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Formulation of Antidandruff Shampoo by Using Fermented Product of *Cajanus cajan*

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Abstract
Dandruff is the common problem of people. The aim of this study was to isolate dandruff causing microorganism, testing the efficacy of fermented product of *Cajanus cajan* against pathogens using and formulation of antidandruff shampoo. The dandruff causing isolates were identified as *Rhodotorulla, Cladosporium, Rhizopus, Acinetobacter, Streptococcus, Aspergillus fumigates, Fusarium oxysporium* and *Malassezia.* Among them *Malassezia is* one of the main causative agent for dandruff, which was isolated on international medical university – *Malasezzia furfur* (IMU-Mf). Fermented product of *Cajanus cajan* showed more antimicrobial activity against *Malassezia spp., Fusarium oxysporium, Aspergillus fumigates* and *Rhodotorulla*. The genera of *Staphylococcus* and *Lactobacillus* were found to be associated with the fermentation of *Cajanus cajan*. Fermented product was tested for total protein content, vitamin B_1, vitamin B_2, vitamin B_3, vitamin C and amino acids content. Fermented product contains all the essential nutrients required for hair growth; hence it was used for the formulation of shampoo, which will not only reduce dandruff but also encourages hair growth.

Keywords
Antidandruff shampoo, *Cajanus cajan*, dandruff causing microorganism, fermentation, *Malassezia*.

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1.0 INTRODUCTION
Dandruff is a skin condition that mainly affects the scalp. Dandruff is a common chronic scalp condition marked by flanking of the skin on scalp. In order to clean the scalp, remove dandruff and oil from scalp it is necessary to wash hair by using shampoo.
In market, most of commercial shampoos are available which causes scalp problem, allergy, hair loss, irritation. Another leading cause of dandruff problem is abundance of *Malassezia* species, affects sensitive skin and causes scalp irritation. *Malassezia globusa* is naturally present on scalp. During dandruff, the level of *Malassezia spp.* increased by 1.5 to 2 times its normal level. It feed on sebum and releases oleic acid. Some people are sensitive to oleic acid. It penetrates in skin and causes inflammation and in response to inflammation, the skin shed large number of skin cells in higher rate. This cells then join together in white flakes this causes dandruff. *Rhodotorula, Acinetobacter, Streptococci, Propionibacterium, Cladosporium, Aspergillus* etc, are also responsible for dandruff problem. So, to deal with this dandruff problem, in India fermented product was used since ancient times. Asian women

used rice water as a hair treatment for centuries. It gives hair shine, strength and elasticity. Bamboo extract contain 70% silica. Bamboo extract is reported to be effective for hair growth, in cleansing hair pollutant and dead skin. *Cajanus Cajan* is crucial for human nutrition because of phytochemicals, bioactive compounds. It belongs to family Fabaceae and dietary legume plant.

The aim of present study was to ferment the *Cajanus cajan* and use that fermented product to treat dandruff causing microorganisms. Fermented product contains water-soluble vitamins mainly vitamin C. Vitamin B help hair cells to produce black pigment and it is more abundant also it contains carbohydrates which protect hair from damage and repairs. Microflora of *Cajanus cajan* help to produce acid during fermentation which is helpful for healthy scalp and hair growth [1].

2.0 MATERIAL AND METHODS

2.1 Isolation and identification of dandruff causing microorganisms: -

The samples were collected from 10 males and 10 females (age is in between 15 years to 25 years) suffering from dandruff problem. For the collection of dandruff causing microorganisms from scalp cotton swab technique [2] was used and cultured on nutrient agar [3] potato dextrose agar and [4] International medical University - *Malassezia furfur* (IMU-MF) medium [5]. The plates were incubated at room temperature for 24 hrs (for bacteria), 48-72 hours (for fungi) and 4-5 days (for *Malassezia*). After incubation desired colonies were selected and subculture on nutrient agar and potato dextrose agar. Isolates were identified based on morphological and biochemical characteristics.

2.2 Fermentation of *Cajanus cajan* (Pigeon pea/Toor dal): -

Pigeon pea (dal) was obtained from local market in (Pune). The pigeon pea was washed thoroughly to remove contaminants by distilled water. Polished and Unpolished Pigeon pea (10 to 20%w/v) was allowed to ferment for 12-24 hrs under sterile and unsterile conditions. Fermented product was centrifuged at 3000 rpm for 15 min. Fermented product was further screened for its composition, associated micro-flora, antimicrobial activity against dandruff causing microorganism and its shampoo-based formulation.

Following combinations was used:

Table 1: - combinations of sample

Sr. no.	Combinations
SKT1	Sterile polish dal(10gm) + Sterile water(100ml)
SKT2	Sterile unpolished dal(10gm) + sterile water (100ml)
SKT3	Unsterile polish dal (10gm) +Unsterile water (100ml)
SKT4	Unsterile unpolished dal(10gm) + Unsterile water (100ml)

All the combinations were allowed to ferment for 12-24 hrs.

