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Formulation of a herbal antifungal cream with *Sansevieria trifasciata* and its efficacy against vaginal candidiasis

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Abstract
Vaginal candidiasis also known as vaginal yeast infection is a disease caused by *candida albicans*, a fungus that is highly resistant after prolonged exposure to antifungals specifically azoles. Vaginal candidiasis is very common and it affects 75% of women. The overgrowth of *candida albicans* causes symptoms like itching, discomfort, soreness, burning sensation and thick white discharge. The effectiveness of antifungals like fluconazole is compromised by the emergence of resistance candida strains, therefore there is a high demand for natural alternative sources and *Sansevieria trifasciata* is a promising drug candidate. *Sansevieria trifasciata* is an evergreen plant found in the tropical and subtropical regions. It known for its medicinal properties and has been used to treat diseases like ear aches, inflammation and it has antimicrobial properties. The ethyl acetate extract of *S T*rifasciata is rich in several phytochemical like saponins, phenols, terpenoids, flavonoids and tannins. These phytochemicals were known to have antimicrobial activity and they were found present in the extract. In this experiment the dried leaves were extracted using the maceration method with ethyl acetate being the solvent of extraction. The extract was quantified UV-VIS and the total phenolic and flavonoid contents were calculated and got 500mgGAE/g and 100mgQE/g respectively. The extract was characterized using FTIR and identified several functional groups that correspond to phytochemicals like phenols, flavonoids and saponins. Bioassays were conducted using the disc diffusion method and the extract got zones of inhibition that were higher than those of the positive control which was clotrimazole the negative control (ethyl acetate) had no zones of inhibition meaning the activity shown by the extract was due to the bioactive compounds found in the extract. Two creams were formulated and they were both stable after the thermal cycle test there was no discoloration or melting. Both creams got pH values that are within the recommended range for vaginal creams which is 3.8 to 5 This study confirms that *Sansevieria trifasciata* extract is a promising antifungal drug that can be used as an alternative to azoles that are in the market.

Keywords: *Sansevieria trifasciata*, vaginal candidiasis, antifungal cream

Introduction

Vaginal candidiasis commonly known as vaginal yeast infection is a fungal infection of the vagina caused by the overgrowth of candida yeast typically *candida albicans*. (Radesca Moncayo, 2024) highlighted that vulvovaginal candidiasis is a common condition affecting at least 75% of women and is one of the leading causes of gynaecological consultations worldwide particularly affecting women post puberty. During pregnancy the prevalence rates can range from 10-50% in different regions (Carvalho de Paula, 2024). The overgrowth of *candida albicans* in the vagina leads to symptoms like itching, discomfort, soreness, burning sensation and a thick white vaginal discharge that may resemble cottage cheese. The predisposing factors of vaginal candidiasis includes use of antibiotics, increased oestrogen levels e.g. during pregnancy and when using contraceptives (Carvalho de Paula, 2024) [6] (Radesca Moncayo, 2024).

In clinical settings the treatment of vaginal candidiasis presents significant challenges due to the limitations of existing therapies. "Antifungals like fluconazole are commonly prescribed but their effectiveness is compromised by the emergence of resistance candida strains. This resistance is primarily attributed to the overuse of antifungal agents, genetic mutations and the ability of *candida albicans* to form biofilms which protect it from drug action. Studies have shown that *candida albicans* is developing resistance to commonly used azoles which leads to treatment failures (Satora *et al*., 2023). Fluconazole which is the first line treatment for vulvovaginal candidiasis improves the quality of life in 96% of women however even 63% of women have ongoing infections after completing maintenance therapy (Satora *et al*., 2023). This high recurrence suggests that current therapies may not adequately address the underlying cause of infection. (Phillips *et al*., 2023) [12] mentioned that among the oral and topical azoles none are approved for recurrent vulvovaginal candidiasis highlighting a significant gap in effective treatment options for this condition. Some patients face adverse effects from azole treatments such as nausea and abdominal pain which can lead to noncompliance with prescribed regimens.

