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Physio and Biochemical Screening of Soap Formulated with Extract of Sodom Apple (*Calotropis procera*) Flower Extract Using Neem Oil (*Azadirachta indica*) as Base Oil

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Abstract*—This research explores several properties of Calotropis procera flower-based toilet soaps developed with neem oil as the base oil and ethanol and ethyl acetate flower extracts. The market need for green antimicrobial cosmetics has motivated researchers to investigate plant resources in soap manufacturing processes. Cold saponification produced soaps using neem oil as the base oil where the product contained Calotropis procera extract at concentrations of 5%, 10%, and 15%. Standard methods were used to evaluate the physicochemical properties of these soaps which included a pH range of 8.5 to 9.2 along with foam stability ratings from 67 to 85% and total fatty matter between 62 and 75% and moisture content at 6–8% and free alkali content at less than or equal to 0.05%. The soap showed strong foaming ability with a gentle alkaline nature and strong cleaning properties. Higher concentrations of extract showed antimicrobial effects that tested effectively against skin pathogens Staphylococcus aureus, Escherichia coli, and Candida albicans through agar well diffusion method while producing inhibitory zones reaching from 14.2 ± 0.3 mm to 22.5 ± 0.6 mm. ANOVA statistical analysis proved through ($p < 0.05$) that varying concentrations exhibited meaningful antibacterial and antifungal activities differences. The independent Sample T-Test (p (0.569) > 0.05) showed no significant difference regarding the mean values of inhibition zone treated with ethyl acetate and ethanol extracts from the flower. The results demonstrate that toilet soap containing Calotropis procera extract and neem oil demonstrates improved antimicrobial capabilities which make it a promising replacement for synthetic toilet soap products. The analysis emphasizes the need to employ natural vegetation as sources for creating sustainable cosmetic solutions*.

***Keywords*—** *Soap, Neem Oil, phytochemicals, antibacterial, Ethanol, Ethyl acetate*.

I. Background of the Study

The investigation follows an increasing movement to develop organic products from local sources intended for body cream and toilet soap production. Current scientific interest in ethnically derived medicinal remedies exists because people are concerned about synthetic drug side effects so researchers investigate them for potential cosmetic and dermatological applications (Portugal-Cohen et al., 2018). The widespread adoption of plant-based soaps comes from their basic nature, cost efficiency, laminal behavior and limited adverse effects which Pardeshi et al (2024) point out.

The research focuses on two thoroughly recognized medicinal plants namely Sodom Apple (Caloptropis procera) and neem oil (Azadirachta indica) because of their traditionally established antioxidant properties in skin disorder treatment (Harkel & Deshmukl, 2024). The plant extracts demonstrate recognized therapeutic applications along with anti-bacterial, anti-fungal, anti-aging potential combined with wound-healing properties. The high drug effectiveness of Sodom Apple makes it an optimal medicinal choice for treating skin disorder along with various skin conditions such as eczema, leprosy and scabies, ringworm, ulcer, and psoriasis (Divived et al., 2024) and cancer (Yaniy & Kottai, 2018) and many other common skin related diseases. Medical experts have identified this double plant therapy as efficient medicine to handle common skin concerns of everyday life.

The target of this research required the collection of flowers from the plant along with drying followed by solvent extraction via maceration using ethanol and ethyl acetate to compare results. Secondary metabolites identification within the extract occurred through phytochemical analysis based on standard laboratory methods. A complete examination of the extract involved GC-MS analysis coupled with metabolite structural identification per Timothy et al., 2023 and Giechaskiel & Clairotte (2021).

The preparation involved different concentrations of plant extracts along with neem oil serving as the base oil to make toilet soap. A standardized testing procedure examined the soap product while a purchased commercial soap served as both the control and standard after its production.

Statement of the Problem

The rise in synthetic drug side effect awareness guides modern scientists to search for natural ethnic medicinal

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remedies in potential new dermatological and cosmetic components. Various parts of C. procera yield extracts that exhibit powerful anti-bacterial performance alongside anti-inflammatory actions and anti-diabetic properties and anti-cancer capabilities (Misra et al., 2024, Alagbe & Chris, 2024, Khan et al., 2024). Additionally, the extracts show protective biological effects against skin inflammation, irritation and function as a skin rejuvenating agent which assists skin chemistry maintenance (Portugal-Cohen et al 2018).