2.2.1pH of sample: -

pH of samples was checked before fermentation and after fermentation. pH of samples was checked by using pH meter.

2.3 Active ingredient present in fermented product:

Fermented product was centrifuged and used for its qualitative and quantitative analysis.

2.3.1 Chromatographic analysis (TLC): -

For detection of amino acid n-butanol: glacial acetic acid: water (8:2:2) was used as solvent system and developer as Ninhydrin in ethanol (0.2%w/v) respectively [6].

Vitamin B_1, B_2, B_3 and vitamin C were analyzed using solvent system n-butanol: Pyridine: Water (5:3.5:1.5) with UV light (360nm) as developer for vitamin B1, B2, B3 and ninhydrin as developer for vitamin C [7].

2.3.2 Determination of total protein and sugar content in fermented product: -

Estimation of protein was done by using Folin-Lowery method [8] and sugar content was determined using Anthrone method [9].

2.3.3 Antioxidant activity: -

Estimation of antioxidant activity of fermented product was done by using Ferric reducing antioxidant power (FRAP) and Free radical scavenging Capacity (DPPH).

a) Ferric Reducing Antioxidant Power: -

The antioxidant capacity of the fermented product was estimated spectrophotometrically following the procedure of Benzie and Strain. The method is based on the reduction of Fe3+ TPTZ complex (colorless complex) to Fe2+-tripyridyltriazine (blue colored complex) formed by the action of electron donating antioxidants at low pH. This reaction is monitored by measuring the change in absorbance at 593 nm. The

Ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300 mM acetate buffer, 10 ml TPTZ in 40 mMHCl and 20 mM FeCl3.6H2O in the proportion of 10:1:1 at37°. Freshly prepared working FRAP reagent was pipetted using 1-5 ml variable micropipette (3.995ml) and mixed with 5 µl of the appropriately diluted fermented product sample and mixed thoroughly. An intense blue color complex was formed when ferric tripyridyltriazine (Fe3+ TPTZ) complex was reduced to ferrous (Fe2+) form and the absorbance at 593 nm was recorded against a reagent blank (3.995 ml FRAPreagent+5 µl distilled water) after 30 min incubation at 37°C. All the determinations were performed in triplicates. Once the calibration curve was prepared by plotting the absorbance at 593 nm versus different concentrations of FeSO4 .The FRAP values were obtained by comparing the absorbance change in the test mixture with those obtained from increasing concentrations of Fe3+ and expressed as mg of Trolox equivalent per gram of sample [10].

b) Free radical scavenging Capacity (DPPH):-
The free radical scavenging activity was measured using the stable free radical DPPH which is one of the main tests used to explore the use of herbal extracts as antioxidant. Briefly, DPPH is solubilized in methanol to have a solution of 0.3 mM. One ml of methanol and 1 ml of the fermented product (at different concentrations 1 mg / ml in methanol) are introduced into tubes and 2 ml of the methanol solution are added to the DPPH. After stirring by a vortex, the tubes were placed in the dark at room temperature for 30min. The reading is performed by measuring the absorbance was measured at 517 nm. The negative control composed of 1 ml of the DPPH methanol solution and 2.5 ml of methanol. Ascorbic acid was used as synthetic antioxidants of reference. The capacity of the antioxidant to scavenge the free radical is estimated as a percentage of discoloration of the DPPH in solution in methanol [11].

The percentage of antioxidant activity was determined according to the following equation:

% Antioxidant activity= (Abs control - Abs sample / Abs control) × 100

The results are the average of two separate measurements ± standard deviation.

2.4 Estimation of vitamin B1 (Thiamine): -
Analysis of vitamin B1was done by using AOAC (2005) method. In 200ml volumetric flask, add 1.5ml of test sample and 100ml 0.1N HCl solution was mixed thoroughly. The mixture was heated in boiling water bath at 100°C for 30 minutes then cool the mixture and add 0.1 N HCl upto the mark of volumetric flask. Mix the mixture rigorously. This solution was filtered using Whatman filter paper no1. First 20ml of filtrate were discarded. Take 2-centrifuge tube of 50ml volume and add 1.25gm flocculent powder in each test tube. The remaining filtrate was added to centrifuge tube vortex it for five minutes. To separate the layers, centrifuge the content at 5000rpm for 5minutes. Supernatant was discarded. In the pellet 5ml absolute alcohol and previously frozen 5ml of 0.1% potassium ferric cyanide solution (potassium ferriccyanide in sodium hydroxide solution was added (15). Mix the solution for 10minutes and visualized for any change in color (pinkish coloration). Then 10ml of toluene solution was added and mixed for 10 minutes by vortexing. Content was centrifuged at 5000rpm for 10 minutes. Pink colour layer was added in toluene layer. Thiamine standard (0.5mg) was prepared and 10ml of thiamine standard solution was treated same as above [6].