There is a growing interest in exploring alternative therapies for vaginal candidiasis particularly those derived from plants and *Sansevieria trifasciata* is one such plant that has the potential. Studies have shown that *S. Trifasciata* contains bioactive compounds like saponins, flavonoids, triterpenoids and phenols which exhibit antifungal properties this makes it a potential natural alternative for treating vaginal candidiasis (Kusumaning Paramastri *et al*., 2022) [11]. (Berame *et al*., 2017) [5] highlights that the phytochemicals exhibit antimicrobial activity and this property suggests its potential as an alternative treatment for infections including vaginal candidiasis due to its effective cytotoxic principles. (*In-Vitro-Testing-of-Emulgel-with-a-Combined-Extract-of-Nt4udophwz*, n.d.) investigated the combined effects of *Sansevieria trifasciata* and Turmeric in an emulgel this shows that *S Trifasciata* as an individual could possess activity against *candida albicans* when the extract is formulated into a cream.

Experimental methods

Procedures

Matured fresh leaves of *Sansevieria trifasciata* were collected from our backyard in Kariba, Mashonaland west province on the 5th day of November. Fresh leaves of the plant were thoroughly washed using distilled water to remove dirty and were cut into small pieces and air dried under shade for two weeks. The dried plant sample was powdered using pulveriser and stored in polyethene plastic bag for future use.

Moisture Content Determination

The moisture content of the airdried plant sample was determined using the drying oven method (Thermogravimetric method) (Reeb & Milota, n.d.).

Plant extraction

The bioactive compounds were extracted using maceration method. 130g of the powdered plant material was placed in a 1000ml conical flask and added ethyl acetate to the flask using a ratio powder: ethyl acetate = 1:7(w/v) for 48hours. The mixture was shaken regularly to optimize extraction. The extract was filtered twice using a funnel and a filter paper under the influence of gravity. The filtrate was concentrated using a rotavapor using a temperature of 35℃. The small amounts of wet extracts left were then stored in small sample tubes and were stored in the refrigerator for future use.

Phytochemical Screening

The phytochemicals of interest in this report were phenols, flavonoids, terpenoids, saponins and tannins. Qualitative tests of these phytochemicals were conducted using the ethyl acetate crude extract of *Sansevieria trifasciata*

Antifungal Testing

Sources of microorganism

Candida albicans was collected from Bindura University of Science Education, Biological sciences laboratory.

Preparation of Potato Dextrose Agar (PDA) plates

Prepared PDA according to the manufactures instructions and poured it into sterile petri dishes, allowed the Agar to solidify.

Inoculation with *Candida albicans*

The Agar plates were inoculated by dipping a sterile cotton swab into the inoculum and streaking the swab in three directions over the entire agar surface and ensured even coverage across the agar surface. Allowed the plates to dry for 15 minutes (Larue, n.d.).

Application of extract

Placed 10µL of the *Sansevieria trifasciata* extract onto the agar surface using a sterile micropipette. Added the positive control and the negative control which was the extracting solvent, ethyl acetate

Incubation

Incubated the plates upside down in an incubator set to 35℃ for 24hours

Quantitative Analysis

UV-VIS Spectrophotometer

UV-VIS spectrophotometer was used to determine the total phenolic content and the total flavonoid content.

FTIR

The method used was described by (Durak & Depciuch, 2020) where the sample's FTIR spectra was obtained using a diamond attenuated total reflection (ATR) accessory. The spectrometer was linked directly to a computer with a range 400-4000cm^{-1}. The number of scans were set at 16 and the resolution at 4.

Formulation

A 100g cream was prepared according to the standard formula in (Baidoo *et al*., 2021) [4] (table 1)**.** The cream base was used to prepare 2 creams constituting of 10% *Sansevieria trifasciata* extract each.

Table 1: Cream formulations

Phase	Ingredients	Formulation1	Formulation2
O/P	Emulsifying wax	30g	30g
O/P	Liquid paraffin	20ml	20ml
O/P	White soft paraffin	50g	50g
A/P	Distilled water	70ml	70ml
Active	*S. Trifasciata extract*	10%	10%
A/P	methylparaben	-	0.3ml
O/P	Baobab oil	-	0.3ml

All ingredients were measured accurately using a top pan balance. Emulsifying wax was firstly crushed using a mortar and pestle. Emulsifying wax, white soft paraffin and liquid paraffin were added in a beaker and was heated at 70℃ until all the ingredients were completely melted. Distilled water was heated separately at the same temperature. The oil phase was then put in a mortar and pestle and the water was slowly added into the oil mixture while mixing vigorously until the mixture began to thicken. Mixture was cooled to about 45℃ and the *Sansevieria trifasciata* extract was incorporated and mixed thoroughly to ensure even distribution (Baidoo *et al*., 2021) [4]. Different ingredients were then added to the cream according to the formula in table1. The creams were transferred into sterilized containers and they were tightly sealed.