Scientific research from Habeeb et al 2024, Safar et al 2024, Aremu 2023, Dividedi et al., 2024 continues to report the poisoning threats of C. procera. Traditional healers Imosemi (2016) along with Yaniv and Kottai (2018) report that different skin conditions including wounds, sores, external infections, swelling, ringworm and eczema receive treatment through C. procera preparations. Research has already documented minimal evidence regarding how to utilize the natural resources in C. procera for developing active components in contemporary skincare formulations. C. procera exists as a highly productive medicinal plant that serves as an environment-dominating weed (Habeeb et al., 2024).

The combined application of C. procera extract with neem oil shows promise for skin well-being because they produce enhanced energy production alongside skin calming effects for skin rejuvenation together with resistance to both skin-aging and dermatophyte infections and bacterial and cancerous cell growth (Harkel & Deshmukl, 2024). Muktar & Usman (2021) established that edible oils have higher prices because their demand for different purposes continues to rise. An upcoming alternative exists because Northern Nigeria is covered with excessive neem tree seeds that produce neem oil which scientists have yet to fully explore (Usman, et al., 2024). The research serves as a significant initiative to convert waste material from the environment into monetary gain.

Significance of the Study

The project serves to advance green chemistry principles and demonstrates a method to generate economic value from scarce environmental waste materials like neem seeds and Sodom Apple plant. These findings will demonstrate the plants' pharmaceutical values to traditional healthcare practitioners and general consumers as well as dermatologists and dieticians and cosmetologists.

II. Literature Review

Phytochemical activities in plants led to their trial testing which eventually led to beneficial applications in health care and drug developments (Siraj et al, 2022, Michalak, 2023 and Khan et al., 2024). The therapeutic properties of neem oil *(Azadirachta indica*) have made this substance one of the most frequently used medicines across Nigeria, Africa, Asia and Europe to treat various illnesses. According to Usman et al (2024) neem trees show strong medicinal properties across Asia, Africa and Europe and Northern Nigeria contains more than four million neem specimens; providing treatments against malaria, typhoid and skin ailments (Harkel & Deshmukl, 2024).

Washing and cleaning require anionic surfactant soap that becomes effective after mixing with water. Abubakar & Usman (2021) state that soap consists of sodium or potassium salts which result from acid-base chemical reactions. The combination of fatty acids with lye produces saponification resulting in the creation of fatty acid and glycerol. Soaps function as emulsifiers to clean up dirt through chemical, thermal and mechanical energies working together during the process. The variety of chemicals in soaps determines their effectiveness for human well-being as noted by Onoicho (2021). Soap manufacturers include particular chemical compounds for achieving their intended target functions. Medicinal soaps exist among different soap types. Soaps incorporate either plant extracts or synthetic/auxiliary materials including heavy metals as well as titanium and silver and nickel and aluminum which help with antibacterial properties and improve their appearance (Atolani, et al., 2019).

This study uses *Calotropis procera* as the analytical plant because it has proven therapeutic benefits throughout international medical practice. *Calotropis procera* grows into an ever-green medium-height shrub reaching 4 -5 meters with middle branches belonging to the Asclepiadean family. The tropical and sub-tropical regions of Asia and Africa maintain arid and semi-arid areas where this plant grows naturally at human settlements. The plant functions as an environmental weed with ever-green characteristics and softwood xerophytic traits throughout worldwide distribution according to Habeeb et al. (2024). The scientific community recognizes Tumfafiya as the Hausa name while Ewe Bomubomu stands as the Yoruba name and Avi-Wara is the Ebira dialect name for this plant alongside its India-based identification as Madar Shrub.

