
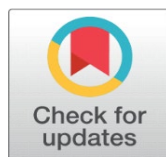
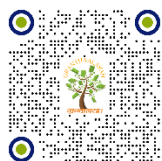


FORMULATION OF CHEMICAL AND CRUELTY FREE VEGAN LIPSTICK AND ANALYZING ITS COMPONENTS

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ABSTRACT

Cosmetics have become a part of everyone's grooming routine, where lipstick promotes the mood of makeup. Around 5000 years ago, the ancient Sumerian and Indus Valley men and women were possibly the first to invent and wear lipstick. Sumerians used crushed gemstones to decorate their faces, mainly on the lips and around the eyes. Egyptians like Queen Cleopatra crushed Red Carmine (bugs) to create a Red Shade.

Keywords: Cosmetics, Red Carmine, Coffee Decoction

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1. INTRODUCTION

Cosmetics have become a part of everyone's grooming routine, where lipstick promotes the mood of makeup.

1.1. HISTORY OF LIPSTICK

Around 5000 years ago, the ancient Sumerian and Indus Valley men and women were possibly the first to invent and wear lipstick. Sumerians used crushed gemstones to decorate their faces, mainly on the lips and around the eyes. Egyptians like Queen Cleopatra crushed Red Carmine (bugs) to create a Red Shade.

1.2. INGREDIENTS USED IN MAXIMUM COMMERCIAL LIPSTICK

Unfortunately, lipstick contains some harmful ingredients like Carmine (Crushed cochineal beetle carcasses), Suet (hard white fat from the kidney and loins of cattle, sheep, etc.), Tallow (boiled fat from beef or sheep carcasses), Lead (optimized) amount, heavy metals such as nickel, copper, chromium, arsenic, and cobalt and some of these toxic substances get absorbed by the lips to stomach during the application of this lipstick. Therefore, people turn towards a solution for well-being and health, and vegan cosmetics are the answer! People tend to entrust plant-based products more than chemical ones because of the severe side effects related to the chemicals.

1.3. SOURCE OF PIGMENTATION

The first lipstick sample was prepared from the Beetroot (*Beta vulgaris*) in the Chenopodiaceae family, recently included in the Amaranthaceae family. The Red beetroot (*Beta vulgaris*) is the best source of betacyanin. The second lipstick sample is based on coffee decoction.

A coffee decoction is an extraction procedure, especially for water-soluble and thermostable constituents.

The third lipstick sample was prepared with yellow pigments of turmeric.

Turmeric has been used in Asia for centuries as a folk medicine, culinary spice, and dye. It has also been a part of Ayurveda, Siddha medicine, Unani, and Traditional Chinese Medicine

2. MATERIALS AND METHODS

2.1. SAMPLE COLLECTION

Beet root extract, Coffee Decoction Turmeric extract, Olive Oil (Moisturizer) Cocoa Butter, Shea Butter Candelila wax, Lavender essence for fragrance.

2.2. FORMULATION OF LIPSTICK

2.2.1. SAMPLE A. BEETROOT EXTRACT

Beetroot was washed thoroughly, cut into tiny pieces, and churned in the mixer grinder.

Now, with the help of a sieve, extract the fresh filtered juice. Then, diminish it by heating it in a pan to get a comparatively thicker solution.

Now, 5 ml of pigmented solution is collected. 2.5 ml of lime juice is added as a mordant. 5 ml of olive oil is added as a moisturizer. 2.5gm of Shea butter and 2.5gm of cocoa butter is added for richness. 1.5 gm of Candelila wax (vegan) is used for binding the pigments and moisturizing agent.

After that, adding all ingredients to a beaker, it was melted in a microwave oven at 70°C for 1 minute, and immediately stirred with Lavender essence extensively with a glass rod, so that all the ingredients mixed homogeneously and let cool.

2.2.2. SAMPLE B. COFFEE DECOCTION

Readymade liquid coffee decoction (Cothas Coffee) is used for the formulation of the second lipstick. Then, diminish it by heating it in a pan to get a thicker solution.

Now, 5 ml of pigmented solution is collected. 5 ml of olive oil is added as a moisturizer. 2.5 gm of Shea butter and 2.5 gm of cocoa butter are added for richness. 1.5 gm of Candelila wax (vegan) is used for binding the pigments and moisturizing agent.