Evaluation Tests

pH test

The pH of the cream was determined using the digital pH meter. A method described by (Desai, A *et al*,2024**)** [8] was used where 5g of cream was dispersed in 50mls of distilled water. The measurement of the pH was carried out in triplicate and the average value was calculated.

Spread ability tests

A method described by (Chauhan & Gupta, 2020) [7] was used where 2g of the cream is put in between two glass slides and a weight of 100g is applied on top of the slides for 5 minutes. Spread ability was calculated using the formula:

$$S = \frac{ml}{t}$$

Where m= weight applied on the slides

I= length moved on the glass slide

t = time taken

Homogeneity and Physical appearance

A method described by (Chauhan & Gupta, 2020) [7] was used where the colour, roughness and homogeneity of the creams were observed by visual appearance.

Thermal cycle test

Bottles of the cream formulation were put at 25℃ for 48 hours and were observed for any change. The creams were then placed in an oven at 35℃ and 45℃ for 2 hours and they were observed.

Results and Discussions

Phytochemical tests

Table 2: Bioactive compounds present in the crude extract

Sample	Flavonoids	Phenols	Saponins	Terpenoids	Tannins
S Trifasciata	+	++	++	++	+

Key:
+ for: flavonoids mean a moderate colour change
Saponins means froth length of 0.5 – 1.0 cm
Tannins means medium colour
++ for: phenols mean very dark colour
Terpenoids means thick precipitate

In vitro* tests against *candida albicans

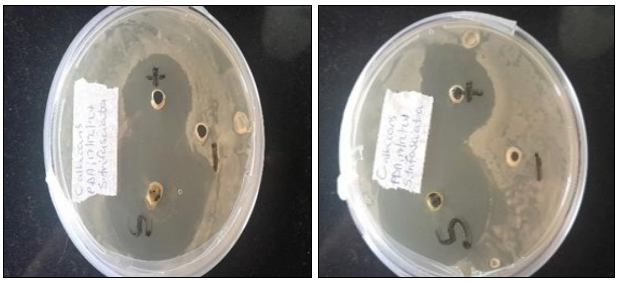

Fig 1: Disc diffusion plates results

Key

PDA=potato dextrose agar
S means sample;
+ means positive control (clotrimazole)
– is negative control (ethyl acetate)

Table 3: Zones of inhibitions from the disc diffusion method

	Zones of inhibition(mm)	Zones of inhibition(mm)
Sample	40	40
Positive control	28	30
Negative control	00	00

In vitro bio assays were conducted using the disc diffusion method. From the results above the negative control which was the extracting solvent (ethyl acetate) showed a zone of inhibition which were 00mm for both plates. This was the expected value as it indicates that ethyl acetate has no activity against *candida albicans*. These results make the experiment valid as it shows that any inhibition that is observed is as a result of the bioactive compounds present in the sample and not the solvent of extraction. The positive control (clotrimazole) used showed zones of inhibition that are 28 and 30mm meaning it can inhibit the growth of *candida albicans*. The positive control helps to confirm that the assay is working. The sample showed zones of inhibition which were 40mm for both discs, this result shows that the bioactive compounds in *Sansevieria trifasciata* ethyl acetate extract possess activity against *candida albicans*. *Sansevieria trifasciata* showed zones of inhibition that are higher than that of the positive control (clotrimazole) meaning that it outperforms the drug that is already in the market, this makes *Sansevieria trifasciata* a potential natural antifungal agent.

