# Formulation and Evaluation of 1 % Shampoo for Antimicrobial Activity Containing Flavonoid Rich Fraction of Aerial Parts of Cassia auriculata

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**ABSTRACT**

Plants produce a wide variety of phytochemical constituents, which are secondary metabolites and are used in the pharmaceutical industry. 'For centuries, man has effectively used various components of plants or their extracts for the treatment of many communicable and non-communicable diseases, including bacterial infections. In the present study extracts of Cassia auriculata leaf were subjected for antimicrobial activity by well-diffusion method against six bacterial strains namely Malassezia furfur (MTCC 1765), Enterococcus faecalis, Escherichia coli, Aspergillus awamori, Bacillus subtillis (MTCC 441), Proteus vulgaris (MTCC 1771), Candida Albicans, Pseudomonas aeruginosa. The results revealed that the extracts exhibited strong inhibitory activity against all the tested organisms. The antibacterial activity of these extracts is possibly linked to the presence of flavonoids, steroid, saponins and / or tannins. Further studies are needed to determine the precise active principles from Cassia auriculata. The study effort was effort to make a multipurpose herbal Shampoo by using Cassia auriculata linn. The task of this work is, to protect the herbal active biomolecules and certify the activity. The herbal Shampoo was prepared by using Cassia auriculata flower extract, it was prepared by using 70 % ethanol. The resultant extract was analyzed by various chemical tests. The results confirms that both the extract have active biomolecules. Cassia auriculata linn flower extract incorporated into inert Shampoo base then the biological activity and Shampoo evaluation were piloted. The result shows alcoholic extract of Cassia auriculata in herbal Shampoo was better results in all standard parameters.

**KEYWORDS:** Cassia auriculata, Herbal Shampoo, antimicrobial Activity of Avaram

## 1. INTRODUCTION

Avaram (Cassia auriculata Linn), family Leguminosae, is also known as Avaram tree, The leaves are alternate, stipulate, paripinnate compound, very numerous, closely placed, rachis 8.8-12.5 cm long, narrowly furrowed, slender, pubescent, with an erect linear gland between the leaflets of each pair, leaflets 16-24, very shortly stalked 2-2.5 cm long 1-1.3 cm broad, slightly overlapping. Chemical constituents: Pod husk contains nonacosane and nonacosan-6-one, chrysophanol, emodin and rubiadin. Medicinal uses: Roots- used in skin diseases and asthma. Flowers used in diabetes, urinary disorders and nocturnal emissions. Its Bark is used as astringent. Leaves and Flowers – Anti-diabetic activity.

Fig. No. 1 Flowers of Cassia auriculata

Fig. No. 2 Leaf of Cassia auriculata

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural products from plants, animal and minerals have been the basis of the treatment of human disease. Today it is estimated that about 80 % of people in developing countries still rely on traditional medicine based larshampooy on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine.

## 2. Definition of SHAMPOO.

A shampoo is colloid that is typically 99% wt. liquid, which is immobilised by surface tension between it and a macromolecular network of fibers built from a small amount of a shampoo containing substance present. The U. S. P. Defines shampoos as a semisolid system consisting of dispersion made up of either small inorganic particle or large organic molecule enclosing and interpenetrated by liquid. The inorganic particles from a three dimensional "house of cards" structures. Shampoos consist of two-phase system in which inorganic particles are not dissolved but the continuous phase, randomly coiled in the flexible chains.

**MATERIALS AND METHOD**

Plant of Cassia auriculata Linn. Was collected from the outskirt of Talegaon village on the way to Amravati road, Nagpur, Maharashtra. The plant materials were authenticated with the help of herbarium sheet by Head of Botany Department, R. T. M. Nagpur University, Nagpur, the specimen numbers given to the authenticated sheet were 9922 for *Cassia auriculata* Linn. Voucher specimen of the plant was deposited in the Department of Botany, R. T. M. Nagpur University, and Nagpur.

## 3. Extraction of Total Flavonoid Rich fraction:

The dried aerial parts of Cassia auriculata were accurately weighed and then they were defatted with petroleum ether ($60^0$-$80^0$) in a soxhlet extractor. The defatted marc was refluxed with 70% ethanol for about 4-6 hrs. to get the desired flavonoid rich fraction.

All the extract was then subjected to specific tests for flavonoids.