Sodom Apple contains four major properties which include anti-aging activity and anti-bacterial and anti-fungal effects as documented by Shinde & Somani (2023). The plant also exhibits skin rejuvenating and wound-healing properties according to Portugal-Cohen et al (2018). Khan et al (2024) identified that the bulbs of Sodom apple produce β-sitosterol which promotes anti-cancer and anti-bacterial and anti-fungal along with anti-diabetic effects and stigmasterol functions as the agent for antimicrobial and anti-inflammatory together with membrane stabilizer properties. Analysis of *C. procera* plant leaf showed that it serves as excellent dietary supplementation due to its numerous nutritious components as alluded to Aremu et al (2023). Alagbe & Chris (2024) confirmed that root and leaf extract molecules function as pharmaceutical compounds which Gambia culture uses as natural medicine. Habeeb et al (2024) describes how the version of its extracts reveals their alkaloids and phenolic components have potential applications in drug synthesis.

III. Materials and Method

Materials: ethanol, ethyl acetate, distilled water, hot water bath, thermometer, Rapid Test, measuring cylinder, viscometer, beakers micro-organism, GC-MS. Cold pressed neem oil was obtained from Gombe Main Market, Gombe, Nigeria and commercial antiseptic soap.

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Collection and Identification of Calotropis procera

The flowers of *Calotropis procera* will be collected in August, 2024 at the premises of Federal College of Education (Technical), Gombe, Gombe State. The plant was identified and authenticated in the Herbarium of the Botany Department of Gombe State University, Gombe.

Preparation of Plant extracts

Both cold extraction method was used for this study for comparison. The *Calotropis procera* flowers was cleaned, air dried and ground to coarse powder using grinding machine. The powder was stored in air tight containers for further use.

Cold maceration extraction of each of the samples will be performed as adopted by Alagbe & Chris (2024) and Khan *et al* (2024) with slight modifications. Ethanol and ethyl acetate were used. The choice of ethanol was due to its safety, environmentally friendly and is a green (renewable) solvent (Nde & Foncha, 2020). The ethyl acetate extracts of the plant parts have been reported to isolate compounds with the highest activity against Escherichia coli, Staphylococcus aurea, Candida albiccaus, Salmonella typhi (Khan *et al*., 2024).

Two hundred grams (200 g) of the powdered samples was soaked into 2 L of ethanol and ethyl acetate. The mixtures were allowed to stand for 3 days at room temperature (28 ±2ºC) with hourly agitations. The extracts were sieved through a muslin cloth, filtered through a Whatman (No.1) filter paper, poured unto a clean evaporating dish and placed on a water bath at 50ºC until all the solvent evaporated.

Chemical analysis of Calotropis procera:

Gas chromatography and mass spectrometry, (GC specification: Agilent GC 7890A coupled to MSD 5975C. Column dimension: Agilent T and DB-35ms, length 30m inner diameter 0.25mm and 0.25µm path film thickness) was used to determine the phytochemicals constituents of extracts of the flower sample following the method adopted by (Malik, *et al*., 2021). The spectra obtained were compared with NIST library (2017) version for the identification using their respective retention times. Their relative abundances determined by the peak areas.

Qualitative Phytochemical of screening ethanolic and ethyl acetate extract

The plant extracts were subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the methods described by Nama dima, *et al*., (2023) and Khan *et al*., (2024). Phytochemical screening was carried out for the presence of carbohydrates using Molish`s reagent, alkaloids (Wagner`s Test), anthraquinones (Borntrager`s Test), cardiac glycosides (Kella-killiani`s Test), flavonoids (Shinoda Test), reducing sugars, saponins (Frothing test), steroids and Triterpenes (Liebermann`s Test), tannins (Ferric chloride test), and terpenes, using standard procedures.

Microbiological analysis - Microbial Bioassay

Antimicrobial activities of the ethyl acetate and ethanol crude extracts were investigated using clinical isolates of some pathogenic microbes such as: *Staphylococcus aureus, Staphylococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans.*

Isolation of Bacteria Species

The samples were grown on sterile blood agar, chocolate agar, and Mac-conkey agar plates at 37°C for 24 hours in an incubator. Individual colonies were selected according to their morphology and subsequently sub-cultured on blood agar and chocolate agar to acquire pure strains. The isolated colonies underwent Gram staining, and based on their Gram reactions, were inoculated onto various selective media — mannitol salt agar, cetrimide agar, and eosin methylene blue agar. Various biochemical tests were performed (catalase, coagulase, and oxidase tests). All the isolates that grow on selected agar media were then be placed on nutrient agar and chocolate agar slants and maintained in a refrigerator at 4°C (Cheesbrough, 2010).