After that, adding all ingredients to a beaker, it was melted in a microwave oven at 70°C for 1 minute and immediately stirred extensively with a glass rod, so that all the ingredients were mixed homogeneously. Let it cool in a mold settle for a day at room temperature.

2.2.3. SAMPLE C. TURMERIC EXTRACT

Turmeric is washed thoroughly, cut into tiny pieces, and churned in the mixer grinder. Now, with the help of a sieve, extract the fresh filtered juice.

Then, diminish it by heating it in a pan to get a comparatively thicker solution.

Now, 5 ml of pigmented solution is collected. 5 ml of olive oil is added as a moisturizer. 2.5 gm of Shea butter and 2.5 gm of cocoa butter are added for richness.

1.5 gm of Candelila wax (vegan) is used for binding the pigments and moisturizing agent.

After that, adding all ingredients to a beaker was melted in a microwave oven at 70°C for 1 minute, and immediately stirred with Lavender essence extensively with a glass rod, so that all the ingredients mixed homogeneously. Let it cool in a mold and settle for a day at room temperature.

3. QUALITATIVE AND QUANTITATIVE ANALYSIS

3.1. PRELIMINARY PHYTOCHEMICAL SCREENING

- **Test for Alkaloids**

2ml of the sample was hydrolyzed with 5ml of 1% v/v Hydrochloric acid. This is then used for alkaloid detection. Hager's Test: Few drops of the saturated aqueous solution of picric acid were added to the sample mixture. The development of a yellow precipitate confirms the presence of Alkaloids.

- **Test for Carbohydrates**

The 2ml of the sample was dissolved in 5ml of distilled water.

Benedict's Test: Few drops of Benedict's reagent were added and kept in a boiling water bath for 10 to 15mins until colour develops. The development of the red colour indicates the presence of Carbohydrates.

- **Test for Proteins**

Xanthoproteic Test: Few drops of concentrated Nitric acid were added to 2ml of the sample. The development of yellow colour confirms the presence of Proteins.

- **Test for Amino Acid**

Ninhydrin Test: 2ml of the sample was treated with 0.25% w/v Ninhydrin reagent and boiled for a few minutes. The development of the blue colour confirms the presence of Amino acids.

- **Test for Glycosides**

2ml of the sample was hydrolysed with dilute Hydrochloric acid. This is then used for glycoside detection. Keller-Kilani Test: 2ml of Glacial acetic acid was added to the hydrolysed sample and 2ml of concentrated sulphuric acid was added on the

sides of the test tube. The development of a brown colour ring at the interphase confirms the presence of Glycosides.

- **Test for Phytosterols**

Salkowski's Test: 10ml of chloroform was added to the sample and filtered. A few drops of concentrated sulphuric acid were added to the filtrate and were shaken well and left to stand. The development of golden yellow colour confirms the presence of Phytosterols.

- **Test for Saponins**

Foam Test: Few drops of sodium bicarbonate were added to 0.5ml of the sample diluted in 2ml distilled water.

- **Test for Phenols**

Ferric chloride test: Few drops of 1% Ferric chloride were added to the sample. The development of bluish/black colour confirms the presence of Phenols.

- **Test for Tannins**

Ferric chloride test: 1ml of 5% ferric chloride solution is mixed with 0.5ml of the extract. The development of brownish colour indicates the presence of Tannins.

- **Test for Flavonoids**

Alkaline Reagent Test: The sample was treated with a few drops of Sodium hydroxide. Development of intense yellow colour and becomes colorless on adding dilute hydrochloric acid confirms the presence of Flavonoids.

- **Test for Diterpenes**

2ml of the sample was diluted in 5ml distilled water and 3-4 drops of copper acetate solution were added. The development of an emerald green colour confirms the presence of diterpenes.

1) Estimation of protein by Lowry's Method

Lowry's method was performed to verify the presence of protein in the plant extracts which are used to formulate the lipstick and the amount present in the lipstick.

2) Estimation of Carbohydrates by Orthotoludine Method

Orthotoludine method was performed to verify the presence of carbohydrates in the plant extracts which are used to formulate the lipstick and the amount present in the lipstick.