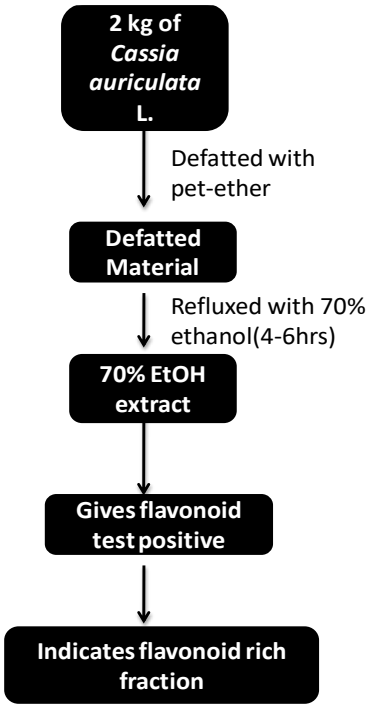



Figure 3: Scheme for extraction of flavonoids

## 4. Determination of Total Flavonoid Content of Flavonoid Fraction of Cassia auriculata L. Extract:

In *C.* auriculata extract the antidandruff activity was shown by the flavonoids. So the total flavonoid content of the extract was determined by the following methods.

a) **Determination of $\lambda_{max}$:** The standard solution of quercetin 100µg/ml was scanned from 800 to 200 nm in UV spectrophotometer taking methanol as blank.

b) **Preparation of standard calibration curve:** From the standard solution, 0.05ml, 0.1ml, 0.15ml & 0.2ml were taken in four separate volumetric flasks and diluted with 10ml methanol to make the solutions of concentration of 1 µg/ml, 2 µg/ml, 3 µg/ml & 4 µg/ml resp. Absorbance of these solutions were taken at its $\lambda_{max}$ 380nm for plotting standard curve of quercetin.

## 5. Formulation of Shampoo:

Development of shampoo was done and three batches of shampoo formulation were prepared. Three shampoo formulations were prepared among which different concentration i.e. 5%, 7.5%, and 10% of extract.

**Table no. 5: Formula for preparation of shampoo**

| Sr. No | Ingredients | Quantity |
|---|---|---|
| 1. | Sodium lauryl ethyl sulphate | 45.0% |
| 2. | Sodium chloride | 2.4% |
| 3. | Methyl paraben | 0.2% |
| 4. | Water | 100.0% |

| | | |
|---|---|---|
| 5. | Colour | q.s |
| 6. | Perfume | q.s |
| 7. | Plant Extract | 5%, 7.5%, 10% |

Sodium lauryl ethyl sulphate, Sodium chloride incorporated in the formulation or should be mixed with the perfume and incorporated in the preparation. Required calculated quantity of preservative was dissolved in water by heating on water bath. The required amount of plant extract was mixed in the above mixture. Finally, all ingredients were mixed properly with continuous stirring in mechanical stirrer.

## 6. EVALUATION.

a) **Physical Evaluation:** Physical parameters such as colour and appearance were inspected visually.

**b) pH:** The pH of the various formulations was determined by using digital pH meter.

c) **Homogeneity:** All developed shampoo were tested for homogeneity by visual inspection after the shampoos have been set in the container. They were tested for their appearance and presence of any aggregates.

d) **Spreadability:** Spreadability was determined by the apparatus which consist of a wooden block, which was provided by a pulley at one end. The spreadability was measured on the basis of slip and drag characteristics of shampoo. An excess of shampoo (about 2g) under study was placed on the ground slide. Another glass slide of the same dimension was placed on the top of the shampoo such that the formulation was sandwiched between the two slides by placing a weight of 100g uniformly on the slides. The weight was removed and the excess of shampoo was scrapped off. The upper slide was then subjected to pull of 20g. The time taken for the upper slide to separate away from the lower glass plate under the direction of the weight was noted. A short interval indicates better spreadability.

Experiment was done in triplicate and spreadability was calculated as follows:

$$S = M \times L / T$$

Where, S = Spreadability (gm.cm/sec) M = weight in the pan (tied to the upper slide)
L= Length moved by the glass slide T= Time (in sec) taken to separate the slide
Completely each other.

e) **Viscosity:** Brookfield viscometer was used to determine viscosity. The spindle used was LV 64. The sufficient quantity of shampoo was filled in wide mouth jar separately the height of the shampoo was filled in the wide mouth jar should sufficiently allow to dip the spindle. The RPM of the spindle was adjusted to 2.5 RPM. The viscosities of the formulations were recorded.

**f) Drug Control Uniformity:** About 0.1g of shampoo was accurately weighed and transferred to 100ml volumetric flask to which about 7ml of methanol was added. After mixing, the volume was made up to 10ml with methanol. The resultant solution was then estimated for the total flavonoid content.