Diffusion method was used for screening the extracts. Mueller Hinton Agar and used as the growth medium for microbes, sterilized at 121°C for 15 min, poured into Sterile Petri Dishes and allowed to cool and solidify. The crude extracts (0.4 g) were dissolved in 10 mL of Dimethylsulphoxide (DMSO) to acquire a concentration of 40 mg/mL. A sterilized medium was then seeded with the standard Inoculum (0.1 mL) of test microbes and spread evenly over the surface of the medium with sterile swabs. Using a 6mm standard cork-borer, a well was cut at the centre of each inoculated medium. A concentration of 5 mg/mL of the already weighed crude extract was then introduced into each well on the inoculated medium. The inoculated medium was incubated at 37OC for 24 hours, after which the medium was observed for the zones of inhibition which was measured with a transparent ruler (Akinyemi *et al*, 2005).

Antimicrobial Susceptibility Test - Preparation of Extract Concentration

This was carried out according to the method described by Srinivasan *et al.,* (2009). Stock solutions of the plant extracts were created by combining 0. 5g of each crude plant extract with 1ml of dimethyl sulphuroxide (DMSO). Concentrations of 500mg/ml, 250mg/ml, 125mg/ml, and 62. 5mg/ml were prepared from each of the stock solutions utilizing the Two-fold serial dilution method.

Standardization of bacterial Inoculum.

Using inoculum loop, over-night grown agar culture (bacteria and fungi) was transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National Committee for Clinical Laboratory Standard (NCCLS, 2008).

Susceptibility Test of Bacterial and Fungal isolates to Different Concentrations of Extracts

The antimicrobial activity of *Calotropis procera* leaf crude extract (ethyl acetate and ethanol) against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Streptococcus pyogenes, Pseudomonas aeruginosa,*

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Aspergillus flavus, Candida albicans, and *Aspergillus niger* were evaluated using agar well diffusion method of susceptibility test (Srinivasan *et al.,* 2009*)*. Mueller-Hinton agar and Sabouraud Dextrose agar plates were inoculated with 0. 1ml of standardized inoculum of each bacterium (in triplicates) using a 0. 1ml pipette and distributed evenly with sterile swab sticks.

Three wells of 6mm size were made with sterile cork borer (6 mm) into the inoculated agar plates. Using micropipette, 0.1ml volume of the various concentrations; 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml each of the crude extracts are dispensed into wells of inoculated plates. DMSO was used as negative control. Commercially available standard antibiotic, ampicillin and fluconazole were used as positive control parallel with the extract.

The prepared plates were then left at room temperature (37 ºC) for 10 minutes, allowing the diffusion of the extracts into the incubation at 37 ºC for 24 hrs in an incubator. The diameter of inhibition zones (DIZ) was measured and expressed.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacteria Concentration (MBC)

These were determined using tube dilution method (Talaro and Talaro, 2002; Betts *et al*., 2012) as reported by Adesokan *et al* (2007). Muller-hinton broth media was used. A serial dilution of the soap made to obtain 125, 62.5, 31.25, 15.75 and 7.87 mg/ml. For the determination of MIC, into the labeled tubes and incubated at 37°C for 24 h. A tube containing Muller-hinton broth, but without an organism is used as control. The tubes were subsequently inspected for the existence or lack of growth using turbidity as the standard. The minimal dilution in the sequence displaying no growth of the test organism was regarded as the MIC. For MBC determination, The MBC and MFC were determined by collecting 1ml of broth culture from the tubes used for the MIC determination and sub culturing into fresh solid nutrient agar plates. The plates are incubated at 37 0C for 24 h. The least concentration that did not show any growth after incubation is regarded as the MBC/MFC.

