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Exploring Ethnopharmacology for Antidandruff Properties in Traditional Herbal Remedies of the Wayanad District Tribes, Kerala

Shibu Prasanth CR[1], Lal Prasanth ML *[2]

[1]Research Scholar, Sunrise University, Alwar, Rajasthan,India

*[2]Professor, Sunrise University, Alwar, Rajasthan,India

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KEYWORDS

Antidandruff, Phyllanthus emblica L., Azadirachta indica A. Cyclea peltate, Ocimum sanctum, Aloe barbadensis miller.

ABSTRACT:

Kerala the Southernmost state of India is known for its biodiversity reserve, high cultural heterogeneity and high rate of literacy. Dandruff, a relatively common non-contagious hair condition, affects a majority of individuals, irrespective of their age. It is scientifically known as pityriasis simplex capillitii, a prevalent scalp disorder that is marked by the shedding of dead skin cells from the scalp, causing itching and if untreated, leads to infections. The clinical application or medical use of zinc pyrithione,steroids,selenium sulphide, imidazole derivatives, salicylic acid, glycolic acid, and coal tar derivatives can be responsible for dandruff. However, it is primarily caused by the overgrowth of a yeast-like fungus called Malassezia, further associated with Propionibacterium acne and Staphylococcus epidermidis. The primary goal of this research was to conduct anti Dandruff activity through anti fungal , anti inflammatory and antioxidant demonstrations, which prompted the current investigation by screening five Medicinal plants extracted from Wayanad, Kerala a place which serves as a dynamic reservoir of potentially valuable biodynamic compounds; the land of paddy fields. These extracts includes: Amla (*Phyllanthus emblica L.*), Neem Leaves *(Azadirachta indica A. Juss),* Patha leaves (*Cyclea peltata),* Tulsi (*Ocimum sanctum*), Aloe vera *(Aloe barbadensis miller).* These experiments were conducted in a potato dextrose agar medium. The strains of *Candida albicans* and *Malassezia furfur* were used for the study of anti-fungal activity. Cyclea peltata showed the presence of the following phytochemicals : alkaloids, flavonoids, terpenoids, steroids, saponin, Gums and Mucilage, Tannins and Phenolic Compounds. The dried leaf samples were crushed in powdered form and were sequentially extracted with petroleum ether, chloroform, and methanol using a Soxhlet apparatus until the solvents became decolorized. *In vitro* anti-inflammatory study was carried out on the freeze dried extracts by HRBC (Human Red Blood Cell) membrane stabilization test. For cross-examination, an antioxidant test was carried out for the freeze-dried extracts by scavenging activity of free radicals of DPPH. These leaf extracts are marked as a potential antifungal, anti-inflammatory and antioxidant agent for treatment of dandruff.

1. Introduction

Kerala, the southernmost part of India, is renowned for its rich cultural diversity, high literacy rate, and wildlife reserve. At 11° 55' N latitude and 75° 59' E longitude, Wayanad is a magnificent hill of Malabar located in the northern region of Kerala State. Its elevation ranges from 950 meters to 1350 meters above sea level. In the winter, the temperature drops to 16°C, while in the summer, it reaches 30°C. Rainfall varies between 300 to 1000 millimeters every year.[1,2] Grasslands, evergreens, and semi-evergreens are the three types of forests.Marked by unique geographic position and distinct physiographic. edaphic and climatic gradients, Wayanad abounds in her vegetational wealth and floristic diversity. The occurrence of diverse types of ecosystems together with an impressive array of species and genetic diversity makes one of the richest centres of biological diversity.[3] A significant portion of Wayanad's plant resource range are medicinal plants.In contrast to other tropical zones with comparable geographic and physiographic features, the medicinal flora of Wayanad has notable intraspecific variability and phytochemical polymorphisms. The occurrence of high genetic variability and a remarkable array of organic compounds

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characterizes the medicinal plants in this region[5]. Therefore, the flora of Wayanad represents a dynamic repository of potentially useful biodynamic compounds, which could pave the way for new drug and pharmaceutical discoveries.The study on medicinal plants in Wayanad and their use in the traditional medicare system has a long and rich history. Ethnobotanical studies conducted in various tribal communities in Wayanad have unveiled crucial information about numerous plants utilized for treating various human ailments. Although the reported usage of many of these ethnomedicinally important plants still requires scientific validation, research conducted on some of them has demonstrated promising pharmacological activities. The extensive and previously unexplored resources of wild medicinal plants, coupled with significant ethnomedicinal findings, provide a tremendous opportunity for developing novel therapeutic agents.