**g) Drug released:** The *in vitro* diffusion studies of the shampoos were performed by using dialysis membrane (Sigma Inc. MO, USA; dry, unwashed, pre-cut and open ended; flat width: 35 mm; inflated diameter, 21 mm; Length: 30 mm). The membrane soaked in phosphate buffer pH 7.4 for 6-8 hr was clamped carefully to one end of the hollow glass tube of dialysis cell (2.3 cm diameter, 4.16 $cm^2$ area). 20ml of phosphate buffer was taken in a beaker, which was used as receptor compartment for the study. 1 gm of each formulation was spreaded uniformly on the membrane. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37±0.50C. The solution on the receptor side was stirred by externally driven Teflon-coated magnetic bars. At pre-determined time intervals, 2 ml of solution from the receptor compartment was pipette out and immediately replaced with 2 ml fresh phosphate buffer solution. The drug concentration of the receptor fluid was determined spectro photo metrically at 272 and 370nm against appropriate blank. The amount of drug permeation of all the formulations was calculated.

## 7. ANTIMICROBIAL ACTIVITY (Microorganisms and Media)

The test organisms used in this study were as followed: Malassezia furfur (MTCC 1765), Enterococcus faecalis, Escherichia coli, Aspergillus awamori, Bacillus subtillis (MTCC 441), Proteus vulgaris (MTCC 1771), Candida albicans, Pseudomonas aeruginosa. These bacteria were obtained from the Rajiv Gandhi, Biotechnology Centre, Nagpur University, Nagpur. Nutrient Broth and Nutrient Agar medium all these Medias were purchased from Hi-media.

**Antibacterial activity testing**

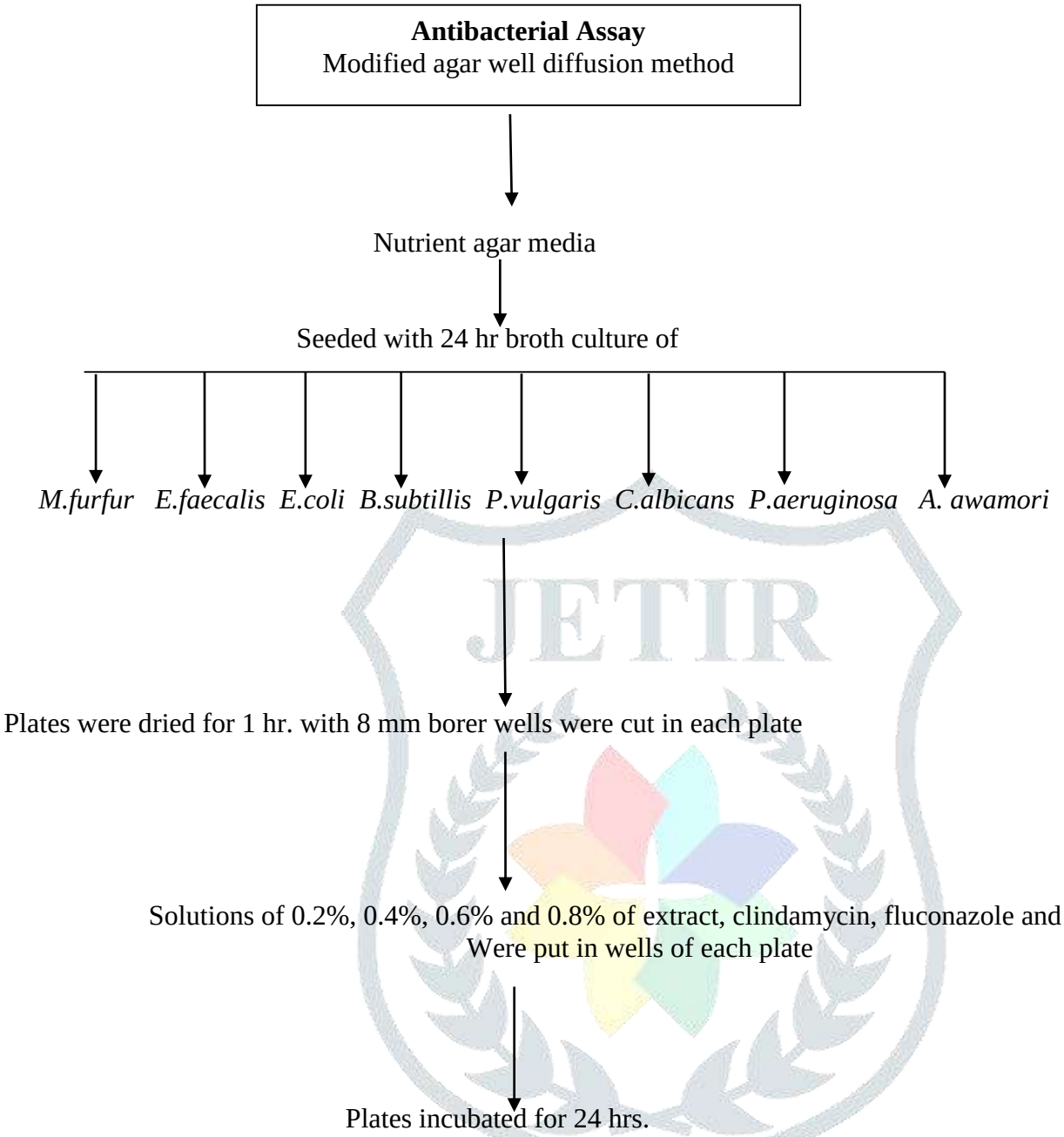
a) **Sample Preparation:** Solutions of extract were prepared using 0.2 mg of extract in 10 ml of water (0.2%). Similarly, 0.4 mg of extract in 10 ml of water (0.4%), 0.6 mg of extract in 10 ml of water (0.6%), 0.8 mg of extract in 10 ml of water (0.8%) was prepared. Clindamycin (10 mg/ ml) and fluconazole (10mg/ml) was used as a standard and distilled water as a control.