Preparation of Toilet Soap with Calotropis procera Extract using Neem Oil

The method used by Mak-Mensah *et al* (2011) was adopted with little modifications. 100g of neem oil was weighed into a 500ml beaker and heated to 100℃ and 20ml of 23.5% NaOH added to initiate saponification. Different concentrations of each of the *Calotropis procera* flower extract were added at 4g/100g, 6g/100g, 8g/100g and 10g/100g of neem oil. To this mixture, 60g of NaOH pellet was dissolved in 100ml of distilled water and added gradually; little at a time with stirring until saponification was complete. About 10ml of distilled water was added to soften the soap while heating. 8g of NaCl dissolved in 30ml of distilled water was added to grain the soap. The salt added helped to separate the spent lye in the bottom, while the saponified mass floats on the surface to reduce the soap viscosity, and to separate the glycol water in the bottom. The glycerol water was isolated by siphoning. The soap paste was then be washed by (5-10%) hot water (90℃) to reduce excess sodium hydroxide and sodium chloride and any other impurities present in the soap paste. The soap was finally washed with 10ml of distilled water, filtered using a linen cloth, air-dried. The soap was then be poured in a cast and allowed to dry. A small portion of each sample was taken for physiochemical tests.

Characterization of soap

All analysis reported was done for three soap samples: the two prepared and the commercial antiseptic soap samples. The procedures were as reported by (Harkel & Deshmukl, 2024)

Physical Test: In this test, the soap was observed for colour, odour, texture and state.

Viscosity Test of Solid Soap Solution using Alpha Series Rotational Viscometer: Viscosity testing helps determine the flow behavior of the soap solution, which impacts its usability.

10 g of the solid soap samples were weighed and dissolved it in 100 mL of distilled water at 40°C. it was stirred until fully dissolved to form a homogeneous solution and allowed to cool to room temperature (around 25°C) unless a specific temperature. LV-1 spindle for low-viscosity samples was fixed to the viscometer and set at 60 rpm and immersed into the soap solution. The viscosity was recorded in centipoise (cP).

Procedure for Determining Foam Generation, Height and Retention of a Toilet Soap Sample

1g of soap was dissolved in 100 mL of distilled water at 25°C to make a 1% soap solution. It was stirred until it completely dissolved.

1. Foam Generation: 50 mL of the soap solution was poured into a 100 mL graduated cylinder and shaken vigorously with a mechanical stirrer for 30 times.
2. Measure Foam Height: Immediately the foam height was measured from the base of the liquid to the top of the foam column.
3. Foam Retention Time: A stopwatch was immediately started after shaking and the time taken for the foam to collapse by half (50%) of its original height was recorded.

All the test were repeated three times for consistency and the average, and standard deviations of the foam retention time and foam height recorded.

Moisture Content Test for Toilet Soap: The moisture content of toilet soap is an important quality parameter, as excessive moisture can affect texture, shelf life, and performance. The Loss on Drying (LOD) method was used to determine moisture content.

A clean, dry crucible or drying dish was weighed (W_1). 5 g of the toilet soap was weighed into the dish (W_2). The sample was placed in a hot air oven at 105°C ± 2°C. and allowed it to dry for 3 hours or until a constant weight was achieved. The dish was removed from the oven and placed in a desiccator to cool for 15 minutes. The procedure was repeated three times The dried sample along with the dish was weighed (W_3). The moisture content was determined using the formula:

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$$\text{Moisture Content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where: W_1 = Weight of empty dish, W_2 = Weight of dish + soap before drying, W3 = Weight of dish + soap after drying. Typical moisture content in toilet soaps ranges between 10–15%. Excessive moisture can cause softening and reduced lathering, while too little can make it brittle.

Hardness test: To determine the hardness of the soap, a needle (6.4 cm in length; 1 mm in diameter) to which a lead fishing weight (130 g) was attached was lowered unto the soap. The distance into which the needle penetrated the soap, after 30 s, was recorded as a measure of its hardness. This procedure was performed three times for each soap sample, and the average and standard deviation were calculated.

pH analysis: The pH values of the soaps produced was analyzed using a pH meter (PHS-3C) E-Tract Instruments England Standard. 2.0 g of the produced soaps were dissolved in 50 ml of deionized water and the pH determined using the meter. This was done three times for each.

Total Fatty Matters (TFM): Total Fatty Matter Content Test: 5gm of soap sample was dissolved in 100ml hot Water. About 40ml of 0.5N HNO_3 was added to make it acidic. The blend was heated until fatty acids began to float as a layer over the solution. It was then cooled in ice water to solidify the fatty acids. The fatty acids were separated and the aqueous solution treated with 50ml chloroform to remove the remaining fatty acids. The separated Fatty matter was mixed together, solvent was then evaporated and the yield recorded. The experiment was performed three times.

