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Antimicrobial Activity of Leaf and Stem Extracts of Sodom Apple (Calotropis Procera) Against Dandruff Causing Organisms (Malassezia Furfur and Staphylococcus Aureus)

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Abstract
Microorganisms are becoming resistant to synthetic drugs. Sodom apple plant has been used in many traditional medicines to treat diseases and infections. Therefore; the aim of this work is to use Sodom apple leaf and stem extract to test it efficacy against dandruff causing organisms (M. furfur and S. aureus). Sample of Sodom apple was collected within Bauchi metropolis and the test organisms were isolated using serial dilution while identification was based on cultural, morphological and biochemical screening. Cold extraction of leave and stem of Sodom apple was obtained by soaking overnight in water while, ethanol extract was obtained using Soxhlet extractor. The phytochemical screening of the extract carried out, revealed the presence of Alkaloids, Saponins, Glycosides and Steroids in both the extracts and absence of Anthraquinones in both the extracts. The antimicrobial activity evaluated through agar well diffusion method showed that, the extracts of leave was the most effective in terms of inhibitory activities, exhibiting 20mm against S. aureus with a Minimum Inhibitory Concentration of 75%. The ethanol extract inhibits 10.2mm against M. furfur with a Minimum Inhibitory Concentration of 50% while the cold extract of the stem has the zone of inhibition of 11.5mm against S. aureus with Minimum Inhibitory Concentration of 100%. The ethanol extract has the least zone of inhibition, 2mm against M. furfur with negative Minimum Inhibitory Concentration. The antimicrobial data assayed against Streptomycin was effectively higher than all the extract with Minimum Inhibitory Concentration of 30mm and 27mm against M. furfur and S. aureus respectively. It could be said that the leaf and stem extracts of Sodom apple was effective against dandruff causing organism in combined forms. With further research on the purification of bioactive component of Sodom apple, it could be used in the treatment of dandruff.

Keywords: Antimicrobial, sodom apple, m furfur, s aureus, dandruff

Introduction

Background to the Study

Some microorganisms have developed resistance to antibiotics that were once commonly used to treat them. For example, Staphylococcus aureus (‘golden staph’ or MRSA) and Neisseria gonorrhoeae (the cause of gonorrhea) are now almost always resistant to benzyl penicillin. In the past, these infections were usually controlled by penicillin (W.H.O, 2016).

Plants have always played a vital role in the treatment of several diseases of man. According to World health organization (WHO) more than 80% of the world population relies on traditional medicine for their primary health care needs (Akinyi *et al.,* 2015) [4]. The practice of herbal medicine in modernized form is now gaining momentum in Nigeria with the various health officials and other personnel realizing the potency and efficacy of some of the indigenous plants (Akinyi *et al.*, 2015) [4]. Moreover, the use of natural products for curing wide variety of human and domestic animal diseases has a long history that goes to human civilization (Debalke *et al*., 2018) [15].

In addition, practice of herbal medicine in modernized form is now gaining momentum in Nigeria, with various health officials and other persons coming to realize the potencies and efficacies of some of the indigenous plants (Nwaogu, 2015). Among the diseases that have been successfully managed traditionally include malaria, epilepsy, indigestion, scurvy, diarrhea, dysentery, gonorrhea, flatulence, worm infections and mental illness. Medicinal uses of these plants range from the administration of the plants root, barks, stem, leaves and seeds, to the use of extracts from a whole plant (Soforowa, 2014).

Sodom apple (Calotropis procera) also called Tumfafiya in Hausa, Achicha in Igbo and Ewe Bomubomu in Yoruba, is a species of flowering plant that is native to North Africa. In Nigeria it is found in almost all the state especially in Kaduna, katsina, Plateau and Bauchi state (Mukharjee *et al*., 2017).

Sodom apple has been shown to be effective against many important human pathogens. Overall, a large number of studies have been published in the last decade on this topic, especially as they relate to the ever-growing number of antibiotic-resistant organisms.

Staphylococcus aureus (*S. aureus*) a commensal microbe in human that can become an opportunistic pathogen, especially in immunocompromise persons, a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies. *Staphylococcus aureus* is one of the leading pathogens for death associated with antimicrobial resistance and the emergence of antibiotic-resistant strains such as methicillin-resistant. *Staphylococcus* aureus (MRSA) is a worldwide problem in clinical medicine. (Valarmathy *et al.,* 2010).

Malassezia furfur (Head and neck dermatitis) is a subtype and difficult to treat form of atopic dermatitis, which is generally seen in post-pubertal atopic dermatitis patients. The clinical manifestations of head and neck dermatitis are typically erythematous involvement of the eyelids, forehead

and neck; Affected areas are itchy and there is often scaling giving the appearances of an eczema flare (Buhman *et al*., 2019).