**b) Antibacterial Assay:** The antibacterial activity of different concentration of extract were determined by modified agar well diffusion method in which nutrient agar plates were seeded with 0.2 ml each of 24 hr broth culture of *M. furfur; A. awamori; E. coli; B. subtillis; P. vulgaris; C. albicans; P. aeruginosa; E. foecalis.* The plates were dried for 1 hr. With a sterile 8 mm borer six wells of equidistance in each of plates were cut into which 0.5 ml of 0.2%, 0.4%, 0.6%, 0.8% of extract, clindamycin, fluconazole and distilled water were introduced at randomly. The plates were incubated at $37^0$c for 24 hr. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition (in mm). The experiments repeated four times.

**c) Determination of minimum inhibitory concentration (MIC):**

The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. In order to determine the relative minimum inhibitory concentration values extract were dissolved in distilled water to make a concentration of 100 mg/ml. The extracts were then diluted with distilled water to make different concentrations. 100 µl of these extract were then added separately to each

cup. The entire test was repeated in duplicates.




## 8. RESULTS AND DISCUSSION

**A. Preliminary Phytochemical Screening of extract:** Phytochemicals investigation of extract of aerial parts of *c. auriculata* showed the presence of flavonoids, tannins, glycosides, strongly positive result for shinoda test indicated that strong presence of flavonoids which shown in Table.

**Table no.2: Phytochemical screening of *Cassia auriculata***

| Sr. No. | Tests | | Observations |
|---|---|---|---|
| | | | C.A extract |
| 1. | Alkaloids | Mayer's Test<br>Dragendroff's Test | _<br>_ |
| 2. | Carbohydrates | Molisch's Test<br>Fehling's Test | ++<br>+ |
| 3. | Glycosides | Modified Borntrager's Test<br>Legal's Test | +<br>+ |

| | | | |
|---|---|---|---|
| 4. | Saponins | Froth Test<br>Foam Test | _<br>_ |
| 5. | Phytosterols | Salkowski's Test<br>Libermann Burchard's Test | _<br>_ |
| 6. | Phenols | Ferric Chloride Test | _ |
| 7. | Tannins | Shampooatin Test<br>Pot. Dischromate Test<br>Bromine water Test | +++<br>++<br>++ |
| 8. | Flavonoides | Alkaline Reagent Test<br>Lead acetate Test<br>Shinoda Test | +++<br>+++<br>+++ |
| 9. | Proteins and amino acids | Xanthoproteic Test<br>Ninhydrin Test | _<br>_ |
| 10. | Diterpenes | Copper acetate Test | _ |

'+++' : Strongly positive '++' : Positive '+' : weakly positive '_' : Negative

## B. PHYSICAL EVALUATION OF EXTRACT:

The characteristics shown by the extract are shown in table no.8.