3) Estimation Of Amino Acid By Ninhydrin Method

Ninhydrin method was performed to verify the presence of amino acids which are used to formulate the lipstick and the amount present in the lipstick.

4) Estimation of Flavonoids

Three test tubes were taken and labeled as U1 and U2 and Blank. The unknown (flaxseed aqueous extract) 1 and 2 ml were added to U1 and U2. A test tube containing 2 ml of distilled water alone served as a blank. To all the test tubes 1 ml of Aluminium chloride and 1 ml, Potassium acetate were added. 2.8 ml of distilled water was added to all the test tubes and incubated at room temperature for 45 minutes. The optical density was read at 420nm. The standard graph was calibrated and the number of carbohydrates in the given unknown solution was calculated.

5) Estimation of Steroid

The unknown 0.5 and 1 ml were added to U1 and U2. 3.5 The unknown (flaxseed aqueous extract) 0.5 and 1 ml were added to U1 and U2. 3.5 and 3 ml of Ferric

chloride Acetic The test tubes were incubated at room temperature for 15 minutes. The optical density was read at 570nm. The standard graph was calibrated and the number of carbohydrates in the given unknown solution was calculated.

6) Estimation of Glycosides

The unknown (flaxseed aqueous extract) 0.5 and 1 ml was added to U1 and U2. 7.5 and 7 ml of Ferric chloride Acetic acid was added to all the test tubes. 1 ml of picric acid and 1ml of 100% Sodium hydroxide were added to all the test tubes. The tubes were incubated at room temperature for 15 minutes. The optical density was read at 520nm. The standard graph was calibrated and the number of carbohydrates in the given unknown solution was calculated.

7) Estimation of Alkaloids

5ml of aqueous extract of flaxseed was taken in a centrifuge tube and 2 ml of Dragendorff reagent was added. This mixture was centrifuged at 10000rpm for 30 minutes. The supernatant was removed, and the pellet was treated with ethanol. This mixture was centrifuged at 10000rpm for 10 minutes. The filtrate was discarded, and the pellet was treated with 2ml of Disodium sulfide solution and a few drops of concentrated nitric acid. This solution was diluted to 10 ml in a standard flask with distilled water. 1 ml was taken from this standard solution and 5 ml of Thiourea solution was added. The optical density was read at 435nm. The standard graph was calibrated and the number of carbohydrates in the given unknown solution was calculated.

8) Estimation of Saponins

The powdered flaxseed was dissolved in 80% ethanol and 2 ml of this filtrate was taken in a test tube. 2 ml of Vanillin was added and mixed well. A few drops of concentrated Sulphuric acid were added. The test tube was kept in a boiling water bath at 60°C for 10 minutes. The optical density was read at 544 nm. The standard graph was calibrated and the amount of carbohydrates in the given unknown solution was calculated.

9) Estimation of Terpenoids

The powdered flaxseed was dissolved in ethanol and 2.5 ml of this filtrate was taken in a test tube. 2.5 ml of Ammonium molybdate and 2.5 ml of concentrated Sulphuric acid were added. The test tube was incubated at room temperature for 30 minutes. 5 ml of Ethanol was added, and the optical density was read at 700nm. The standard graph was calibrated and the amount of carbohydrates in the given unknown solution was calculated.

4. ANTIOXIDANT ASSAY

1.5 ml of 0.1mM DPPH solution and add 1.5 ml of extract. The mixture was shaken vigorously. Incubate at room temperature for 30 minutes in the dark. The reduction of DPPH free radical as measured by reading the absorbance at 517nm by a spectrophotometer. (-) Control: Without any extract, DPPH and Ethanol (+) Control: Ascorbic Acid The inhibition of the DPPH free radical in % was calculated by:

The absorbance of Control -Absorbance of INHIBITION/ Absorbance of Control *10

5. ANTIMICROBIAL ACTIVITY

5.1. MATERIALS

The Petri plates were prepared with 20 ml of sterile Muller Hinton media. The strains (E. Coli, Klebsiella Pneumoniae, and Staphylococcus Aureus (Candida Albicans) that had been incubated for 24 hours were used for the assay. A sterile cotton swab was dipped into the bacterial suspension and then evenly streaked over the entire surface of a sterile Muller Hinton Agar plate to obtain uniform inoculum. Samples were added in different concentrations. The plates were incubated overnight at 37 C. The antimicrobial activity was determined by measuring the diameter of the zone of inhibition(mm).