Most people, regardless of age, suffer from dandruff, a fairly prevalent non-contagious hair condition. Shedding of dead skin from the scalp is known medically as pityriasis simplex capitis. Depending on whether it's oily or dry, greasy flakes might smell bad and have a pale yellowish appearance, whereas dry dandruff is silvery and white.Pityriasis simplex and pityriasis capitis (relating to Pityrosporum) and furfuracea (referring to Malassezia furfur) are two other descriptive names that were previously employed to describe the fungal etiology of this ailment. 5% of people worldwide suffer with this prevalent and humiliating condition. Itching and aesthetic value are both negatively impacted by dandruff, and keratinocytes are crucial for the expression of immune responses during dandruff production. Seasonal variations in dandruff severity are common, with wintertime seeing an increase in cases. Dandruff is a common scalp ailment that causes itchy, unpleasant white flakes, excessive skin dryness, and seborrhea, or overactive oil glands. The therapeutic usage of selenium sulphide, zinc pyrithione, salicylic acid, imidazole derivatives, glycolic acid, steroids, and coal tar derivatives are currently accessible treatment options for the management of seborrheic dermatitis. These drugs do, however, have considerable drawbacks, either because of low effectiveness in clinical trials or because of problems with adherence. These medications also can't stop reappearance. Thus, an attempt has been made to assess the antidandruff efficaciousness of plants that the tribes of the Wayanad district customarily employ in order to counteract all of these adverse effects. In addition to this, the antioxidant and anti-inflammatory activity shown by these extracts may enhance the antidandruff effectiveness and reduce the inflammation associated with it.

2. Methodology

A. Procurement of Herbs

Amla (*Phyllanthus emblica L.*)[6]

In March, the amla fruit was procured from the Kalpetta, Wayanad, Kerala, regional market. In short, to get rid of any dust or other particles that were accumulated on the amla fruit, they were gently rinsed under running water. The seed present inside the amla fruit were carefully removed. 1 kg of clean amla fruit were cut into small pieces and then dried using hot air oven at 40 ^{0}C for a period of 72 h . The dried material was powdered and stored in air tight container for further use.

Neem Leaves *(Azadirachta indica A. Juss)*

The neem leaves were collected from the plants in Wayanad, Kerala in the month of March. [7-8]Briefly, the collected leaves were washed systematically under running water to remove soil and other debris adhered to them. 1 kg of clean leaves were dried using hot air oven at 40 ^{0}C for a period of 24 h . [9-10]The dried material was powdered and stored in air tight container for further use.

Tulsi (*Ocimum sanctum*)

The aerial parts of tulsi were collected from various parts of Wayanad, Kerala in the month of March. [11-12]Briefly, the collected plant parts were washed systematically under running water to remove soil and other debris adhered to them. 1 kg of clean plant parts were dried using hot air oven at 40 ^{0}C for a period of 24 h .[12-15] The dried material was powdered and stored in air tight container for further use.

Patha leaves (*Cyclea peltata)*

The patha leaves were collected from various parts of Wayanad, Kerala in the month of March.[16-19] Briefly, the collected leaves were washed systematically under running water to remove soil and other debris adhered to them. 1 kg of clean leaves were dried using hot air oven at 40 ^{0}C for a period of 24 h . [20-22]The dried material was powdered and stored in air tight container for further use.

Aloe vera *(Aloe barbadensis miller)*

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(1), 1303-1315 | ISSN:2251-6727

The matured leaves of aloe vera were collected from Wayanad, Kerala in the month of March. [23-26] Briefly, the collected leaves were washed systematically under running water to remove soil and other debris adhered to them. 1 kg of fresh clean leaves were cut into small pieces and then grinded using a mixer. [27] Finally, the juice was collected by squeezing the solid and straining the liquid through a strainer.

B. Extraction

The plant materials (amla, neem leaves, patha leaves, and tulsi) which are cleaned, dried and finely powdered separately were used for extraction. The extraction is done by using the Soxhlet apparatus. [28]In the extraction processes, 1 L of distilled water is introduced to the round bottom flask and 100 g of the powdered drug was weighed and added to the thimble. Then thimble is settled in the extraction chamber of the Soxhlet apparatus. The condenser is also fitted to the Soxhlet apparatus (Figure1). The RB Flask is warmed using a heating mantle, which causes the solvent to boil. The solvent's vapours then pass through a vapour duct to the condenser, where they are condensed, before falling on the drug material in the extraction chamber. Solvent steadily fills the extraction chamber until it is leveled with the syphon tube, at which stage it flows through the syphon tube back to the RBF and the entire process is repeated. The plant extracts were collected and stored in the dark for further use. When opposed to the conventional soaking method, it has the benefits of low solvent flow rates, high efficacy in extraction, and total extraction. It also has a straightforward design, a continuous production procedure, easy to visually monitor, and can be reused following separating and the process of distillation.

Figure1 Extraction process using Soxhlet apparatus

C. Preliminary Phytochemical Evaluation

Various standard qualitative chemical tests [29]were carried out in the total aqueous extract of amla, neem leaves, patha leaves and tulsi as well as aloe vera juice to identify the existence of various phytochemical contents such as alkaloids, glycosides, flavanoids, steroids, triterpenoids, saponins , tannins, carbohydrates, aminoacids and proteins.