**Table no.3: Characteristics of Extract**

| Sr. No. | Characteristics | *C. auriculata* |
|---|---|---|
| 1. | Colour | Dark red |
| 2. | Consistency | Solid |
| 3. | Odour | Characteristic |
| 4. | Extractive value(%w/w) | 5.34% w/w. |

## C. Thin Layer Chromatography Of Extract:

Flavonoid rich fraction was isolated from the c. auriculata extract and subjected to TLC. Various solvent systems were tried to find the satisfactory developing systems. Satisfactory resolution was obtained in the following system:

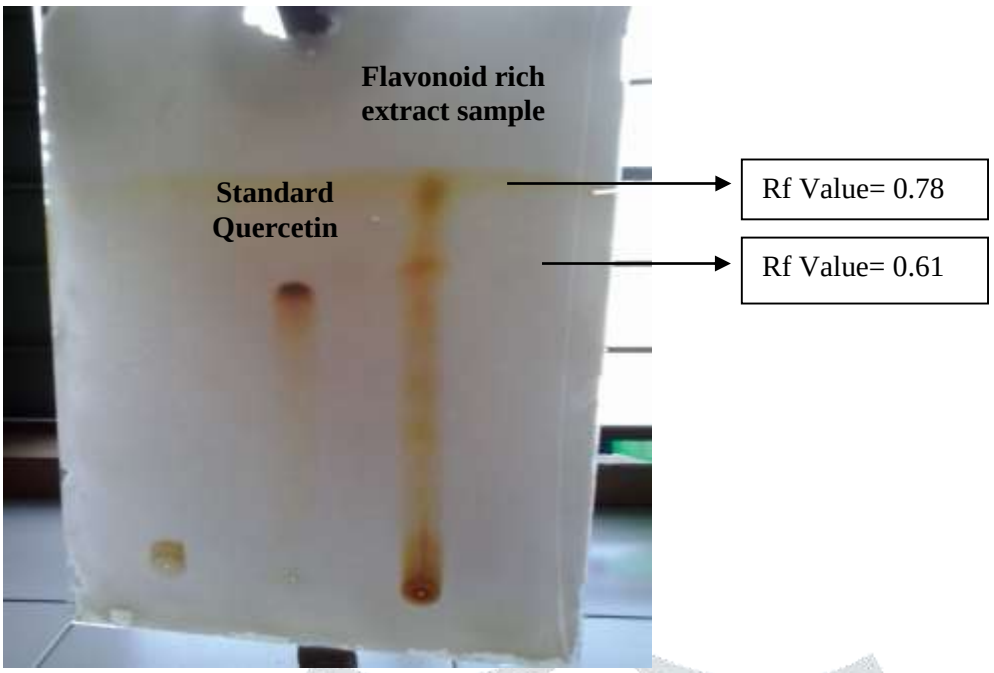



**Fig no.5: Comparative TLC of standard Quercetin and Flavonoid extract.**

**Table no.4: Solvent System Developed for Isolated Flavonoid Rich Fraction**

| Sr. No. | Solvent System | No. Of Spot | RF Value |
|---|---|---|---|
| 1 | Toluene: Ethyl acetate: Acetone (40: 25: 35) | Two | 0.78 and 0.61 |