6. ANTICANCER ACTIVITY

6.1. MATERIALS AND METHODS

6.1.1. PRELIMINARY SCREENING

The preliminary screening for in vitro cytotoxicity was carried out in the Vero cell line with the synthesized nanoparticles, plant extracts, and lyophilized nanoparticles. Based on the initial assessment of non-toxicity, the assay was further checked with varying concentrations of the nanoparticles. The absorbance was read at 590nm with a reference filter of 620nm. The percentage of cell viability was calculated.

The formula for cell inhibition = (O.D. of treated cells/O.D. of control) 100.

Formula to check cell viability = (100 - cell inhibition)

6.1.2. IN VITRO CYTOTOXICITY

The in vitro cytotoxicity of the 15 nanoparticles was tested in an epithelial cell lineage such as the Vero cell line through MTT assay. Further, the nanoconjugates NCC - (formulated based on the anticancer activity of NPS) nano-drug conjugates (NDC) and Chitosan Nano-drug were screened for in vitro cytotoxicity as per a similar protocol.

The absorbance was read at 590 nm with a reference filter of 620 nm.

The percentage of cell viability and IC₅₀ were calculated.

The formula for cell inhibition = (O.D. of treated cells/O.D. of control) x 100

Formula to check cell viability (100-cell inhibition) (Loosdrecht et al., 1994)

% of inhibition= (Absorbance of control-Absorbance of treated cells)/absorbance of control *100

7. RESULT AND DISCUSSIONS

7.1. QUALITATIVE ANALYSIS

1) Test for Alkaloids

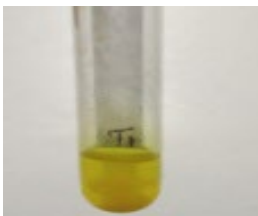
Alkaloids is present in the Lipstick Sample A from Beetroot extract as the yellow precipitate is observed.



Alkaloid is present in the Lipstick Sample B from Coffee Decoction as the yellow precipitate is observed.

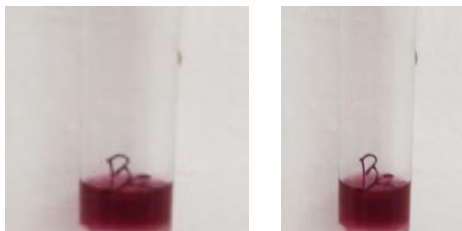


Alkaloid is present in the Lipstick Sample C from Turmeric extract. as the yellow precipitate is observed.



2) Test for Carbohydrates

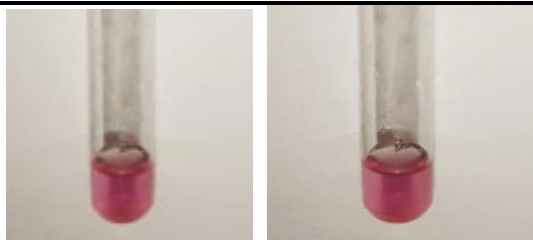
Carbohydrates are present in sample A from the Beetroot extract.



Carbohydrates are present in the Lipstick Sample B from Coffee Decoction

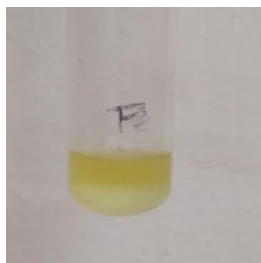


Carbohydrates are present in the Lipstick. Sample C from Turmeric extract.

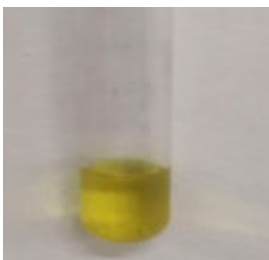


3) Test for Proteins

Proteins are present in the Lipstick Sample A from Beetroot extract as the yellow colour is observed.