Quantitative analysis using UV-VIS spectrophotometer Total phenolic content

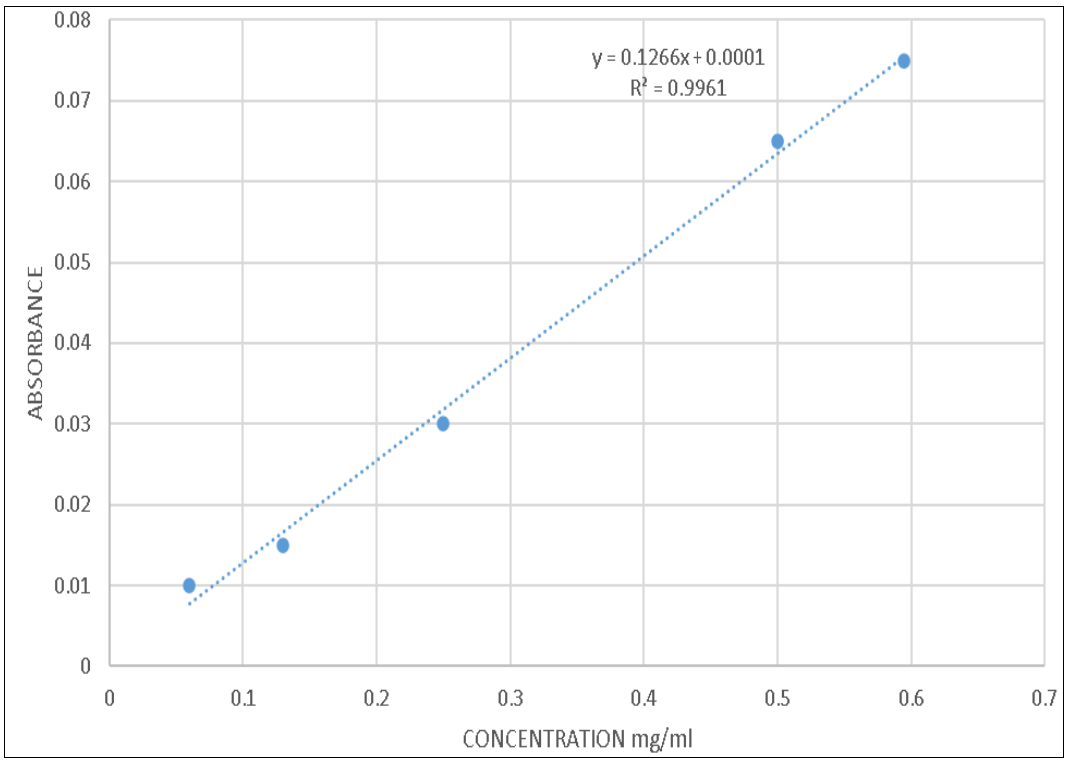

Fig 2: Calibration curve for Gallic acid.

Total flavonoid content

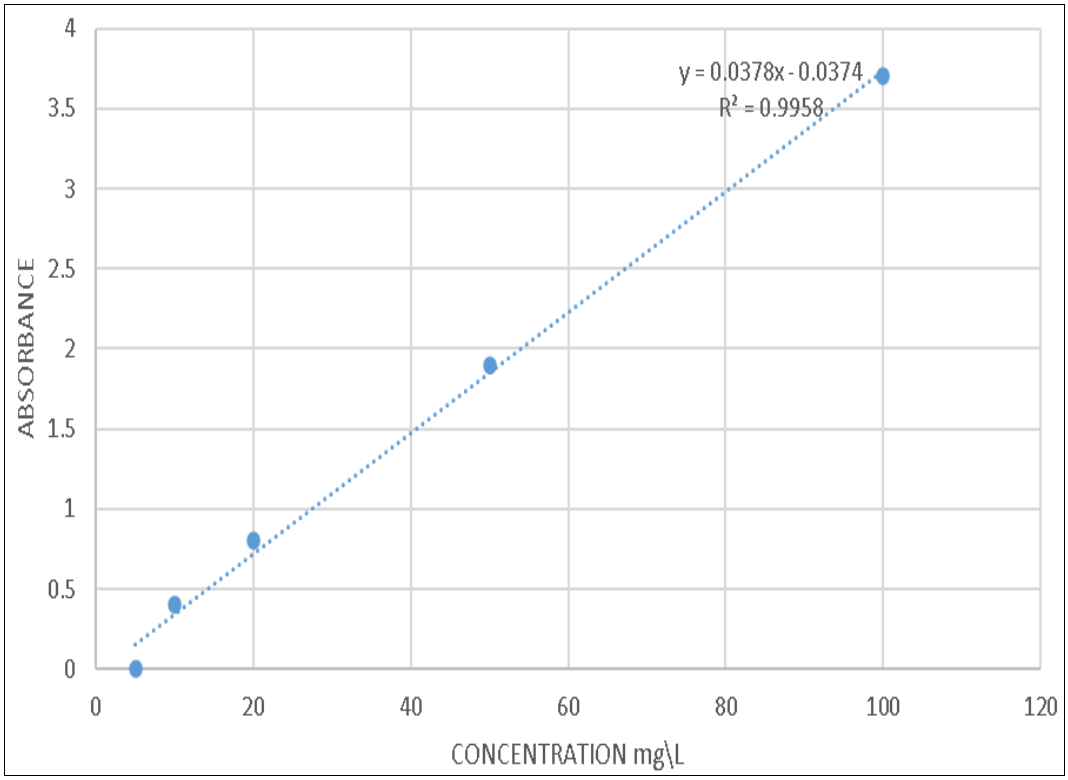

Fig 3: Calibration curve for Quercetin.