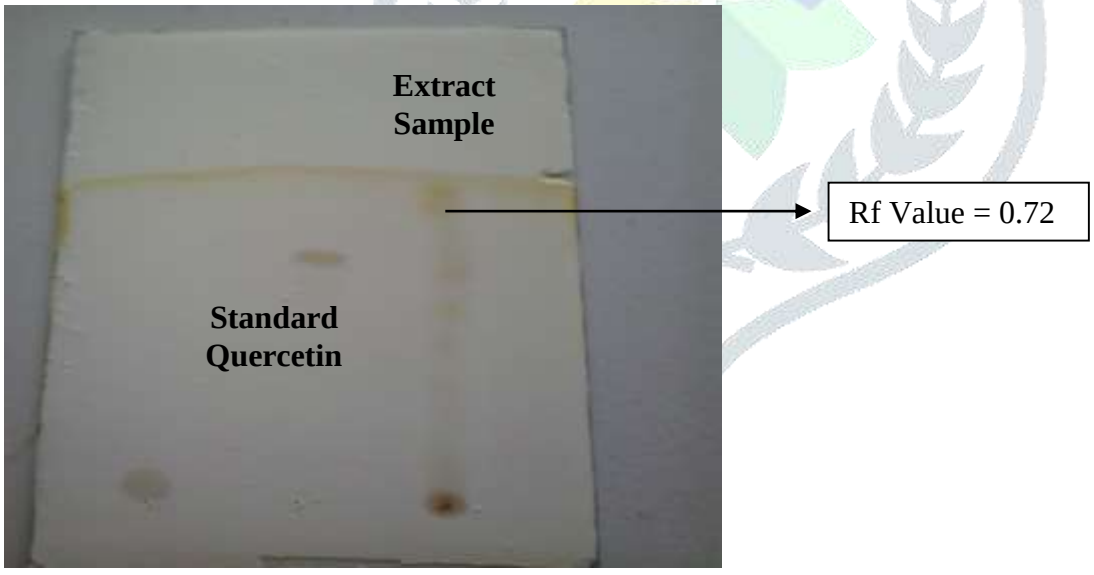



**Fig no.6: Comparative TLC of standard Quercetin and Flavonoid extract.**

**Table no.5 : Solvent System Developed for Isolated Flavonoid Rich Fraction**

| Sr. No. | Solvent System | No. Of Spot | RF Value |
|---|---|---|---|
| 1 | Ethyl acetate: Formic acid: Glacial acetic acid: Water (100: 11: 11: 26) | One | 0.72 |

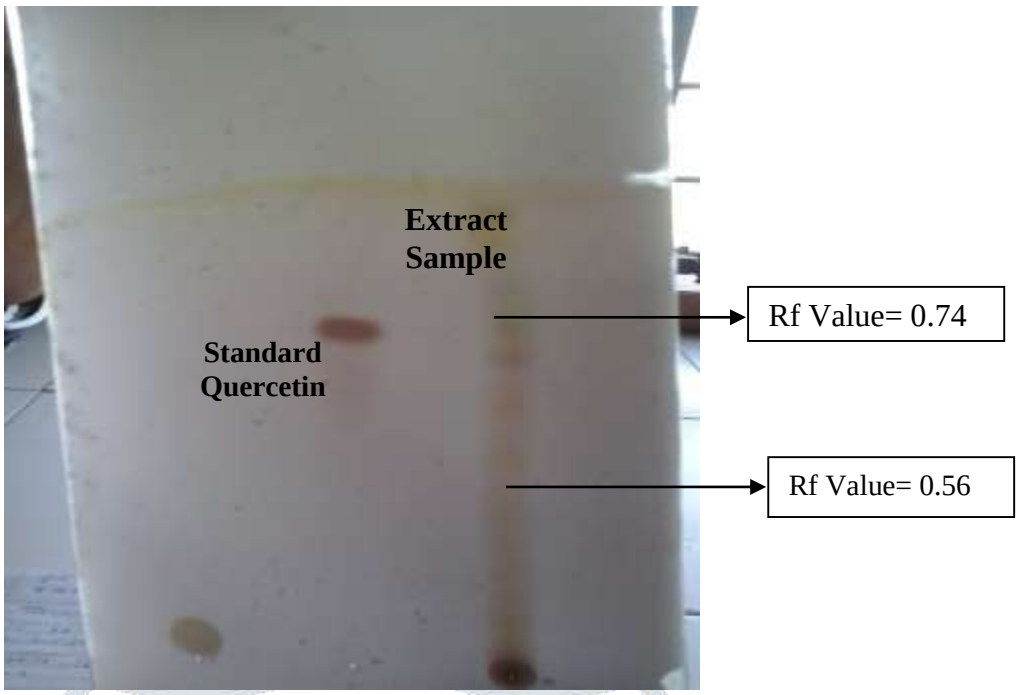
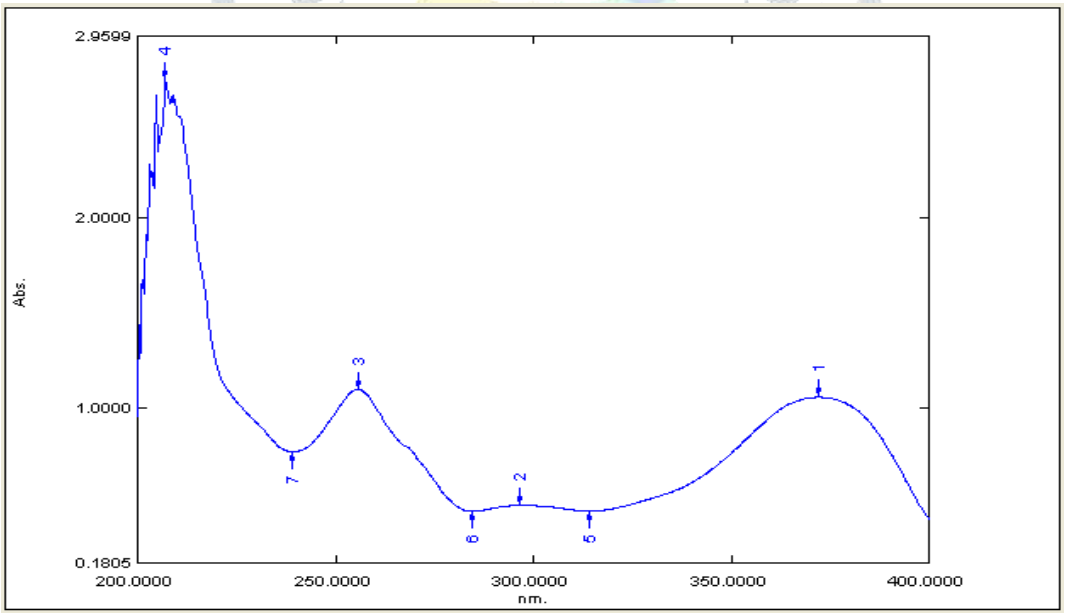