1. Justification

Dandruff is a common condition that causes the skin on the scalp to flake, with high occurrence rate seen in most half of the population of the world (Begun, 2019) [9]. According to reports by Rudramuthy *et al.,* (2014) that dandruff causes irritation, extending to nasolabial folds, eyebrows, headache in some condition.

2. Objectives of the study

The specific objectives of the study wereto:

1. Determine the active compounds of Sodom apple extracts through Phytochemicals screening.
2. Isolate and identify the test organisms (Malassezia furfur and staphylococcus aureus).
3. Determine the antimicrobial activity of leaf and stem extract of Sodom apple against Malassezia furfur and Staphylococcus aureus.
4. Determine the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Sodom apple extract against M. furfur and S. aureus.

Medicinal Plants

Plants produce an extensive range of phytochemical components which are secondary metabolites (Ojewume *et al*., 2017). These secondary metabolites work uniquely and are used directly or indirectly in the pharmaceutical industry. Phytochemicals have the ability to act as antioxidants by preventing cell damages which is usually caused by free radicals such as those associated with heart disease and cancer. Examples of phytochemicals with antioxidant activity are allyl sulphides, flavonoids, polyphenols and caternoids. Phytochemicals are also known to possess the ability to interfere with enzyme action. For instance, indoles stimulate enzymes that make estrogen less potent, and thus could lessen the risk of cancer of the breast. The ability of Phytochemicals to interfere with DNA replication has been documented; in cases where harmful cells are presenting a biological system, phytochemicals have prevented the replication of those cells (Agarwal, 2018). Phytochemicals have the ability to act like hormones. For example, of flavones can imitate human estrogen and help decrease menopause symptoms and osteoporosis. Phytochemicals are known to possess the capability to destroy or impede the growth of bacteria. For instance, allicin is one of the phytochemicals known to possess antibacterial properties. Phytochemicals help in the prevention of the adhesion of pathogens to cell walls. For example, proanthocyanidins have anti-adhesion tendencies which help reduce the risk of urinary tract infections and enhance dental health. Due to the presence of diverse phytochemicals in essential oils and other plant extracts, there is high potential for extracts from different plants to have anti-microbial properties. The varied phytochemical constituents and unique functioning of each phytochemical, if properly investigated and harnessed could lead to discovery of novel substances and active compounds that are effective against seemingly drug resistant microorganisms while simultaneously alleviating several of the side effects that usually accompany synthetic antimicrobials. Many plant essential oils have been reported to have significant inhibitory effects on pathogenic microorganisms, and it has been shown that most herb essential oils obtained from medicinal herbs have antifungal, antiparasitic, antibacterial, and antimicrobial properties; therefore, plant essential oils have been greatly utilized in some fields such as pharmacology, herbal pharmacology, pharmaceutical/clinical microbiology, and phytopathology, as well as maintenance of food, fruits, and vegetables. These herbal medicines are popular among people (Beigomi *et al*., 2021).

1. Sodom apple

Sodom apple is used medicinally by the East African Maasai, as well as other indigenous communities in the region. Pulp from the sodom apple is often applied directly to aching teeth and gums, acting as a pain reliever, and the plant's stem is used as a tooth brush due to its known anti-bacterial properties (James *et al*, 2017). Not only that the stems are boiled and consumed to relieve stomach pains, and the plant is also known to be applied directly to external wounds to aid healing. While the plant is used in a variety of ways, it is highly recommended that one never swallows or consumes the Sodom apple fruit.

2. Sodom Apple Chemistry

The chemicals that have pesticidal activity can most efficiently be extracted from Sodom Apple seed kernels. Sodom Apple trees begin their productive stage at about eight months to one year of age but do not become a fully reproductive until they are one and half year old. From this time on, the tree yields an average of about 20.5 kilograms of fruit per year, with maximum production reaching 50 kilograms per year. Of the fruit yield, only about ten percent is attributed to seed kernels, and desired biologically active compounds comprise only ten grams per kilogram of kernel weight. This means that an adult Calotropis procera tree will only produce about 20grams of pesticidal compounds in a season (Schmutterer, 2001). The milky sap of Calotropis procera contains a complex mix of chemicals, some of which are steroidal heart poisons known as "cardiac aglycones". These belong to the same chemical family as similar ones found in foxgloves. The plant contains steroidal components that are the cause of its toxicity. In the case of the Calotropis glycosides, their names arecalotropin, calotoxin, calactin, uscharidin and voruscharin. (United States Department of Agriculture, 2010)

Materials and Methods

1. Study Area

The study was carried out within Bauchi LGA located in Bauchi state. Bauchi is located between latitude 9 degree and 12-degree north and longitude degree and 11 east equators. The state shares border with Kano and Jigawa to the north, Taraba and plateau to the south, Gombe and Yobe to the east, and Kaduna to the west. Bauchi state occupies a total land area of 49,11km2 representing about 5.3 percent of Nigerians total landmass.