2.5 Estimation of vitamin B_2 (Riboflavin): -
Analysis of vitamin B_2 was done by using AOAC (2005) method. In 200ml volumetric flask 1.5ml of test sample and 100ml of Acetic acid: Water (50:50) solution was added. The mixture was heated in boiling water bath at 100°C for 30 minutes. The mixture was cooled to room temperature (20°). The solution of acetic acid: Water (50:50) was added up to the mark of flask. The mixture was stirred for 10 minutes and filtered in the dark. First 20ml was discarded and remaining solution was used as sample. Absorbance was determined at 460nm in UV spectrophotometer. Same protocol was repeated for 0.5mg of standard riboflavin [6].

2.6 Estimation of vitamin B_3 (Niacin): -
Analysis of vitamin B_3 was done by using AOAC (2005) method. In 200ml volumetric flask1.5ml of test sample was added. 5N of 5ml HCl solution, 5ml of dichloromethane and 90 ml of deionized water was taken. The mixture was stirred. The mixture was heated in a boiling water bath at 100°C for 30 minutes. After cooling to room temperature (20°C) distilled water was added up to the mark. Filtration was done using Whatman filter paper no. 1. First 20

ml of filtrate was discarded and remaining solution was used as sample. Absorbance was taken at 460nm in UV spectrophotometer. Standard riboflavin (0.5mg) solution was prepared and treated same as sample above (6).

2.7 Determination of functional groups based on FTIR analysis:-

Fermented product and washed water of *Cajanus Cajan* was dried in oven at 60°C for 5 hours. Dried powder was analyzed for differences in the functional product using Perkin Elmer FTIR.

2.8 Antidandruff activity of fermented product of *Cajanas cajan*: -

The antidandruff activity against all isolates was done by using well plate method. The isolated organisms were grown on Mueller Hinton agar, potato dextrose agar and IMU-Mf medium and incubated at room temperature. The organisms were suspended in saline (for bacteria) and teepol saline (for fungus) and cell count of 10^6 -10^8 was monitored. 0.1 ml suspension was spread on respected medium with the help of glass spreader. Well of 8mm diameter was made using cork borer with the help of well borer cut the well. Fermented product of 100µl was added. The plate was kept at 4°C for 1-2 hours for pre-diffusion and incubation was carried out as per the requirement of microorganisms.

2.9 Bioautography

Bioautography was carried out by using Immersion method (12).

Immersion method: - For detection of antimicrobial compound agar overlay method (immersion) was used.10µl of fermented product was loaded on TLC plate (Merck Silica Gel F_{254}). Solvent system used was n-butanol: glacial acetic acid: water (8:2:2). For evaporation of solvent chromatogram was kept at room temperature. Developed chromatogram was placed on sterile Potato dextrose agar plate (for antifungal activity) and on Mueller Hinton agar (for detection of antibacterial activity). Sterile Potato dextrose agar 5ml seeded with 1ml of spore suspension (0.2 A_{540} =10^7 spores/ml) was poured on chromatogram for detection of antifungal activity. Sterile Muller Hinton agar 5ml seeded with 1ml of cell suspension of respected bacteria (0.1 A_{540} =10^7 cells/ml) was poured on chromatogram for detection of antibacterial activity. After solidifying the plates were kept at 4°C for diffusion for 3hours. The plate were incubated at room temperature for 24 hours for bacterial culture and 48-72 hours for fungal culture. After incubation plates were checked for zone of inhibition.

2.10 Development of antidandruff shampoo from the fermented product of *Cajanas cajan*

2.10.1 Shampoo formulations

The shampoo was formulated by using following ingredients [1].

Table 2:- Formulation of Shampoo

Ingredients	Functions	Quantity
Sodium dodecyl sulphate	Detergent	20gm
Cocamidediethanolamine	Foam builder	5ml
Glycerol	Humectant	2ml
NaCl	Thickening agent	2gm
Tween80	Solubilizing agent	1ml
Rice bran oil	Emollient	2ml
Flakes of egg albumin	Conditioning agent	1gm
Capsule of vitamin	Antioxidant	1.5
Citric acid	Preservative, pH modifier	0.5gm
Fermented Product	Diluent	25ml

2.10.2Characterization of shampoo formulations:-

a) Physical appearance: -

For the determination of external physical appearance 5g of shampoo was used for color, odor, and homogeneity [1].

b) Determination of foaming ability and foam stability:-

Foaming ability was carried out by using the cylinder shake method. One gm of shampoo was added in50 ml of deionized water. The Cylinder was covered with paraffin film and shaken ten times. The foam volume was recorded immediately as flash foam. Maximum foam was reported after 4 minutes. The experiment was performed in triplicate [1].

c) pH measurement: -

The shampoo was diluted to 10% (v/v) (1gm of sample in 100 ml water). pH was recorded by using

pH meter. The experiment was carried out in triplicate [1].

d) Determination of wetting time:-

The paper was cut into one inch square. Paper was placed in aqueous solution of 1% (w/w) shampoo. The time was recorded when a paper become wet and reported as wetting time [1].

e) Determination of solid content:-

In an evaporating dish 4gm of shampoo was added and total weight was recorded. The evaporating dish was placed in water bath at 100°C for evaporating complete evaporation and again evaporating dish was weighed. Calculate the % of solid content by using following equation [1].