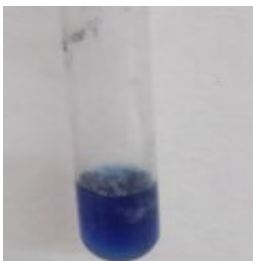
Proteins is present in the Lipstick Sample B from Coffee Decoction as the yellow colour is observed.



Proteins are present in the Lipstick Sample B from Turmeric Extract as the yellow colour is observed.

4) Test for Amino Acid

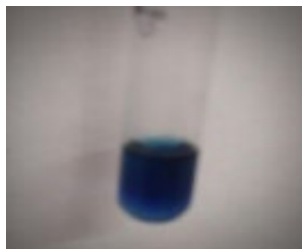
Amino acid is present in the Lipstick Sample A from Beetroot extract as the blue colour is observed.



Amino acid is present in the Lipstick Sample B from Coffee Decoction extract as the blue colour is observed.



Amino acid is present in the Lipstick Sample C from Turmeric extract as the blue colour is observed.



5) Test for Glycosides

Glycosides are present in the Lipstick Sample A from Beetroot extract as there is the development of a brown colour ring at the interphase.



Glycosides is present in the Lipstick Sample B from Coffee Decoction as there is the development of a brown colour ring at the interphase.

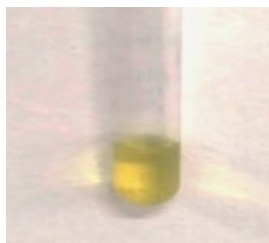


Glycosides are present in the Lipstick Sample C from Turmeric Extract as there is the development of a brown colour ring at the interphase.

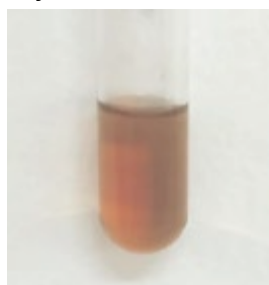


6) Test for Phytosterols

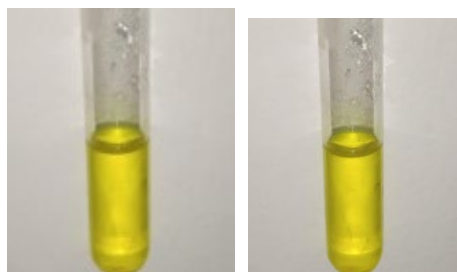
Phytosterols are present in the Lipstick Sample A from Beetroot Extracts the golden yellow colour is observed.



Phytosterols are present in the Lipstick Sample B from Coffee Decoction as the golden yellow colour is observed.



Phytosterols are present in the Lipstick Sample C from Turmeric Extract as the golden yellow colour is observed.

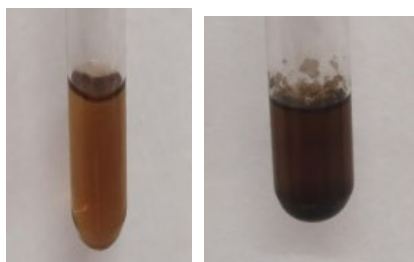


7) Test for Saponin

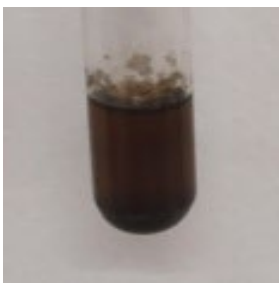
Saponin is absent in Lipstick Sample A from Beetroot Extract
Saponin are absent in Lipstick Sample B from Coffee Decoction
Saponin are absent in Lipstick Sample C from Turmeric Extract

8) Test for Tannin

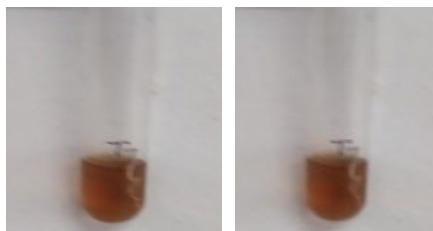
Tannins is present in the Lipstick Sample A from Beetroot Extract as the development of brownish colour is observed.



Tannins is present in the Lipstick Sample B from Coffee Decoction the development of brownish colour is observed.