2. Sterilization of Glass wares and Equipments

All glass wares/equipment used were washed, rinsed with clean water, cleaned and sterilized in Hot Air Oven at 160ºC for 1 hour. All chemicals and reagents were prepared accordingly.

3. Collection/Processing of Plants Material
Leaf and stem of Sodom apple was collected from the federal polytechnic, Bauchi metropolis and transfer to laboratory of microbiology, science laboratory technology department in federal polytechnic, Bauchi. Sample was washed and air dried at a room temperature for seven days. The dried leaves and stem samples were grinded into fine powder using mortar and pestle in the Laboratory. The powdered leaves samples were sieved, packed in a seal for further analysis (Iwelewa, 2015).

4. Extraction of Plants Materials
The Ethanol and cold-water extraction of the plant was carried out according to the method of Olayemi and Opaleye (2018).

a. Ethanol extraction of leaves and stem of Sodom apple
Forty grams (40g) of the Sodom apple (leaf and stem) powder was measure on an electronic weighing balance and poured in to thimble with a lower part covered in to cotton wool. 500ml of ethanol was poured in to the flask which has clamped to the retort stand and a set up was mounted on a heating mantle and heated for 6hours to extract the ingredients. After 6hours the sample was removed and concentrated in a water bath at 75ºC to evaporate the ethanol.

5. Phytochemical Screening of Plant Extract
Phytochemical analysis including test for alkaloids, saponins, tannins, flavonoids, glycosides and steroids in the plant in accordance with the standard procedures as described by Standard methods Association of Analytical Collaboration (AOAC, 2003).

a. Test for Alkaloids
Two milliliter (2 ml) of each of the extract added to 2 ml of 10% Hydrochloric acid (HCl) and mixed in test tube. 1 ml from the above mixture was treated with few drops of Wagner's reagent and another 1 ml treated with few drops Maye`s reagent. Turbidity with both of the two reagents was considered as an indication for the presence of alkaloids.

b. Test for Saponins
Four milliliters (4 ml) of each of the extract was placed in a test tube followed by 4 ml of distilled water. The mixture was shaken vigorously. Froth formation indicate the presence of Saponins.

c. Test for Tannins
Ferric Chloride solution (5%) was added drop by drop to 2 ml of each of the extract in test tube and the color produced was noted. Condensed tannins usually give a dark green color, while hydrolysable tannins give blue-black color.

d. Test for Flavonoid
Three milliliter (3 ml) of each of the extract was added to 1 ml of 10% Sodium hydroxide (NaOH) and mixed in test tube. Yellow color formation indicates the possible presence of flavonoid compounds.

e. Test for Glycosides
Five milliliters (5ml) of each of the extract was placed in a test tube, 2.5 ml of 50% H2SO4was added and mixed. The mixture was heated in boiling water for 15 minutes. After cooling, the mixture was neutralized with 10% Sodium Hydroxide (NaOH). From the above mixture, 5 ml was mixed with 5ml of Fehling's solution and the resultant mixture was boiled in a water bath. A brick-red precipitate indicates the presence of glycosides.

f. Test for Steroids
Five milliliters (5ml) of each of the extract was dissolved in 2 ml of chloroform in a test tube. 2 ml of concentrated sulphuric acid was carefully added down the side of the test tube to form two layers. Reddish-brown color at the interface indicates the presence of a steroidal ring.

6. Collection of Test Organisms (M. furfur and S. aureus)
The sample of Staphylococcus aureus was collected from Bayara Hospital and confirmed by sub-culturing on a fresh nutrient agar.

7. Media Preparation
Nutrient Agar, Sabouraud Dextrose Agar, Sabouraud dextrose Broth and Mueller Hinton agar was prepared according to the manufacturing standard and sterilized using autoclave according to (Udosen, 2018).