The Total fatty matter was calculated using the following method using: % of fatty mater = $\frac{y-x}{weight\ of\ soap\ (g)}$ x 100 = %.

Where Weight of the China dish = (x), Weight of China dish + Soap after drying = (y) and Weight of soap sample = g

Sensitivity of the Soap Samples

The agar well diffusion method was used. 20 ml of freshly prepared Muller-hinton agar was poured into sterile Petri-dishes and the agar and allowed to solidify (gel). Using sterile syringe, 0.2 ml of each of the standardized organisms was inoculated into two Petri-dishes. Four (4) wells each of diameter 6 mm are made into each agar plate using a cork borer and the plate are labeled. Unto each plate, 0.2 ml of appropriate soap dilution was placed in appropriate wells, that is, 500, 250, 125 and 62.5 mg/ml, respectively. The plates were left for about 1 h for the soap to diffuse into the agar and then were incubated at 37°C for 24 h. After incubation, the plates were observed for evidence of inhibition which appeared as a clear zone completely devoid of growth around the well (zone of inhibition). The diameters of the wells are measured using a calibrated ruler in millimeters. The experiment was performed in duplicates and the mean of the zone of inhibition computed (Baba, *et al.*, 2017).

Statistical Analysis Tests

Analysis of Variance (ANOVA) has been conducted to determine whether there is significant difference between the means of the Zone of Inhibition exhibited by the groups of the ethyl acetate and ethanol varying concentrations of the plant extracts against the levels of microbial inhibition with the following hypotheses:

Null Hypothesis (H_0): There is no significant difference between the group means.

Alternative Hypothesis (H_a): At least one group mean is significantly different from the others.

Independent T-Test was also performed to determine significant difference between the Zones of Inhibition (ZoI) exhibited by the ethyl acetate extract and the ethanol extracts of the plant using the hypothesis:

Null Hypothesis (H_0): There is no significant difference between the ethyl acetate extract means of ZoI and those of the ethanol.

Alternative Hypothesis (H_a): At least one group mean of ZoI is significantly different from the other.

IV. RESULTS

Phytochemical Analysis

The result of the qualitative analysis of the flower sample is as shown in Table 1.

Table 1: Result of Qualitative Determination of Phytochemicals in *Calotropis procera* Flowers:

S/No.	Analyzed Phytochemical Factor	Ethanol	Ethyl acetate
1	Carbohydrates	+	+
2	Saponin	+	++
3	Flavonoids	++	+
4	Alkaloids	+	+
5	Steroids	-	+
6	Triterpenes	+	+
7	Cardiac Glycoside	-	+
8	Tannins	+	+
9	Anthraquinones	-	+

Note: + means positive activity, - means negative activity

GC-MS Analysis: The result revealed that Ɣ-sitosterol, stigmasterol, methyl ester, ȧ-amyrin acetate, β-amyrin, camp sterol, hexadecenoic acid, methyl ester, methyl stearate, phytol etc.

The results of the quantitative analysis of the phytochemicals are presented in Table 2.

Table 2: Quantity of Phytochemicals recorded in the Analysis of *C. procera* Flower:

S/No.	Phytochemical	Ethyl acetate (mg/100mg)	Ethanol (mg/100mg)
1	Carbohydrates	0.0058±0.016	0.0047±0.01
2	Saponin	0.638±0.006	0.453±0.23
3	Flavonoids	0.4448±0.16	0.431±0.1
4	Alkaloids	0.068±0.23	0.018±0.02
5	Steroids	0.008±0.02	0.001±0.02
6	Triterpenes	0.688±0.01	0.611±0.16
7	Cardiac Glycoside	0.0058±0.21	0.001±0.01
8	Tannins	0.855±0.26	0.638±0.23
9	Anthraquinone	0.002±0.18	0.0013±0.26

Anti-microbial Analysis

Below is the result of the anti-microbial tests performed on the formulated soap.

