agar well diffusion techniques were used to determine the antimicrobial activity of the laboratory formulated *Aloe vera* black soap and that of the Aloe vera extracts. The selected strains of bacteria *Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa*were inoculated into 5ml of sterile nutrient broth and incubated at 37^{0}C for 24 hours. Using sterile cotton swab, the broth cultures were swabbed on the surface of sterile Mueller Hington Agar (MHA) plates, after making the "wells". The varying concentrations of the produced soap 10%, 30%, 50% and 70% and 10mg/ml, 30mg/ml, 50mg/ml and 70mg/ml of the Aloe vera ethanol leaf extract

and the gel extract, were then added to each of the "well" contain in separate plates and the plates were then incubated in an upright position at 37^0C for 24 hours. This was done in duplicate. The diameter of the zone of inhibition was measured in millimeters and the results were recorded. Pure Egyptician liquid soap was used as control [27].

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the produced *Aloe vera* antiseptic soap against the clinical test bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*) was determined according to the method purposed by [27] using tube dilution with a set of twelve test tubes.

Results and Discussions

Determination of MIC and MBC of the Aloe vera extracts

Table I: Antibacterial Activity of *Aloe vera* Organic soap produced against Bacteria Isolates obtained from skin origin

	Mean Zone of inhibition (MM)/Concentration of the Extract (%/mg/ml)				
Extracts / TestBacteria	**10**	**30**	**50**	**70**	**control Pure Egyptician soap**
Aloe vera Formulated Soap					
Staph aureus	10.0	14.0	20.0	20.0	24.0
Strep pyogenes	14.0	12.0	20.0	20.0	24.0
Ps. aeruginosa	6.0	10.0	14.0	16.0	22.0
Aloe vera gel extract					
Staph aureus	6.0	6.0	12.0	12.0	20.0
Strep pyogenes	6.0	6.0	14.0	14.0	20.0
Ps. aeruginosa	10.0	10.0	16.0	18.0	18.0
***Aloe vera* ethanol extract**					
Staph aureus	6.0	6.0	12.0	12.0	16.0
Strep pyogenes	6.0	6.0	6.0	6.0	16.0

Ps.	*aeruginosa*	10.0	10.0	12.0	18.0	16.0

Key: MM = Millimetre, mg/ml = Milligram/m, Ps= Pseudomonas, 6.0mm= no activity

Note: The concentrations of the *Aloe vera* produced soap and that of the Gel extract are in % while that of the *Aloe vera* ethanol leaf extract are in mg/ml.

Table II: Result of MIC and MBC of Aloe vera Organic Soap Produced, Aloe vera gel and Aloe vera ethanol extract against Bacteria isolates from skin origin

Test/ Bacteria	**Concentration in (mg/ml)**					
	Lab produced Organic Soap		**Gel extract**		**Ethanol extract**	
	MIC Values	**MBC Values**	**MIC Values**	**MBC Values**	**MIC Values**	**MBC Values**
S. aureus	4.375	8.75	2.1875	4.375	4.37	8.75
S. pyogenes	4.375	8.75	2.1875	4.375	-	-
Ps.aeruginosa	2.1875	4.375	2.1875	4.375	4.375	8.75

Key: mg/ml = milligram/ml, MIC= Minimum inhibitory Concentration, MBC =Minimum Bactericidal

Table III: Phytochemical Compounds of Aloe vera

Components	**Level of Presence**	
	E. extract	**Gel extract**
Tannins	+	++
Alkaloids	+	+
Flavonoids	++	++
Glycosides	+	+
Saponins	+	+
Steroids	+	+
Tevpeuse	+	+
Anthraquibone	+	+

Key: ***++ = Highly present, + = Slightly present - = Absent***

Discussion

The Antibacterial Potency of the formulated black soap from *Aloe vera* and the *Aloe vera* extracts were determined against some bacteria isolates (*Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa*) from skin origin, using agar "well" diffusion method. The Bacteria are clinical isolates obtained from the hospital, which were confirmed using morphological and biochemical characteristics. Table I, indicates the mean zone of inhibition of the formulated black soap from *Aloe vara* and the activities of 10.0, 14.0, 20.0 and 20.0mm, 14.0, 12.0, 20.0 and 20.0mm and 66.0, 10.0, 14.0 and 16.0mm at all the concentration of 10, 30, 50 and 70% used against *S aureus, S pyogenes and Ps aeruginosa* while the *Aloe vera* gel extract did not produce activity at lower concentration of 10, and 30 mg/ml against *S aureus and S pyogenes* only at high concentration of 50 and 70mg/mi with activities of 12.0mm against *S aureus* and 14.0mm against *S pyogenes* at both concentrations and activity of 10.0, 10.0, 16.0 and 18.0mm against *Ps aeruginosa.*

The ethanol extract did not produce any activity against *S pyogenes* at all the concentrations used. It also did not produced activity at lower concentration of 10 and 30 mg/ml against *S. aureus.* It was only at higher concentrations of 50 and 70mg/ml, the following activity of 10.0, 10.0, 12.0 and 18.0mm were recorded against *Ps. aeruginosa* at all the concentrations used. This research work is in line with the work of [28] who reported *Aloe vera* to have antiseptic effect against fungi, bacteria and viruses. Table II, indicates the survey of the minimum inhibitory concentration and minimum bactericidal concentration of the formulated laboratory black soap and that of the *Aloe vera* extracts against the clinical test bacteria isolates of the skin origin *S aureus, S pyogenes* and *Ps aeruginosa*, the survey indicates that both the formulated soap from *Aloe vera* and *Aloe vera* extracts has bacteriostatic and bactericidal potentials except for *Aloe vera* ethanolic extract that fails to show any antibacterial potency against *S pyogenes*.

The Phytochemical screening of *Aloe vera* extracts carried out as seen in Table III, revealed the presence of important bioactive compounds such as tannins, alkaloids, flavonoids, glycosides, saponins, steroids, terpenes and anthraquinones. This was also reported by [6] that *Aloe vera* contains around 75 potentially active constituents.

Conclusion

This research work indicates that the *Aloe vera* soap formulated in the laboratory from *Aleo vera* and the *Aloe vera* extracts have some antiseptic and antibacterial potential against the clinical bacteria isolates (*S. aureus, S. pyogenes and Ps aeruginosa*) from skin origin. The responses of

these clinical bacteria isolates against the *Aloe vera* extracts and the formulated soap and the presence of many important bioactive compounds in the *Aloe vera* extract; suggest the possibility of using *Aloe vera* extracts for formulation of skin care, skin lotions or antiseptics to take care of the body hygiene need and even infections or diseases or disorders that might arise from the bacteria under study.

Recommendation

Further research should be conducted on the following area

1. Physicochemical parameters of the produce soap should be determined.
2. The material analysis such viscosity and others should be determined

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