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Shampoo production as an antimicrobial agent from avocado (*persea americana*) pawpaw (*carica papaya*) and neem seeds (*azadirachta indica*) extract against dandruff causing organism (*malassezia furfur*)

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Abstract
Dandruff is a very common contagious hair problem that affects people irrespective of age. The aim of this work is to produce shampoo as antimicrobial agent from avocado, pawpaw and neem seeds against dandruff causing organism (*Malassezia furfur).* Samples of avocado, pawpaw and neem seed were collected within Bauchi metropolis and the test organism was isolated using serial dilution while identification was based on cultural, morphological and biochemical screening. Extract from the samples was obtained using soxhlet extractor with n-hexane. The phytochemical screening of the extract carried out revealed the presence of Alkaloids, Saponins, Glycosides and Steroids in pawpaw and Neem seed while Glycoside was absent in avocado extracts. The Minimum Inhibitory Concentration (MIC) carried out using agar well diffusion method showed that the shampoo produced inhibits the test organism (*Malassezia furfur*) with the highest zones of inhibitions at 12mm in 100% mg/ml and the lowest is at 7mm in 25%mg/ml while the commercial shampoo (control) has the highest zone of inhibition at 20mm in 100% and the lowest at 13mm in 25%mg/ml. The shampoo has the pH of 6.78. The foam value (mm) was 350, and the solid content (%) was 22.1. The patch test for sensitivity testing was also carried out and it shows no evidence of skin irritation and allergic reaction. In conclusion herbal shampoo formulated with avocado, pawpaw and neem seed was effective against *Malasszie furfur.* Thus, it can be used in the treatment of dandruff.

Keywords: Antimicrobial, avocado, pawpaw, neem seeds, dandruff, *malasszie furfur*

Introduction

Dandruff is a very common contagious hair problem, nearly affecting persons irrespective of age (Szepietowski, 2009) [23]. Dry dandruff appears silvery and white while greasy flakes appear pale yellowish and may have an unpleasant smell and excessive shedding of dead skincell from scalp. It's an apparently caused by a fungus called Malassezia restricta, Malassezia globosa and Malassezia furfur (Del Rosso, 2011).

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. (Kohsaka *et al*., 2018). The Malassezia (antigen) proteins are found in sweat and the disease is therefore triggered by sweating (sometimes referred to as sweat allergy) (Maarouf *et al.,* 2018). An IgE antibody against Malassezia is found in up to 27% of children and 65% of adults with atopic dermatitis (Glatz *et al*., 2015).

Shampoo is a liquid or cream soap or detergent that is used to wash the hair. Shampoo is a hair washing product that comes in the form of a thick liquid. Shampoo's purpose is to remove the undesired build-up between the hairs without removing so much sebum that the hair becomes unmanageable. The most popular type of hair treatment is shampooing. Shampoos are made up of a range of herbs and their extracts, although most shampoos in the market today are surfactant-based. Surfactants are included for their cleansing properties, but their continued usage can cause eye irritation, hair loss, and hair dryness (vijayalakshmiel *et al*., 2018). The interest of people towards herbal product is increasing day by day due to their fewer side effects. Liquid shampoos most commonly available in markets are synthetic and contains many harsh chemicals that are associated with different health problems when used repeatedly and continuously (Arora *et al.,* 2011) [1]. This led to the dryness of hair, hair loss and dandruff itself. To reduce this and other related side effects, an attempt has been made to formulate herbal shampoos containing easily available herbal plant extracts. These plant products can be powdered, crude, pure extracts, or derivatives.

Avocado, (Persea Americana) also called alligator pear, it belongs to the family of Lauraceous and it's edible. Avocados are native to the Western Hemisphere from Mexico south to the Andean regions, middle belt region in Nigeria and are widely grown in warm climates. Avocado fruits have greenish or yellowish flesh with a buttery consistency and a rich nutty flavor. They are often eaten in salads with bread, and in many parts of the world they are eaten as a dessert. Mashed avocado is the principal ingredient of guacamole, a characteristic sauce like condiment in Mexican cuisine. Avocados provide thiamine, riboflavin, and vitamin A, and in some varieties the flesh contains as much as 25 percent unsaturated oil (Chaudhary et al., 2015) [4]. There are two types of avocado flowers, A and B, depending on the cultivar. These flowers are dichogamous (male and female parts mature separately), and each flower opens only twice. Type A flowers are functionally female in the morning, close at midday, and then reopen as functionally male in the afternoon of the following day. Type B flowers are functionally female in the afternoon, close in the evening, and then reopen the following morning as functionally male. When the two flower types are grown together, this temporal overlap of mature male and female parts encourages cross-pollination and, thus, greater fruit production (Aghel, 2007). The fruit's outer skin is sometimes no thicker than that of an apple and