**Fig no.7: Comparative TLC of standard Quercetin and Flavonoid extract**

**Table no. : Solvent System Developed for Isolated Flavonoid Rich Fraction**

| No. | Solvent system | No. Of Spot | RF Value |
|---|---|---|---|
| 1 | Toluene: Ethyl acetate: Formic acid (50: 40: 10) | Two | 0.74 and 0.56 |

## C. Estimation of Total flavonoid contents of Cassia auriculata:



| No. | P/V | Wavelength nm. | Abs. |
|---|---|---|---|
| 1 | ↑ | 372.0000 | 1.0543 |
| 2 | ↑ | 296.6000 | 0.4842 |
| 3 | ↑ | 255.6000 | 1.0968 |
| 4 | ↑ | 207.0000 | 2.7283 |
| 5 | ↓ | 314.2000 | 0.4528 |
| 6 | ↓ | 284.4000 | 0.4525 |
| 7 | ↓ | 239.0000 | 0.7676 |

**Fig. no.8: UV Spectrum of standard Quercetin scanned from 800 to 200 nm and its absorbance.**

**Standard calibration curve of Quercetin**

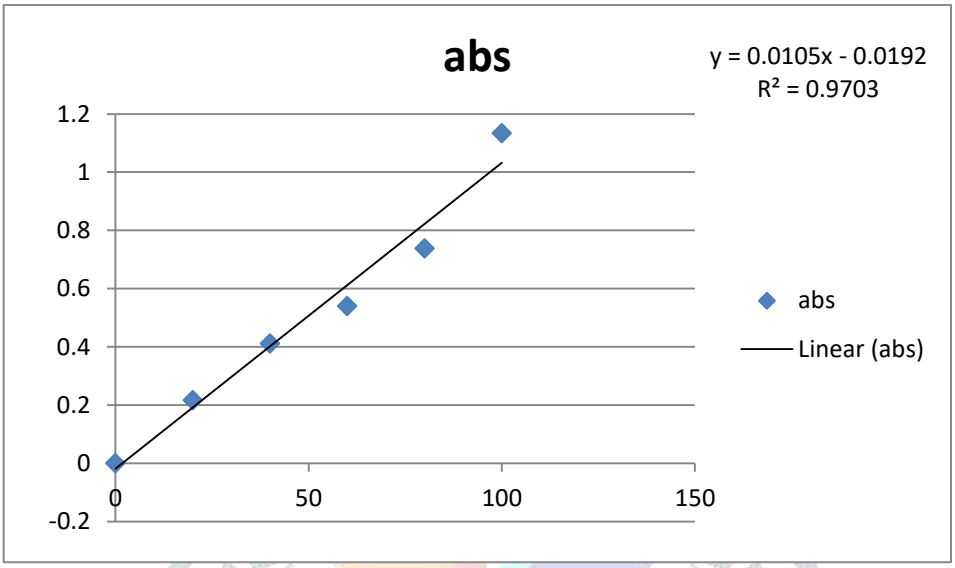



**Figure no.9: Quercetin standard curve**

**Table 7: Results from calibration curve**

| Type of the Extract | Absorbance | Concentration, µg/ml |
|---|---|---|
| 70% EtOH Extract of Cassia auriculata L. | 0.513 | 56 µg/ml |

**Table no.14: % Total flavonoid content of different extract**

| Type of the Extract | % TFC in grams |
|---|---|
| 70% EtOH Extract of Cassia auriculata L. | 14.00% |

TFC = Total Flavonoid Concentration was expressed in terms of quercetin equivalent per 100 grams of plant material.

**Discusion:** The results of these studies revealed that, 70% ethanolic extract of Cassia auriculata showed the presence of significant amount of flavonoid i.e. 14.00% TFC in grams respectively.