Where,

A= Total weight of shampoo and evaporating dish after evaporation.

B= Total weight of shampoo and evaporating dish before evaporation.

f) Evaluation of conditioning performance of shampoo formulations:-

10ml of shampoo added in 30gm of distilled water in Erlenmeyer flask. Hair was placed in the solution for 2 min. After 2min remove the tress of hair and rinsed with deionized water until clean. The hair was kept at room temperature for complete dry.

The effect of shampoo on hair was observed under stereomicroscope and questionaries were asked for conditioning effect of shampoo in terms of smoothness, hairs shine and frizz reduction. The questionaries' were answered by 30 volunteers. The effects were rated from 1to 5 (1= very poor, 2= poor, 3= moderate, 4= good, 5= excellent) [1].

2.11 Antidandruff activity of formulated shampoo:-

The antidandruff activity of shampoo was determined against dandruff causing microorganisms by using well plate method. The isolated organisms were grown on Mueller Hinton agar, potato dextrose agar and International medical University - *Malassezia furfur* (IMU-MF) medium and incubated at room temperature. The organisms were suspended in saline (for bacteria) and teepolsaline (for fungus). Spread the 1ml suspension on respected medium with the help of glass spreader. Cut the well with the help of well borer. Add 100µl of formulated shampoo (diluted 10^2 in the well. Incubate the plates at 4°C for 1-2 hours for pre-diffusion and incubated at room temperature [1].

2.12 Stability test of shampoo formulations:-

The stability test was done after every 15 days. Take 5gm of sample placed at room temperature. After 15 days antidandruff activity and physical appearance was checked [1].

3.0 RESULT AND DISCUSSION

3.1 Identification of microorganisms present in dandruff:-

Table 3:- Colony morphology and biochemical characteristic of dandruff causing microorganisms.

Isolates / Biochemical Test	TSK1	TSK2
Gram staining	Gram negative cocci	Gram positive cocci
Motility	Nonmotile	Motile
Glucose	-	-
Sugar fermentation		
Sucrose	-	-
Lactose	-	-
Fructose	-	-
Mannitol	-	-
Indol	-	-
Methyl red	-	-
Voges- prauskauer	-	-
Nitrate reduction	+	-
Nitrate assimilation	-	-
Oxidative fermentative(aerobically)	+	+
Oxidative fermentative(anaerobically	+	+
Starch	-	-

Gelatinase	-	-
Catalase	+	-
Oxidase	-	+
Blood agar	-	+
Growth on 10% NaCl	-	+
Simmons Citrate	+	-

Keywords- "+" Positive test, "-" Negative test

Table 4:- Colony morphology and biochemical characteristic of dandruff causing microorganisms.

Isolates / Biochemical Test	TSK3	TSK4
Lactophenol cotton blue staining	Yeast	Yeast like fungus
Sugar fermentation		
Glucose	-	+
Xylose	-	+
Sucrose	-	-
Lactose	-	--
Fructose	-	-
Mannitol	-	-
Indol	-	-
Methyl red	+	-
Voges- prauskauer	-	-
Nitrate reduction	-	-
Nitrate assimilation	+	-
Oxidative fermentative(aerobically)	+	-
Oxidative fermentative(anaerobically	+	-
Starch	_	-
Gelatinase	-	+
Catalase	ND	+
Oxidase	-	-
Simmons Citrate	-	-

Keywords- "+" Positive test, "-" Negative test, "ND" not detected

The isolated microorganisms present in dandruff were identified using Bergey's manual of bacteriology are *Rhodotorula, Acinetobacter, Streptococcus*.

The isolated fungus present in dandruff was identified using Identification of imperfect fungi by Burnett. The identified fungus was *Cladosporium spp., Rhizopus spp., Aspergillus fumigates, Fusarium oxysporium.*

Malessezia spp. was identified by using IMU-Mf medium and biochemical test [13].