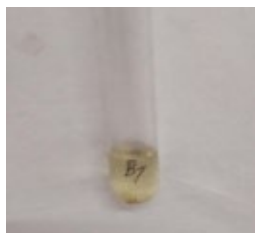


Tannin is present in the Lipstick Sample C from Turmeric Extract as the development of brownish colour is observed

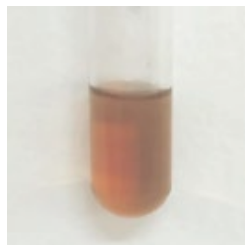


9) Test for Flavonoids

Flavonoids are present in the Lipstick Sample A from Beetroot Extract



Flavonoids are present in the Lipstick Sample B from Coffee Decoction



Flavonoids are present in the Lipstick Sample C from Turmeric Extract

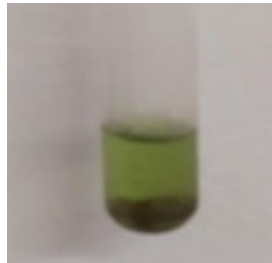


10) Test for Diterpenes

Diterpenes are present in the Lipstick Sample A from Beetroot Extract as emerald, green colour is observed.



Diterpenes are present in the Lipstick Sample B from Coffee Decoction as emerald, green colour is observed.



Diterpenes are present in the Lipstick Sample C from Turmeric Extract as emerald, green colour is observed.

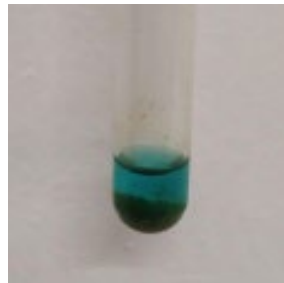


Figure 1

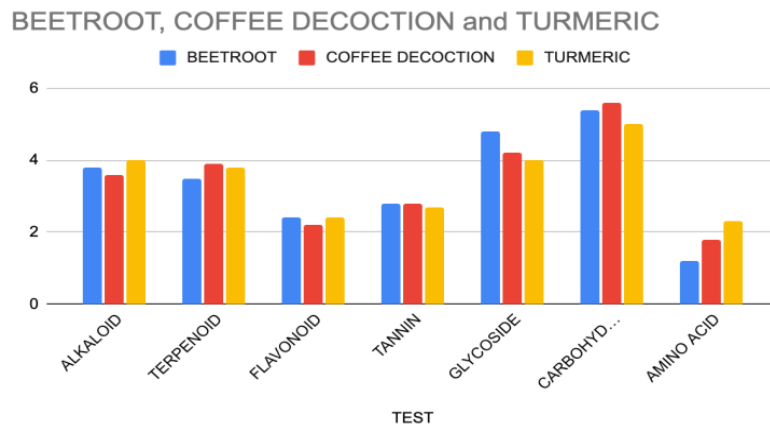


Figure 1 Research Gate by Samiullah, Farida Behlil, Farrukh Bashir (March 2015)

Table 1

Table 1 Qualitative Analysis			
TEST	Beetroot	Coffee Decoction	Turmeric
ALKALOID	+	+	+
PHENOLS	-	-	-
TERPENOID	+	+	+
SAPONIN	-	-	-
FLAVONOID	+	+	+
TANNINS	+	+	+
GLYCOSIDES	+	+	+
PHYTOSTEROLS	+	+	+
CARBOHYDRATE	+	+	+
PROTEIN	+	+	+
AMINO ACID	+	+	+
DITERPENES	+	+	+

Research gate by Chika C. Nwankwo, Daodu Bamidele T (June 2021)

Table 2

Table 2 Quantitative Analysis			
TEST	Beetroot	Coffee Decoction	Turmeric
ALKALOID	3.8	3.6	4
TERPENOID	3.5	3.9	3.8
FLAVONOID	2.4	2.2	2.4
TANNIN	2.8	2.8	2.7
GLYCOSIDE	4.8	4.2	4
CARBOHYDRATE	5.4	5.6	5
AMINO ACID	1.2	1.8	2.3