Total phenol content

The R^2 value obtained is 0.9961 which is greater than 0.95 and it shows good linearity**.** In natural sources phenolic compounds are recognized as potential antifungals. Phenolic compounds disrupt the cell membranes of fungal, inhibits key enzymes and inhibits the formation of biofilms and morphogenesis (Teodoro *et al*., 2015). The total phenolic content in the extract is 500mgGAE/g. There are no significant studies that calculated the total phenolic content using the ethyl acetate extract of *Sansevieria trifasciata* leaves however (Riza Marjon *et al*., n.d.) calculated it using other solvents like ethanol and methanol and got a value of 271 ± 0.026mgGAE/g and 1124 ± 0.041mgGAE/g respectively. The difference in the values could be as a result of different polarities of the solvents. The high concentration of phenolic compounds in the extract suggests a strong potential for antifungal activity. (Teodoro *et al*., 2015) has shown that various plants that are rich in phenolic compounds inhibit the growth of *candida albicans* at relatively low concentration with the efficacy being influenced by concentration. The high zone of inhibition recorded could have resulted from the high concentration of phenolic compounds. The total phenolic content was calculated using the formula:

$$TPC = \frac{CV}{M}$$

Where: C is concentration of extract in mg/ml, V is volume of extract used in ml, M is weight of extract in grams

$$TPC = \frac{\frac{296.33mg}{ml} \times 0.5ml}{0.29633g}$$

= 500mgGAE/g

Total flavonoid content

The R^2 value of the curve (0.9958) is within the recommended range. Flavonoids are polyphenolic compounds that are abundant in the *Sansevieria trifasciata* extract but its quantity is lower than those of phenols. There is limited data on the quantification of flavonoids in *Sansevieria trifasciata* ethyl acetate extract, however most studies quantified other extracts from different solvents like methanol, ethanol and acetone. (Riza Marjoni *et al*., n.d.) quantified the methanolic extract and got a total flavonoid content of 342,15mgQE/g. The obtained total flavonoid content in the *S Trifasciata* ethyl acetate extract was 100mgQE/g, this value is lower than that of the methanolic extract. The difference in the total flavonoid contents could be because of the difference in the polarities of the solvents. Studies have shown that flavonoids possess antifungal activity against *candida albicans* (Aboody & Mickymaray, 2020) [2]. Flavonoids interrupt the fungal cell membranes and inhibit the synthesis of nucleic acids. The antibiofilm properties of flavonoids are crucial because biofilm formation is a major factor in candida pathogenicity and antifungal resistance (Aboody & Mickymaray, 2020) [2]. The significantly high concentration of flavonoids in the extract likely contributes to the antifungal properties (Kaleena *et al*., 2011) [10]. The total flavonoid content was calculated using the formula:

$$TFC = \frac{CV}{m}$$

Where C is concentration of extract in mg/ml, V is volume of extract used in ml and M is mass of extract in grams