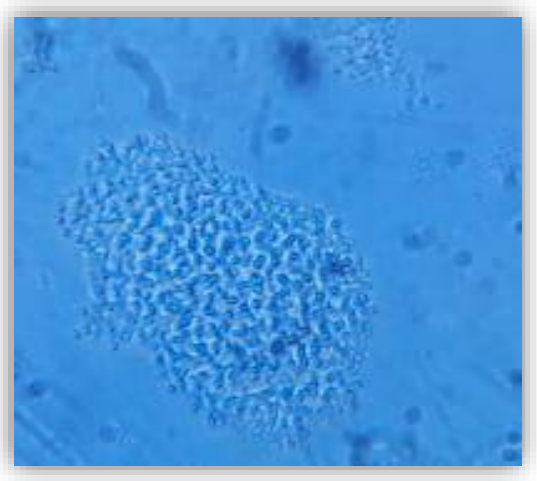

Figure1:- Microscopic observation of *Malassezia spp.*

3.2 pH measurement of fermented product: -
After fermentation, slightly decrease in pH was observed. It was within 5.5-5.8, close to pH of hair. As a result, slightly acidic pH plus the added nutrients through the fermentation process helps restore hairs pH balance, nourish hair follicles to promote healthy hair growth and improve general hair condition.

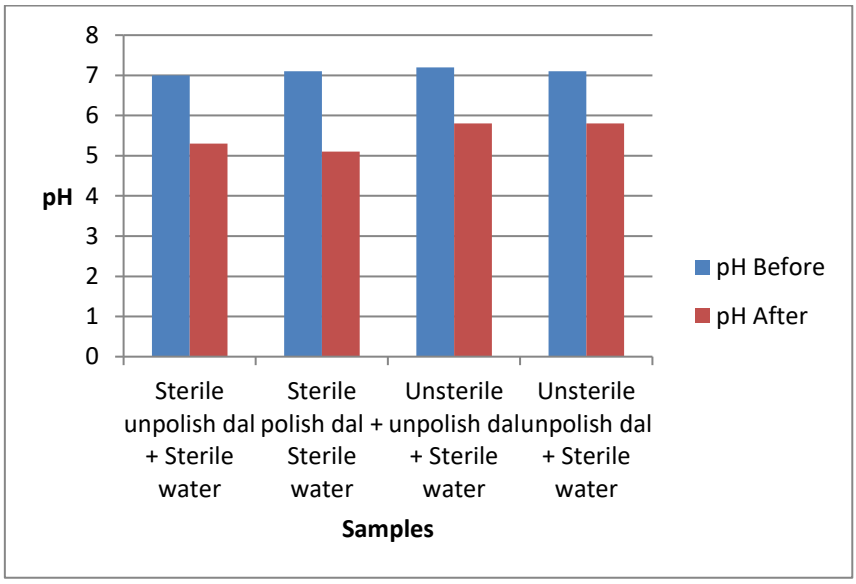

Figure 2:- pH of samples

In fermentation process there is decrease in pH after fermentation (as shown in figure). The pH will decrease because of protein and vitamin present in fermented Product.

3.3 Identification of microorganisms in the fermented product:-
According to morphological and biochemical characterization the isolated organisms present in fermented product are *Lactobacillus spp.* and *Staphylococcus spp*.

Table 5: - Colony morphology and biochemical characteristic of bacterial spp. isolated from Fermented Product of *Cajanus cajan* (pigeon pea).

Biochemical Test	MRS	NA
Gram staining	Gram negative Rod	Gram positive cocci
Motility	Motile	Nonmotile
Glucose	+	-
Sucrose	+	-
Lactose	-	-
Fructose	-	-
Mannitol	+	-
Indol	+	-
Methyl red	+	-
Voges- prauskauer	-	-
Nitrate reduction	+	+
Nitrate assimilation	+	-
Oxidative fermentative(aerobically)	+	+
Oxidative fermentative(anaerobically	+	-
Starch	-	-
Gelatinase	+	-
Catalase	+	+
Oxidase	-	-
Blood agar	-	+
Growth on 10% NaCl	-	+
Simmons Citrate	+	-

Keywords- "+" Positive test, "-" Negative test

3.4 Chromatographic analysis (TLC):-

Based on TLC analysis, fermented product found to contain glutamic, tyrosine, alanine Cysteine, and methionine amino acids and Vitamins B2, B3 and Vitamin C.

Cysteine present in fermented product which gives strength to hair and provide sulfur to hair cells which can improve texture, elasticity and strength. Methionine helps to make pre collagen and works as an antioxidant to help an imbalance called oxidation stress. Glutamic acid promotes hair growth. Tyrosine yields melanin.