## D. ANTIMICROBIAL ACTIVITY OF EXTRACTS:

The *Cassia auriculata* extract, was examined for antimicrobial activities against microorganisms frequently involved in antidandruff activity, *M. furfur; P. aeruginosa; B. subtilis; C. albicans; P. valgaris; E. coli; E. faecalis; A. awamori.*

From the result below it was observed that the extract shows the minimum zone of inhibition as compared to standard antibiotic clindamycin and antifungal fluconazole.

**Table no.7 : Antimicrobial Activity of Medicinal Plant Extract**

| Microorganisms | Zone of Inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Plant extracts | | | | Standards (10mg/ml) | |
| | 0.2% | 0.4% | 0.6% | 0.8% | Clindamycin | Fluconazole |
| *M.furfur* | 19 | 20 | 23 | 25 | _ | 29 |
| *P.aeruginosa* | 15 | 17 | 26 | 29 | 24 | _ |
| *B.subtilis* | 24 | 20 | 25 | 25 | 31 | _ |
| *C.albicans* | 21 | 19 | 18 | 22 | _ | 24 |
| *E.coli* | 14 | 16 | 20 | 22 | 20 | _ |
| *E.faecalis* | 13 | 20 | 26 | 27 | 24 | _ |
| *A.awamori* | 15 | 14 | 21 | 25 | 31 | _ |
| *P.vulgaris* | 17 | 18 | 23 | 25 | 30 | _ |

## E. EVALUATION OF FORMULATIONS:

The various physical properties of the prepared shampoo formulations are shown in table.

**Table no.8: Physical evaluation of shampoo formulation**

| No. | Physical evaluation | Prepared shampoo | | | Plain shampoo |
|---|---|---|---|---|---|
| | | 5% | 7.5% | 10% | |
| 1. | Colour | Brown | Brown | Brown | White |
| 2. | Appearance | Coloured | Coloured | Coloured | Transparent |
| 3. | Homogeneity | Good | Good | Good | Good |
| 4. | Viscosity (cps) | 1456 | 1328 | 1764 | 1781 |
| 5. | Spreadability (gm.cm/sec) | 11.54 | 11.62 | 11.76 | 12.14 |
| 6. | pH | 6.68 | 6.72 | 6.78 | 6.89 |
| 7. | Drug content | 93.621± .22 | 94.645±1.43 | 96.651±1.56 | _ |

From the results it is clearly evident that the shampoo formulation showed good elegancy and good homogeneity with absence of lumps. The values of spreadability indicate that the shampoo is easily spreadable. The pH of the formulations was 6.68, 6.72, 6.78 and 6.89 which lies in normal pH range of the

skin. A comparative study of viscosity and spreadability showed that, increase in viscosity of the formulations, the spreadability is decreased and vice versa which is shown in table no.8.

## F. STABILITY STUDY OF FORMULATIONS:

Stability study of shampoo formulation was carried out at $40^0C\pm 2^0C$/ 75% ± 5% RH temperature and relative viscosity for the period of 30 days. The different parameters which were recorded for the change during this period were as follows:

a) Change in appearance

b) Change in pH

c) Change in viscosity

d) Change in spreadability

e) Change in drug content

**Table no.9: Stability study of shampoo at $40^0C \pm 2^0C$/ 75% ± 5% RH for 30 days.**

| Sr. No. | Evaluation | Observation |
|---|---|---|
| 1. | Appearance | Brownish |
| 2. | pH | 6.7 |
| 3. | Viscosity (cps) | 1568 |
| 4. | Spreadability (gm.cm/sec) | 11.54 |
| 5. | Drug content (%) | 90.124± 0.19 |

## 9. CONCLUSION

In the present investigation, it was concluded that the aerial parts of *Cassia auriculata* exhibited the abundant presence of flavonoid in the 70% alcoholic extract. The percentages of total flavonoid were found to be 14.0% in the flavonoid rich fraction of aerial parts of *Cassia auriculata* (i.e., 70% alcoholic extract). The flavonoid rich fractions have shown a significant antimicrobial activity with special emphasis on *M. furfur*. The antidandruff activity of the flavonoid rich fraction of *Cassia auriculata* was reported as a first time. The antifungal and antibacterial activities were attributed to the rich amount of flavonoid present in the 70% alcoholic extract of *Cassia auriculata*. The flavonoid rich fraction was formulated into a shampoo and shampoo preparation. The drug released (flavonoids) was studies with the help of franz diffusion cell with the help of Qurecetin as standard. The formulations were evaluated with the help of various parameters.

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