sometimes is coarse and woody in texture (Sharma 1998) [20]. Avocados provide thiamine, riboflavin, and vitamin A, and in some varieties the flesh contains as much as 25 percent unsaturated oil (Kumar and Mali, 2010) [9]. It's used in the treatment of anti-hyperglycaemic, anticancer, anti-hypercholesterolemia, antioxidant, anti-inflammatory, and anti-neurogenerative effects are clearly demonstrated how these properties can be used to formulate or fortify food.

Objectives of the study

The specific objectives were to

1. Carryout phytochemical analysis on avocado oil, Neem oil and Pawpaw oil extract.
2. Isolate and identify the test organism
3. Produce shampoo from Avocado seed, Pawpaw seed and Neem seed
4. Evaluate the antimicrobial activities of the herbal and commercial shampoo

Hairs

Hairs are the integral part of human beauty. Hair is a protein filament that grows from follicles on the dermis or skin. Scientific name of hair is pili or pilus. Hair is a component of the integumentary system and extends downward into the dermal layer where it sits in the hair follicle (Kumar, 2010) [9]. The presence of hair is a primary differentiator of mammals as a unique class of organisms. In humans, it is a cherished and highly visible indicator of health, youth, and even class. It has a sensory function, protects from cold and UV radiation, and can have a significant psychological impact when its growth or structure is deranged (Kumar, 2010) [9]. At a microscopic level, the variety in length, color, diameter, and cross-sectional shape of each hair creates the characteristic profiles seen across ethnic groups and among individuals (Kumar, 2010) [9].

1. Parts of the Hair

Dermal papillae: The dermal papilla is responsible for regulating the hair cycle and hair growth, and is also comprised of androgen receptors that are sensitive to the presence of DHT. Matrix: The matrix surrounds the dermal papillae and contains all the active cells needed for hair growth and for the development of the different parts of the hair, particularly the outer root sheath, the inner root sheath and the hair shaft. Combined, the matrix and the dermal papillae make up the hair both PDA and SDA (Pooja *et al*., 2010).

Outer root sheath: The outer root sheath, or trichelemma, is the outermost part of the hair and is keratinized. It covers the entire hair follicle inside the dermis and then transitions through to the epidermis, providing the hair follicle with an opening from which to surface from. Inner root sheath: inner root sheath is comprised of three parts: the Henley layer, Huxley layer, and cuticle. This, together with the capsular layers that make up the Henley's and Huxley's layers, secures the hair and allows it to grow in length (Pooja *et al*., 2010).

Hair shaft: The hair shaft is the solitary part of the hair follicle that fully exits the surface of the skin. The hair shaft is made up of three layers: the medulla, cortex, and the cuticle.

2. Dandruff

Dandruff is a very common contagious hair challenge, nearly affecting person irrespective of age. Medically it is defined as pityriasis simplex capitis – shedding of dead skin from the scalp. It may be – dry or grease. Dry dandruff appears silvery and white while greasy flakes appear pale yellowish and may have an unpleasant smell. Historically there have been multiple other descriptive names reflecting the fungal cause of this condition, such as pityriasis simplex and pityriasis capitis (referring to Pityrosporum) and furfuracea (referring to Malassezia furfur) (Kal, 2017). It is a common disorder which affects 5% of the global population. Dandruff is common scalp condition that producing the irritating white flakes and itchy scalp. Excessive drying of skin and over-activity of oil gland known as seborrhoea (Randall, 2010).

a. Types of Dandruff

Dandruff can be classified as disorders of the sebaceous gland or skin scaling disorders. Dandruff can be of two types. They are:

1. Oily dandruff
2. Dry dandruff

Oily dandruff (Pityriasis Steatoides): On the scalp, waxy, greasy, yellowish, thick scales crusts are present. Beneath the crusts, the scalp is red or pale but dry. The hair may be dull and flat without shine. There may be slight itching. If irritated eczematization complicates the condition to produce seborrhoeic dermatitis. Patients with Pityriasis steatoides usually develop thing and later loss of hair.