8. ANTICANCER TEST

CONTROL

BEETROOT EXTRACT=0.110

COFFEE DECOCTION=0.099

TURMERIC EXTRACT=0.112

SAMPLE

BEETROOT LIPSTICK=0.105

COFFEE DECOCTION LIPSTICK=0.111

TURMERIC LIPSTICK=0.104

BEETROOT LIPSTICK 95.45%

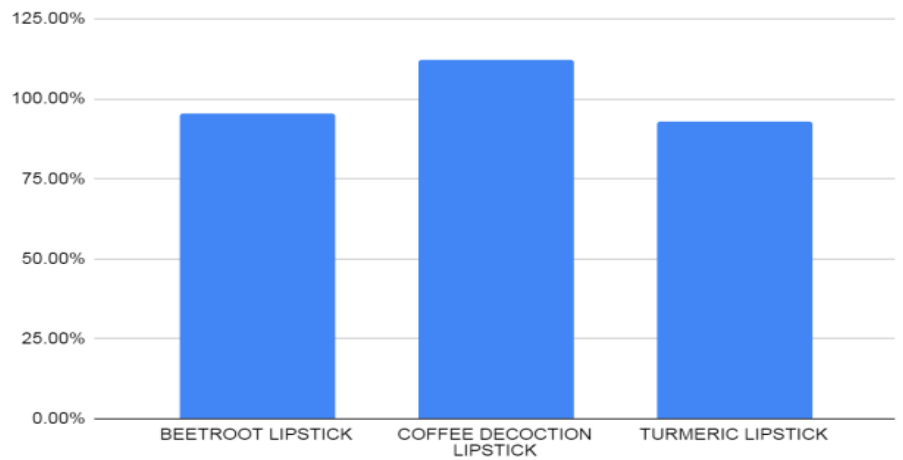
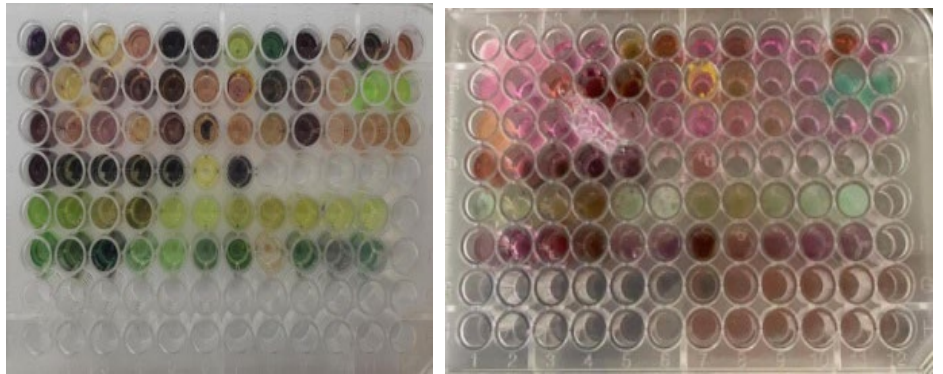
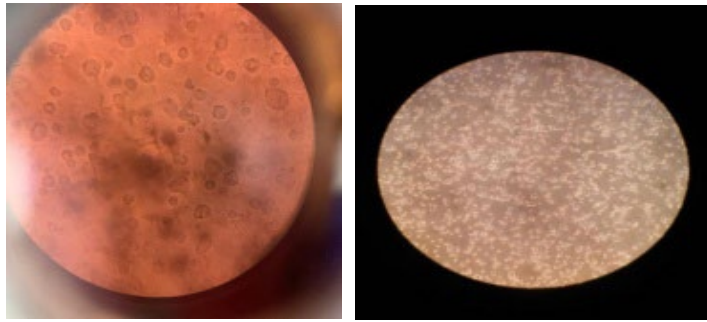
COFFEE DECOCTION LIPSTICK 112.12%