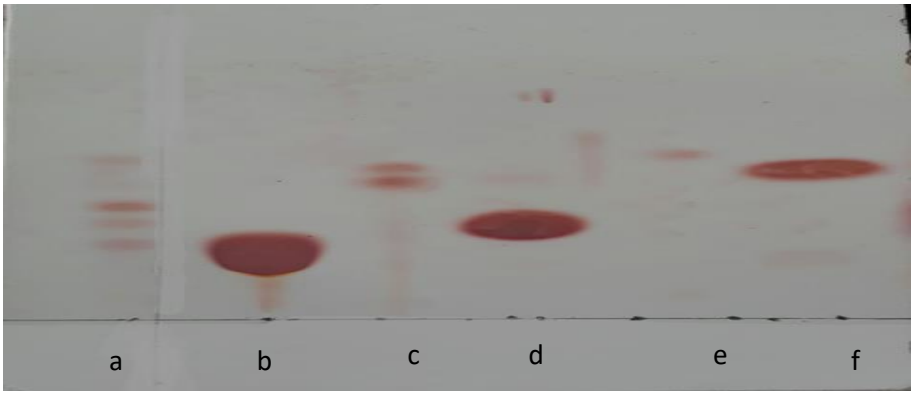

Fig 3: - TLC analysis of amino acid using Butanol: Acetic acid: water (8:2:2) with ninhydrin as locating reagent. From left to right a) fermented Sample, b) Standard Glutamic acid, c) Standard Tyrosine, d)Standard Alanine, e) Standard Cysteine and f) Standard Methionine

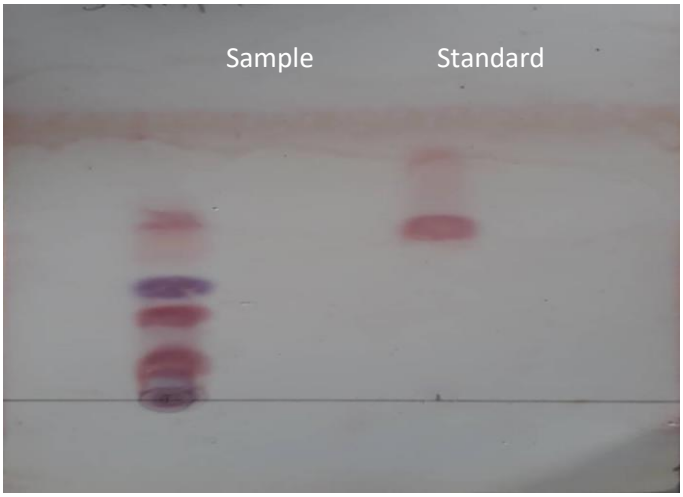

Figure 4: - TLC analysis of Vitamin C using n-butanol:Pyridine:water. (5:3.5:1.5) with ninhydrin as locating reagent.

3.5 Ingredient analysis of fermented Product:-

Table 6:- Quantitative estimation of active ingredient in fermented broth.

Name of Ingredients	Quantity
Protein	0.143mg/ml
Total carbohydrate	0.28mg/ml
Vitamin B2	50mg/ml
Vitamin B3	98mg/ml
Anti-oxidant by DPPH	71.79%
Anti-oxidant by FRAP	30mg of Trolox equivalent/gm

3.6. Determination of functional groups based on FTIR analysis:-

Fourier transform infrared spectroscopy is valuable tool for the identification of functional groups present in the sample or molecule. It helps for identification and structure determination of the molecule. FTIR of fermented product were helps in the identification of functional group present in it. The peak pattern represents in figure3 and 4before fermentation and after fermentation gives difference in between them.