Dry dandruff (Pityriasis sicca): The scales are fine, thin, furfuraceous, white or grayish and dry or only slightly greasy. The hair is dry and lusterless. There is mild to moderate itching. The scales fall freely on the shoulders. This type of dandruff is more common in winter than in summer. It signifies exaggeration of normal exfoliation of the horny layer of the epidermis. It usually affects people with dry integument and scalp. In nutritional disorders, scaliness of the scalp is exaggerated (Randall *et al.,* 2010)

b. Causes of dandruff

One explanation for dandruff is that the fungus Pityrosporumovale, which is naturally present on the scalp and other parts of the skin. Typically, this fungus causes no damage. However, with the weather changes, hormonal, and stress, the scalp will produce more oil, causing the fungus P. ovaleto proliferate. The Malassezia fungus breaks down sebum to oleic acid by using these enzymes. The oleic acid then penetrates the top layer of skin and causes increased skin cell turnover in susceptible people. This, in turn, causes dandruff flakes and sometimes itching and redness (Randall *et al*., 2010).

Dandruff has been shown to be the result of 3 required factors.

- Skin oil commonly referred to as sebum or sebaceous secretions.
- The metabolic byproduct of skin micro-organisms (most specifically Malassezia yeast, andlipophillic fungus).
- Individual susceptibility against presence of Malassezia species.

Candida a-PDA and SDAicans is one of the major causes for dandruff together with the fungus. There may be some bacterial infestation on scalp wound by nail scratching.

Materials and Methods

1. Study Area

This research was carried out at the Federal Polytechnic Bauchi, Microbiology laboratory. Bauchi state is located between latitude 9° 3' North and longitude 8°50' and 11° East. Rainfall in Bauchi state ranges between 1300mm per annum in the South and only 700mm per annum in the extreme North. The weather experienced in the South and North varies considerably. While it is humidly hot during the early part of the rainy season in the South, the hot, dry and dusty weather lingers up North (Wikipedia, 2019).

2. Sterilization of Glass wares and Equipment's

All glass wares/equipment's 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 of Seeds Samples

Avocado (Persea Americana), Pawpaw (carica papaya) and Neem (Azadirachta indica) were purchased from Yelwa market, Bauchi, Bauchi state.

4. Preparation of Samples

The Avocado (Persea Americana), Pawpaw (Carica papaya) and Neem (Azadirachta indica) seeds were air dried for five (5) days, the sample was pounded and sieve. Aloe-vera jell was extracted from aloe-vera, fenugreek was pound and dissolve in water, onion water was extracted from onion.

5. Extraction of avocado, pawpaw, and Neem seeds using N-hexane

40g of each sample was poured and inserted into the soxhlet extraction apparatus and fixed into a round bottom flask containing 250ml of N-hexane. The flask was subjected to heat using heating mantle for 4 hours. The resultant mixture of solvent and extract passed through a lie big condenser cooled by a continuous flow of fresh water. The oil extract was separated using water bath. (Tan & Ghazali., 2019)

6. Phytochemical Screening of Plant Extract

Phytochemical analysis including test for alkaloids, saponins, tanins, flavonoids, glycosides and steroids in the plant in accordance with the standard procedures as described by Standard methods Association of Analytical Collaboration (AOAC, 2010).

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 Maya's reagent. Turbidity with both of the two reagents was considered as an indication for the presence of alkaloids.

b. Test for Saponins

Four milliliter (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 indicates 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.

7. Media Preparation

Sabouraud Dextrose Agar, Sabouraud dextrose Broth and potatoes dextrose agar was prepared according to the manufacturing standard and sterilized using autoclave according to (Udosen, 2018) [26].

8. Isolation of Malassezia furfur

The isolation was conducted according to the method described by Begum *et al.*, (2019). One gram (1g) of the scrapping was mounted in 2ml of 10%KOH, 1ml of glycerol was added, park solution and observed in microscope using lacto phenol cotton blue. The fresh sample was inoculated in 5ml Sabouraud Dextrose broth and incubated overnight. After incubation 1ml of the suspected colony was streaked on Sabouraud Dextrose Agar (SDA) containing commercial shampoo of (0.05%) to inhibit bacterial growth and incubated at 30ºC for 72 hours.

9. Determination of Minimum Bactericidal Concentration (MBC)

The tubes that showed no turbidity in the MIC test was taken and a loop-full from each tube was streaked on SDA and nutrient agar plate. The plates were incubated for 24h at 37°C and the absence of growth was observed (MBC) Owoseni and Aayi, (2012) [13].