$$TFC = \frac{0.10268mg/ml \times 0.1ml}{0.00010268g}$$

= 100mgQE/g

Characterization using FTIR

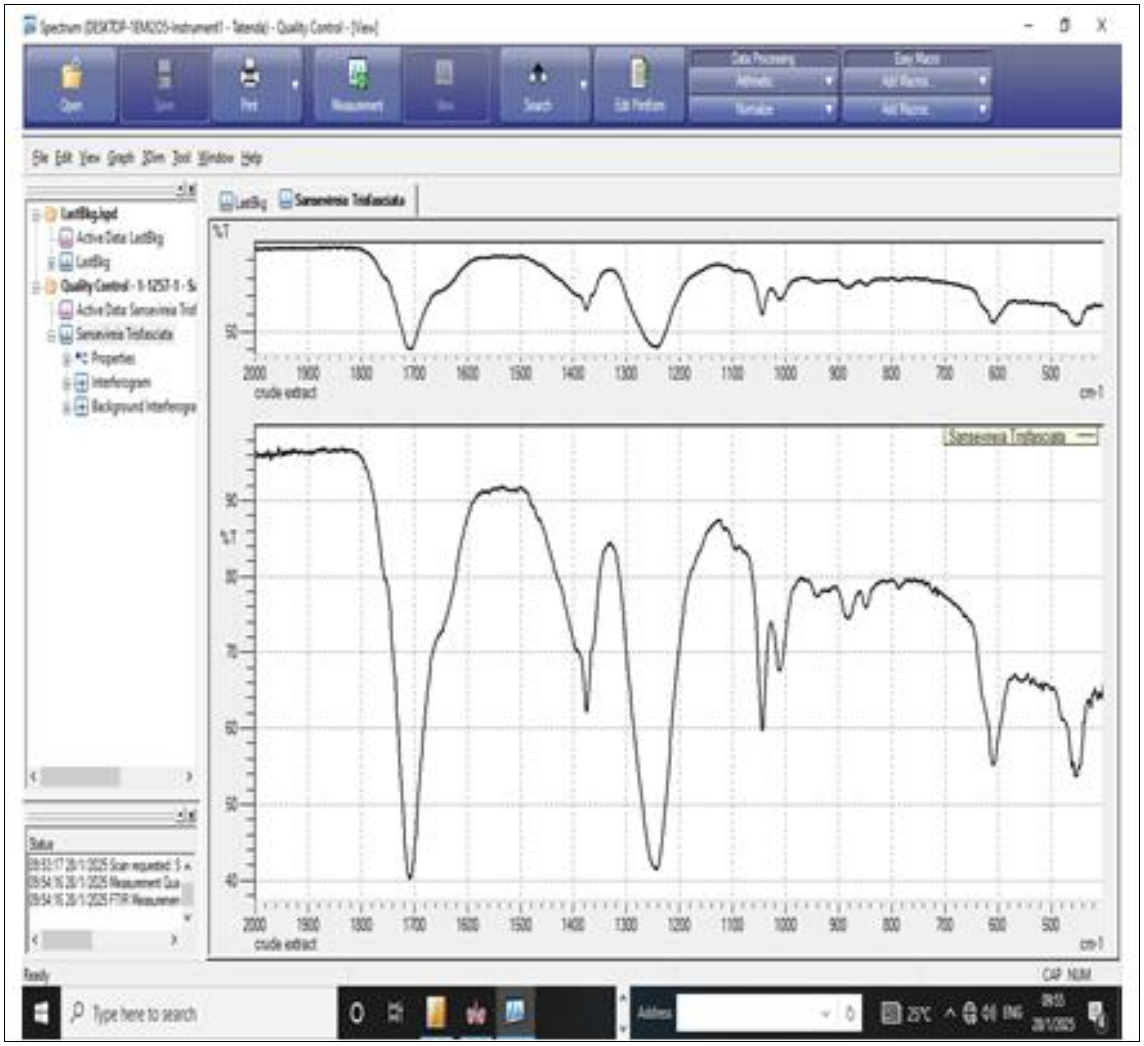

Fig 4: FTIR results

Figure4 shows the FTIR analysis of the crude extract of *Sansevieria trifasciata* showing the characteristic absorption peaks that correspond to specific functional groups in the plant's chemical constituents. The strong absorption peak at around 1710cm^{-1} indicates the presence of C=O stretching vibrations likely carboxylic
acids, esters, carbonyl containing compounds (aldehydes and ketones). The prominent absorptions at approximately 1600-1500cm^{-1} suggests aromatic C=C bonds and N-H bending vibrations indicating the presence of phenolic compounds, flavonoids and alkaloids". The fingerprint region (1400-600cm^{-1}) shows multiple peaks characteristic of various C-O, C-N and C-H bending vibrations that are present in plant's secondary metabolites.

Formulation evaluation tests

Table 4: Results for formulation cream tests

	Cream1			**Cream 2**		
Colour	Greenish			Greenish		
Homogeneity	Homogenous			Homogenous		
Ph	3.98			4.35		
Stability	25℃	35℃	45℃	25℃	35℃	45℃
	stable	stable	stable	stable	stable	stable
Spreadability	48gcm/min			52gcm/min		

Both cream 1 and cream 2 remained stable after the thermal cycle test where they were subject to different temperatures, both creams did not change colour or melt, however cream 1 is expected to have a shorter shelf life because it does not contain a preservative in the formulation and cream 2 is expected to have a longer shelf life because of the preservative that was added to the formulation. The creams are both greenish in colour and by looking at them they are both homogenous.

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