TURMERIC LIPSTICK 92.85%

10) Anti-Cancer Activity of Beetroot Extract=95.45%

11) Anti-Cancer Activity of Coffee Decoction=112.12%

12) Anti-Cancer Activity of Turmeric Extract=92.85%



9. ANTIOXIDANT ASSAY

ASCORBIC ACID	CONTROL	SAMPLE	INHIBITION%
0	0.414	0.315	23.91304348
20	0.414	0.376	9.178743961
40	0.414	0.308	25.60386473

Figure 2

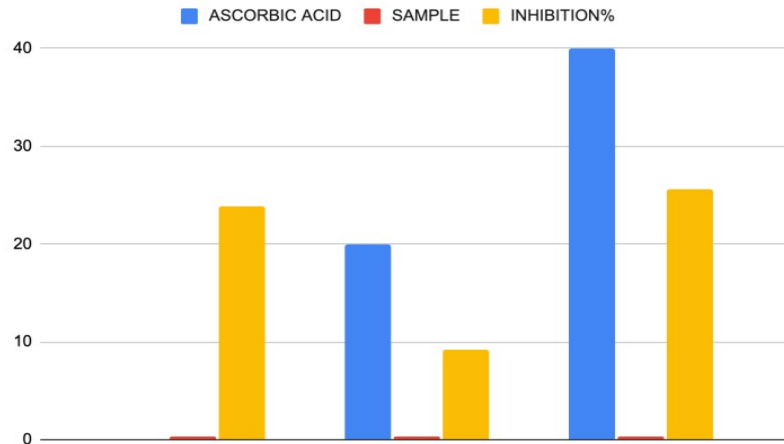


Figure 2 Research Gate by Chika C. Nwankwo, Daodu Bamidele T (June 2021)

10. ANTIMICROBIAL ACTIVITY (DISC METHOD)

- E. Coli - NO antimicrobial activity is seen
- Klebsiella - NO antimicrobial activity is seen
- Staphylococcus- NO antimicrobial activity is seen
- Candida - NO antimicrobial activity is seen

11. STORAGE TEST

• STORAGE TEST FOR LIPSTICK SAMPLE A. (BEETROOT EXTRACT)

- The colour diminishes after 20 days. Risks of contamination prevail if not kept under refrigeration.

• STORAGE TEST FOR LIPSTICK SAMPLE B (COFFEE DECOCTION)

Being a Nude Shade, the colour stays intact for a minimum of 35-40 days at room temperature.

• STORAGE TEST FOR LIPSTICK SAMPLE C (TURMERIC EXTRACT)

- The colour stays intact for 35-40 days at room temperature. (Formulation and Evaluation of Herbal Lipsticks from Research Journal of Pharmacy and Technology by Nuha Rasheed,)

Syed Abdul Rahman, and Samreen Hafsa was also referred (Volume - 13, Issue - 4, Year - 2020)

11.1. SENSORY TEST

1) SENSORY TEST FOR LIPSTICK SAMPLE A. (BEETROOT EXTRACT)

ODOUR-Smell of lavender Essence. No displeasing odour.

TOUCH- Smooth, semi-viscous.

PIGMENT- PINK SHADE

2) SENSORY TEST FOR LIPSTICK SAMPLE B (COFFEE DECOCTION)

ODOUR-Smell of Cocoa Butter. No displeasing odour.

TOUCH- Smooth, semi-viscous.

PIGMENT- NUDE SHADE

3) SENSORY TEST FOR LIPSTICK SAMPLE C (TURMERIC EXTRACT)

ODOUR-Smell of Lavender Essence. No displeasing odour.

TOUCH - Smooth, medium firm.

PIGMENT- LEMON YELLOW SHADE

12. CONCLUSION

The best thing about vegan or natural products is they are cruelty-free which intends products are free from animal testing and kill several thousand living creatures every year! Uric acid, insect secretion, diary extracts, and other undesirable chemicals like mica, and lead eventually get absorbed in the skin after application, which may lead to severe skin diseases, cancer, etc. In today's harsh atmospheric conditions, many prefer organic, healthy ingredients. Plant extracts retain most of the nutritional value which benefits the skin. But on the grey side, chemicals, may not be conducive to the skin. By choosing a safer product like this we can actually contribute to the environment. Another significant aspect of the product is that it is pocket-friendly with better benefits. According to Future Market Insights report on Vegan Cosmetics Market, the vegan cosmetics market will exceed US \$20 Bn by 2025. As the Veganism movement gains momentum in 2022, established firms will move toward the vegan cosmetic market. Some celebrities like, Harry Styles launched his gender-inclusive vegan beauty brand "Pleasing" in 2021. Currently, the company specializes in vegan nail polishes along with skincare products.

CONFLICT OF INTERESTS

None.

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