Table 1: Phytochemical Test, Result of Avocado, Pawpaw and Neem seed extract

Plant Chemical	N- hexane		
	Avocado oil	Neem oil	Pawpaw oil
Alkaloids	+	+	+
Saponin	+	+	+
Tannin	+	+	+
Flavonoid	+	-	+
Glycoside	-	+	+
Steroid	+	+	+

Key: + = present, - = Absent

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

Cultural Characteristics			Biochemical Test										Sugar Fermentation		Suspected Organism
COL	EL	SH	Gram Stain	Catalase	Coagulase	Oxidase	Indole	Growth & 41°C	Maltose	Glucose	Mannitol	Lactose	Sucrose		
Cr	C	O	+	+	+	−	−	−						A	A
A	−	−	*Malasezia Furfur*												

Key: + = Presence, A = acid production, COL = Color, E = Elevation, SH = Shape, C = Convex, - = Absence, A/G = acid and gas, = Colorless, O = Ova

Table 3: Antimicrobial Activity of formulated Shampoo and Commercial Shampoo *Malassezia furfur* in Percentage (%)

Zone of inhibition (mm) at different concentration (MG/ML)

Test Organism	Concentration (%)	Commercial Shampoo (Control)	Formulated Shampoo
M. furfur	100	20	12
	75	18	10
	50	15	8
	25	13	7

Table 4: Minimum Inhibitory Concentration (MIC) Of the Extract against the test Organism in Mg/Ml

Organism	25%	50%	75%	100%	Control	MIC	MFC	Control
Malassezia	+	+	-	-	-	75	100	-
furfur								

Keys: (+) = Turbidity, (-) = Clear, Control = Commercial Shampoo

Table 5: Result of Minimum Bactericidal Concentration (MBC) of the Extract against the test organism in mg/ml

Organism	25%	50%	75%	100%	Control	MIC	MFC	Control
Malassezia	+	+	-	-	-	75	100	-
furfur								

Keys: (+) = Turbidity, (-) = Clear, Control = Commercial Shampoo

Table 6: Physiochemical Evaluation of the Formulated and Commercial Shampoo

Test	Formulated Shampoo	Commercial Shampoo
pH Value	6.78	6.80
Foam Value (mm)	350	348
Solid Content (%)	22.11	23.12
Surface Tension (dynes/cm)	28.76	29.00

Discussion

The result of this study showed that, the phytochemical screening revealed the presence of Alkaloid, Saponin, steroid, Glycisides, and Tanins, whereas Flavonoid, was not detected in Neem, Findings from this work agreed with the work of Shinkafi (2013) [22]. Who reported that plants containing tannins, saponins, glycoside, sterold and Anthraquinone are reported to have antimicrobial activity.

The antimicrobial activities show the heighest zone of inhibition at 20mm in 100% mg/ml and the lowest zone of inhibition at 13mm in 25% mg/ml. This is in line with the findings by Shahtalebi *et. al.*, (2013) [19]. Who reported that study on commercial shampoo and formulated shampoo is effective against the test organism Malassezia furfur. Some of these secondary metabolites have been previously report as having antimicrobial activity Saleh *et al.*, (2015) [17]. The pH value for formulated shampoo was 6.78 while that of commercial shampoo was 6.80. Medical plants used in the formulation of herbal shampoo were found as rich source of useful chemical component. Plants extract such as onions extract, Avocado, pawpaw and neem seed extract Fenugreek, Aloavera ginger, Cloves were used to formulate herbal shampoo for hair growth, antidandruff, cleansing and conditioning without any site effect unlike commercial shampoo that has site effect such as grey hair, dandruff and hair breakage.

Conclusion

In conclusion the herbal shampoo produced from neem, pawpaw and avocado seed was effective against *Malasezia Furfur* with a Minimum Inhibitory Concentration (MIC) of 12mm in 100% mg/ml and lowest at 7mm in 25%mg/ml. The efficacy of neem, pawpaw and avocado seed extract against this microorganism provides a scientific ground for the application of the herb in the prevention and treatment of fungi infections caused by various pathogenic bacteria that have the ability of developing resistance to antibiotics.

Recommendations

1. The shelf life of herbal shampoo should also be determined, in order to assess their rate of deterioration.
2. Pawpaw and avocado seed extract could have greatest potential use as a food preservative since they have been known to inhibit bacteria, fungi and yeast, therefore research on this could be carried out.

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