Table 1: Phytochemical test of Ethanol and Cold extract of Sodom apple

Plant Chemical	Leaf Extract		Stem Extract	
	Cold	Ethanol	Cold	Ethanol
Alkaloids	+	+	+	+
Saponin	+	+	+	+
Tannin	-	-	-	-
Flavonoid	-	-	-	-
Glycoside	+	+	+	+
Steroid	+	+	+	+
Anthyraquinone	-	-	-	-

Key: + = present - = Absent

Table 2: Cultural, Morphological and Biochemical Characteristics of the Isolates

Cultural Characteristics COL EL SH	Biochemical Test	Sugar Fermentation	Suspected Organism
	raGin Stain, Catalase, Coagulase, Oxidase, Indole, Growth & 41ºC	Maltose, Glucose, Manitoles, Lactose, Sucrose	
BG C R	- + - + - -	A - - - A/G	*Staphylococcu aureus*
CLL, C O	+ + + - - -	A A A - -	*Malasezia Furfur*

Key: + = Presence, A=acid production, COL=Color, EL=Elevation, SH=Shape, BG=Blue green, C=Convex, =Absence, A/G=acid and gas, CLL=Colorless, R=Rod, O=Oval

Table 3: Anti-microbial activity of Ethanol and Cold extract sodom apple on *furfur* and *Staphylococcus aureus* with zone of inhibition (in mm)

Conc. (%)	Ethanol Extracts				Cold Extracts			
	M. furfur	S. aureus	M. furfur	S. aureus	M. furfur	S. aureus	M. furfur	S. aureus
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
100	15	10	20	7	10.2	5	10.5	11.5
75	5	10	15	3	7.5	3	10	-ve
50	2	-ve	-ve	-ve	-ve	-ve	-ve	-ve
25	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Control	30	17	20	21	21	20	27	21

Key: = present -ve = Absent, Control = Streptomycin and Fluconazole

Table 4: Result of minimum inhibitory and Bactericidal concentration of the leaf extract against the test organism in percentage (%)

Extract	Organism	25%	50%	75%	100%	MIC	MBC
Cold	S. aureus	+ + +	—	—	—	50	75
	M. furfur	+ + +	+ +	—	—	75	—
Ethanol	S. aureus	+ +	+	—	—	75	100
	M. furfur	+ +	+	—	—	75	100

Key: MIC = minimum inhibitory concentration, MBC= minimum bactericidal concentratiuon
(-) = no growth, (+) =turbidity, (++) =moderate turbidity, (+++) = highly turbidity

Table 5: Shows the minimum inhibitory and bactericidal concentration of the stem extract against the test organism in percentage (%)

Extract	Organism	25%	50%	75%	100%	MIC	MBC
Cold	S. aureus	+ + +				50	75
	M. furfur	+ + +				50	75
Ethanol	S. aureus	+ +	+			75	100
	M. furfur	+ +	+			75	100

Key: MIC = minimum inhibitory concentration, MBC= minimum bactericidal concentratiuon
(-) = no growth, (+) =turbidity, (++) =moderate turbidity, (+++) = highly turbidity

Discussion

The result of this study showed that, the phytochemical screening revealed the presence of Alkaloid, Saponin, steroid, Glycisides, Tanins whereas Flavonoid in both whiles, anthraquinone were not present in both the cold and ethanol extracts This is in agreement with the report of Akanmu *et al.,* (2021) carried out a similar work on phytochemical screened of Solanum incanum.

The observation of bactericidal concentration was observed at 100% and 75% concentration on M. furfur and S. aureus in which other concentrations shows no colony, is in lined with the work of (Saeidi *et al*., 2013), in his work on important Human pathogen.

The effect of cold-water extract on S. aureus and M. furfur was lower as shown by their zones of inhibition, this may be attributed to the solvent (water) that was used. This observation is in agreement with the work of (Saeidi *et al.*, 2013), which states that Ethanol are better solvent than water for the extraction of active ingredients of the plants

In antimicrobial activity, the highest zone of inhibition was observed at 100% concentration with the 20mm 15mm zone of inhibition and the least zone of inhibition was observed at 75% with zone of 2mm and 3mm for M. furfur and S. aureus in ethanol and cold respectively. This agrees with the work of Waithaka *et al.,* (2019) which showed that ethanol extract have highest antimicrobial activity wth 30mm in diameter than cold extract with 20mm in diameter.

Conclusion

Based on this research, both of the extract of leave and stem of Sodom apple was found to be effective dividually and in combined form with an MIC of 20mm at a concentration of 75% against *Staphylococcus aureus* and a MIC of 10.2mm at a concentration of 50% against *Malassezia furfur*. Therefore, it could be said that Sodom apple can be used as an effective antimicrobial agent against *Staphylococcus aureus* and *Malassezia furfur* infections as well as other related diseases.

Recommendations

Being that Sodom apple has a percentage of active ingredients on leave and stem which is the characteristics for medicinal activity against different disease-causing organisms. Further research work should be carried out to explore the phytochemicals constituent by purification, toxicological evaluation with a view of formulating a new chemotherapeutic agent in order to provide curative remedy to resistance pathogenic microorganisms to synthetic antibiotics.

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