Physical Properties of Soap Analysis Result

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The physical properties of the soap formulated with the extract of the flower and neem oil as base oil is shown in the table below:

Table 3: Antimicrobial Activity of Ethanol Extract of *C. procera* Flower:

	Concentration/Zone of Inhibition (mm)								
Clinical Isolates	**125**	**62.5**	**31.25**	**15.75**	**Amp**	**Fluc**	**Distilled Water**	**MIC**	**MBC/MFC**
Klebs pneumoniae	13	9	6	8	6	0	0	8	15.75
Pseudomonas aeruginosa	11	8	7	6	6	0	0	8	15.75
Esherishia coli	4	0	0	0	8	0	0	8	15.75
Strep pyoenes	14	14	11	10	8	0	0	6	15.75
Staph aureus	4	0	0	0	8	0	0	8	15.75
Aspergillus flavus	14	13	10	6	0	8	0	0	0
Aspergillus niger	14	12	10	8	0	8	0	8	15.75
Candidas albicaus	12	10	8	6	0	6	0	8	15.75

Table 4: Antimicrobial Activity of Ethyl acetate Extract of *C. procera* Flower:

	Concentration/Zone of Inhibition (mm)								
Clinical Isolates	**125**	**62.5**	**31.25**	**15.75**	**Amp**	**Fluc**	**Distilled Water**	**MIC**	**MBC/MFC**
Klebsiela pneumonia	12	10	7	6	6	*	0	6	15.75
Pseudomonas aeruginosa	12	9	8	6	6	*	0	8	15.75
Esherishia coli	3	0	0	0	6	*	0	8	15.75
Streptococcus pyoenes	14	14	11	10	8	*	0	6	15.75
Staphylococcus aureus	14	12	9	8	8	0	0	8	15.75
Aspergillus flavus	13	12	8	7	*	6	0	0	0
Aspergillus niger	14	12	10	8	*	10	0	6	15.75
Candidas albicaus	13	10	7	6	*	8	0	8	15.75

* = Nested with the respective anti-biotic antibiotic not tested for. 0 = No inhibition at lowest concentration. Amp = Ampicillin, Fluc = Fluconazole

Table 5: Results of the Physical Examination carried on the Soap Sample:

Parameter	**Ethyl acetate Extract Soap**	**Ethanol Extract Soap**	**Commercial Soap**	**NIS 4:2017 Standard**
Colour	Creamy	Creamy	Light Yellow	NA
Odour	Pleasant	Pleasant	Pleasant	NA
Appearance	Good	Good	Good	NA
pH	10.2±0.02	9.7±0.02	9.16	9-11
Foam Height	110±0.002mm	110±0.002mm	25.25	100-200mm
Foam Retention	7±0.01mininutes	5±0.01mininutes	9minutes	≥15 mins
Stability	Melted at 68.4±0.19℃	Melted at 63.4±0.19℃	75℃	NA
Hardness	4.69±0.029 grains/gallon	4.29±0.029 grains/gallon	5.80±0.27 grains/gallon	NA
Total Fatty Matter (TFM)	63.4±0.003%	68.4±0.003%	79%	≥76%
Moisture Content	9.86±0.1%	10.86±0.1%	11.89%	≤15%
Viscosity	16.66±0.0029mPas	14.6±0.0025 cP	12.56 cP	NA

NA = Not Available

V. DISCUSSION

The result of the phytochemical analysis of the flower samples of Sodom Apple revealed a number of very important phytochemicals that useful to both human and animal. The result is presented in Table 1 above. The number of phytochemical compounds unearthed from the use of ethyl acetate was found to be more in number compared to those of ethanolic extraction. They both were positive for carbohydrates, saponin, flavonoids, triterpenes and tannins. The ethanolic extraction was not positive for anthraquinones, steroids, alkaloids, and cardiac glycosides.

The GC result of the analysis revealed that Ɣ-sitosterol and stigmasterol were present. The two most prominent among the compounds have been identified and in a number of plants and are found to possess anti-inflammatory and anti-diabetic activities, β-amyrin has antifungal and anti-inflammatory properties, while campsterol is reported to be anti-cancer compound (Malik *et al*., 2021). The result revealed that Ɣ-sitosterol, stigmasterol, methyl ester, ἀ-amyrin acetate, β-amyrin, campsterol, hexadecenoic acid, methyl ester, methyl stearate, phytol etc among other twenty compounds that have either prophylactic or/and therapeutic properties.