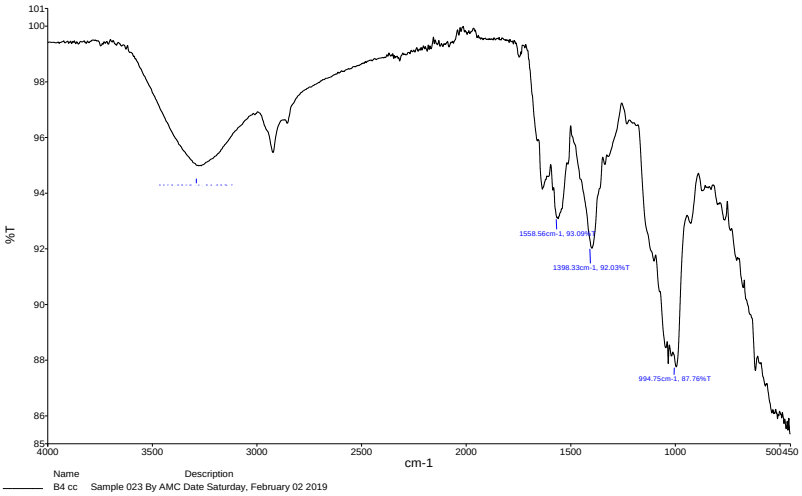

Fig 4:-Figure 5:- FTIR before fermentation

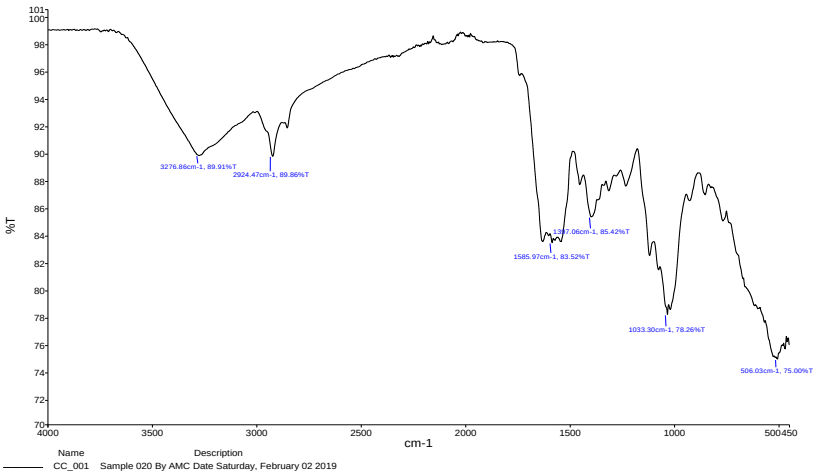

Figure 6:- After fermentation

Table 7:- Before fermentation FTIR analysis

Frequency ranges (cm-)	Functional groups
3279.0	O-H stretching vibrations presence of alcohol and phenol
1558.56	N-O asymmetric vibrations stretching presence of nitro compounds
1398.33	Isopropyl groups
994.75	Aromatic compounds

Table 8:- After fermentation FTIR analysis

Frequency ranges (cm-)	Functional groups
3276.86cm	O-H stretching vibrations presence of alcohol and phenol
2924.47	C-H stretching vibration presence of alkene
1585.97	N-O asymmetric vibrations stretching presence of nitro compounds
1397.06	Isopropyl groups
1033.30	Polysaccharide C-N stretching vibrations
506.03	Halogen compounds

FTIR analysis before fermentation shows the presence of alcohol, phenol, nitro, isopropyl, aromatic compounds and after fermentation shows the presence of alcohol, phenol, alkene, nitro, isopropyl, nitro, isopropyl, polysaccharide, halogen compounds. Polysaccharide and halogen compounds were additional functional group present in fermented product after fermentation.

3.7Antidandruff activity of cell free fermented product:-

Antidandruff activity of fermented product was carried out by well plate method. Zone of inhibition was measured. Fermented Product (unsterile dal + unsterile water) showed higher antimicrobial activity against all isolated microorganism. Polish dal and unpolished dal gives nearly equal inhibition zone.

Table 9:- Antidandruff activity of cell free fermented product.

Sample / Pathogen	Zone of Inhibition (mm) ± SD											
	B	SKT1=P	SKT1=UP	B	SKT2=P	SKT2=UP	B	SKT3=P	SKT3=UP	B	SKT4=P	SKT4=UP
Rhodotorula	-	-	-	-	25±0.5	24±0.5	-	-	-	-	20±1.1	21±0.5
Cladosporium spp.	-	20±1	20±0.5	-	21±0.5	25±1	-	-	17±0.5	- -	20±0	24±0
Rhizopus	-	18±1.1	17±0.5	-	12±0.5	17±1.5	-	12±1	15±1	-	19±0.5	16±0.5

Acinetobacter	-	20±1	-	-	24±1.1	22±0.5	-	-	-	-	20±0	17±1
Streptococcus	-	-	-	-	19±0.5	17 ±0.5	-	-	-	-	25±1.1	30±0.5
Aspergillus Fumigates	-	24±1.1	21±0.5	-	16±1	18±0.5	-	27±1	30±0.5	-	25±0.5	28±0.5
Unidentified	-	18±0.5	15±1.5	-	18±0.5	25±0.5	-	20±2	24±2	-	20±1	30±0.5
Cladosporium spp.	-	12±1.5	12±1.5	-	24±1	20±1	-	20±2	18±0.6	-	21±1	20±1
Fusarium Oxysporum	-	-	20±1.5	-	30±2	29±1.5	-	18±1.5	20±1	-	23±0.5	20±1.5
Malassezia	-	-	20±0.5	-	40±0.5	41±0.5	-	20±0.5	21±0.6	-	39±0.8	40±0.5

SKT1= Sterile dal+ Sterile water, SKT2= Unsterile dal++ Unsterile water, B= Blank, SKT3= Sterile dal+Unsterile water, SKT4= Unsterile dal+ Sterile water

Fig 7:-Antidandruff activity of fermented product against dandruff causing organism are *Cladosporium spp.*

3.8 Bioautography:-

Bioautography is a technique that combines thin Layer chromatography with bioassay in situ. It is one of the simplest and cheapest methods for detecting antimicrobial compounds in partially purified extracts because the method is easy to run, reproducible and requires less equipment. Hence, bioautography was used as a tool to screen out the production of antimicrobial compounds.