Table 2 provides the quantity of the phytochemicals with the ethyl acetate generally higher in output compared to ethanol. The concentrations follow the following order: Tannins (0.855mg/100mg), triterpene (0.688mg/100mg), saponins (0.638mg/100mg), flavonoids (0.488mg/100mg), alkaloide (0.068mg/100mg), steroids (0.008mg/100mg), cardiac glycoside (0.0058mg/100mg) and anthraquinone (0.002mg/100mg) respectively. This result above agreed with the findings of Habeeb *et al* (2024) on the leaves of the plant and particularly, the high content of tannins is not surprising as a number of researchers have reported the compound as foremost in antibacterial, antioxidant activities (Malik *et al*., 2021, David *et al*., 2022, Namadina *et al*., 2023).

The sensitivity test result shown in Tables 3 and 4 revealed the activities of varies concentrations of the for the determination of MIC and MBC. The antibacterial and antifungal activities strength of the toilet soaps was performed with different concentrations of the soap using disc diffusion method with ampicillin (antibacterial), fluconazole (anti-fungi) as positive controls and distilled water as negative

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controls. The result showed enough antibacterial and antifungal properties as exhibited in the respective zones of inhibition (ZOI). Even as the ethanolic extract did not exhibit significant inhibition against E. coli and A. flavus, the ethyl acetate extract demonstrated reasonable exhibition against the bacteria, including *S. aureus* and fungi. This development is in agreement with Khan *et al* (2024) findings reported in one of his studies; stating that ethyl acetate extracts show inhibition against a wide range of microbes.

The soap quality was also screened through foam height, foam retention time, hardness, total fatty matters, pH and found to meet soap quality requirement by Nigeria Industrial Standard for toilet soap. (Table 5). These results are also in consonant with the findings of Ameh *et al*., (2013) whose combination of neem oil and shea butter oil demonstrated antibacterial activity in soap.

Statistical Analysis Results

A one-way analysis of variance (ANOVA) was conducted to evaluate the effect of different concentrations on the zones of inhibition for Ethyl Acetate and Ethanol extracts. There was a statistically significant effect of concentration on the zones of inhibition for Ethyl Acetate [$F(3, 28) = 4.049$, sig 0.016, $p < 0.05$]. This means that the plant extract at higher concentration levels is more likely to be effective against the microbes. However, the ethanol extracts [$F(3, 28) = 2.098$, sig 0.123, $p > 0.05$]. This indicates that there is no significant difference among the different concentration levels and therefore, the concentrations are effective at all levels. Tukey's post-hoc test revealed that each increase in concentration resulted in a significant increase in the zone of inhibition ($p < 0.05$).

An independent samples t-test was conducted to compare the antimicrobial activity between Ethyl Acetate and Ethanol extracts at each concentration. The null hypothesis is accepted with the result $t (0.05) = 0.567$; $p = > 0.05$. There was no significant difference in the zones of inhibition at all concentrations. Ethyl Acetate extract did not exhibit larger zones of inhibition compared to Ethanol extract at all concentrations.

VI. Conclusion

The *C. procera* flower extract-enriched soaps satisfy the essential requirements of NIS 4-2017 toilet soap standards because they display desirable physical traits. The extract material shows positive responses when tested against regular bacteria and fungi thus forming a strong potential base for use as antiseptic toilet soaps in both residential and workplace settings.

The ANOVA and Independent Samples T-Test results showed significant antimicrobial effects for the two types of extracts which demonstrated comparable zone of inhibition levels at every concentration. The tested differences were insufficient to prove statistical significance indicating solvent extraction technique selection has no impact on plant extract antimicrobial effectiveness.

Recommendations

In light of the findings above, the study recommends as follows:

1. Pharmaceutical and cosmetic industries should take advantage of the availability and abundance of the plant and incorporate its extracts in formulating medicated soap.
2. More studies should be carried out to identify, isolate and eliminate the anti-nutrition component of the flower of the plant.
3. Further research is recommended for antioxidant and cytotoxic properties of the flower.

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