Bioautography of fermented product was done against *Malassezia* spp.Solvent system of amino acid was used. R_f value of the active principle found to be 0.8 which was responsible for 20 mm zone of inhibition.

Figure 8:- Bioautography of fermented product against dandruff causing *Malassezia* spp. (immersion).

3.9 Evaluation of formulated shampoo:-

a) Physical appearance:-

The physical appearances of formulated shampoo are listed in table9. The formulated shampoo had good characteristics with respect to foaming ability and foaming stability.

b) Foaming ability and foaming stability:-

There is no correlation between detergency and foaming but it is important for consumer and

therefore it is important criterion in evaluating shampoo. The foam ability was shown in table was 45ml and maximum foam stability is 40ml. Foaming ability was important for consumer.

c) pH measurement: -

For enhancing and improving the qualities of hair pH of shampoo play important role, it minimizing irritation of eyes and stabilizing ecological balance of scalp. Lower pH shampoo is the current trend to minimizing damage to the scalp and hair. Mild acidity prevents swelling and promotes tightening of the scalp there by inducing shine. As shown in table1 shampoo formulation has the range between 6.1-6.5.

d) Wetting time:-

The wetting time of shampoo formulation was determined by paper method. The wetting time of paper was in range between 11-13 seconds

e) Percentage of solid content:-

If the shampoo has too many solids it will be hard to work into the hair or too hard to washout. The solid content was in range between 20-25% therefore they were easy to washout.

f) Conditioning performance:-

Conditioning performance evaluated by 30 volunteers give good result for shampoo. The effect of shampoo on hair was observed under stereomicroscope (Fig).

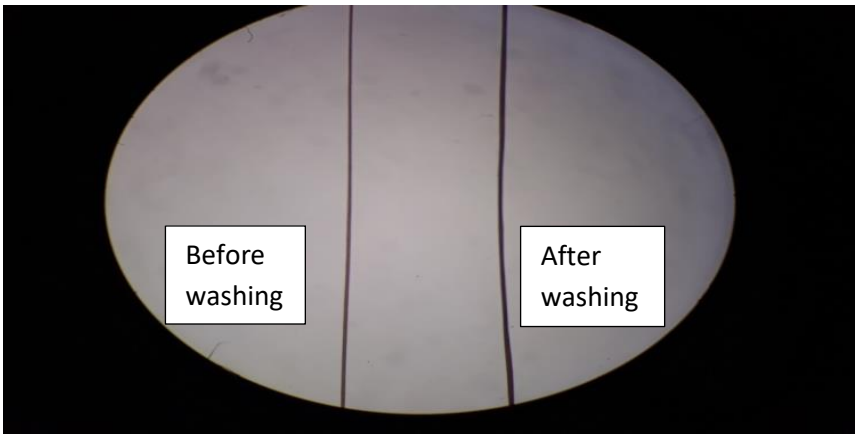

Fig 9: - Stereomicroscopic image of hair strand before and after treatment of formulated shampoo.

Figure 10: -Effect of formulated shampoo on reducing the fizziness.

g) Antidandruff activity of shampoo: -

The antifungal activity of shampoo was done by using well plate method. The zones of inhibition of formulated gives significant inhibition zone against isolated microorganisms (Table-11).

h) Stability of shampoo:-

The stability was checked after 15 days. After 15 days the shampoo shows good stability by observing physical appearance and antidandruff activity (Table-10and 11).

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Table 10:- Evaluation of formulated shampoo.

Characteristics	Observation on Zero day	Observation on 15th day (stability)
Color	Creamy brown	Creamy brown
Odour	coffee odour	coffee odour
pH	6.1	6.1
Flash foam	45cm	45cm
Foam stability	40cm	40cm
Total solid content	25%	25%
Wetting time	11.16 seconds	11.16 seconds

Table11:- Antidandruff activity of formulated shampoo by using well plate method.

Isolated microorganisms Pathogen	Zone of inhibition(mm)± Standard deviation On zero day	on15th days (Stability)
Rhodotorula	19±1	25±1.1
Cladosporium	21±1	24±1.1
Rhizopus	23±1	23±0.5
Acinetobacter	19±1	21±0.5
Streptococcus	32±1.5	32±0.5
Aspergillusfumigatus	17±1	17±1
Cladosporium	24±1	24±1.1
Fusariumoxysporum	20±1.7	19±1.5
Malessezia spp.	35±1	36±1.1

4.0 CONCLUSION

This study proved that fermented product of *Cajanus cajan* (pigeon pea) and shampoo containing fermented product could be used to conflict dandruff. Use of fermented product is not only cost effective but also with negligible side ffects. Further research is need to understand active principle from these fermented products which is directly involved in the inhibition of dandruff causative agents. Toxicity studies of formulated shampoo needs to be done so that it will have commercial value.

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