D. Freeze drying

In a lab-scale freeze dyer (Christ Alpha, Germany), several herbal extracts and aloe vera juice were freeze dried. [30] Until the processing step, the obtained

aqueous extract/juice of herbs was frozen in static air in the freezer at a temp. of -80 °C. The freezing substance was then stored in a freeze dryer for 72 hours at -50 °C with a pressure less than 0.110 mbar. (Figure 2) The dried product was ground into a fine powder by a mortar and pestle then preserved in an airtight container in a desiccator until use.

Figure 2 :Freeze Dryer for freeze drying of herbal extracts

E. Evaluation of Antifungal Potential

The strains of *Candida albicans* and *Malassezia furfur* for the study of anti-fungal activity were collected from the Microbiology lab of Dr Moopen's Medical College Wayanad. [31] In 100ml of distilled water, 20 grammes of potato-based influsion, 2 gramme of dextrose, and 1.5 gramme of agar were dissolved to produce the potato dextrose agar media. The diluted media was placed in an autoclave for 15 minutes at 121 °C and 15 pounds of pressure. While still molten, the autoclaved media was well blended and put onto 100 millimetres petri dish plates.Various quantities of freeze dried extracts of Amla, Tulsi, Neem, Aloe vera, and Patha (100, 50, 25 and 12.5 g/ml) were transferred to petri plates having 20 ml potato dextrose agar media, which had been inoculated with a 72-hour culture of fungal strains. After that, the plates underwent incubation for 72 hours at 28 degrees Celsius. By determining the diameter of the inhibition zone that developed within the wells, the anti-fungal efficiency was evaluated. The successful control was amphotericin B. Software from the USA named Graph Pad Prism 6.0 was used to calculate the numbers.

F. Evaluation of Antioxidant Potential

According to Gornas et al. (2014), the DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free radical scavenging activity was used to determine the total antioxidant capacity of the freeze-dried herbal extracts.[33] In short, 4 mg of DPPH was dissolved in 100 ml of methanol, and the mixture was homogenised for thirty minutes in an ultrasound bath.

The required quantity of freeze dried herbal extact was dissolved in 1.6ml distilled water to obtain the various concentrations and 2.4 ml of DPPH solution was added to the solution, and the mixture was stirred thoroughly before incubating it for thirty minutes at ambient temperature in darkness. The solution's absorbance was then determined at 517 nm using a UV-visible spectrophotometer compared as std Ascorbic acid . The following formula was used to determine the scavenging activity:

$$\% \text{ Scavenging activity} = \frac{(A517 \text{ control} - A517 \text{ sample})}{(A517 \text{ control})} \times 100$$

G. Evaluation of Anti-inflammatory Activity

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(1), 1303-1315 | ISSN:2251-6727

In vitro anti-inflammatory study was carried out on the freeze dried extracts by the HRBC (Human Red Blood Cell) membrane stabilization test using the blood bank of Dr. Moopen's Medical College, Wayanad. Blood suspension (10% v/v) was made using regular saline. One millilitre of different freeze-dried extract strengths and one millilitre of a 10% Red Blood Cell suspension constitute the reaction mixture.[35] In this case, ordinary saline was put to the test control tube in lieu of the test sample. The typical medication was diclofenac (200 g/mL). Each the reaction solutions underwent a 30-minute incubation in a water bath at 56°C. After the incubation phase, the test containers were allowed to cool using water flow and underwent centrifugation for five minutes at a speed of 2500 rpm. The percentage suppression of cell membrane lysis was estimated using the coloured supernatants' absorbance at 560 nm.[36] For each test sample, the study was carried out three times. Using the formula, the percentage of membrane stabilisation activity was determined. [Chioma AA, et.al].

$$\text{Percentage Inhibition} = \frac{(\text{Control} - \text{test})}{\text{Control}} \times 100$$

3. Results

A. Procurement of Herbs

The plant materials required for the present study were collected from the local farm of Meppadi and local market of Kalpetta, Wayanad District (India) in the months of April and May 2023. All of the plant components were recognised and verified, and then they were all thoroughly rinsed with the flowing water to get rid of any dust or other dirt that had become stuck to them.

B. Extraction

The plant materials (amla, neem leaves, patha leaves, and tulsi) which are cleaned, dried and finely powdered separately were used for extraction. The extraction of is done by using the Soxhlet apparatus.

C. Priliminary Phytochemical Evaluation

Qualitative chemical tests were carried out in the aqueous extract of herbs collected. The result of the chemical test for extracts was tabulated in the following table 1.

Sl. No.	Phyto constituents	Aloe vera	Neem	Cyclea	Tulsi	Amla
1)	Glycosides	+	+	-	-	-
2)	Steroids	-	+	+	+	-
3)	Saponins	+		+	+	-
4)	Alkaloids	-	-	+	-	-
5)	Flavanoids	-	+	+	+	+
6)	Triterpenoids	-	+	+	+	-
7)	Tannins & Phenolic Compounds	-	+	+	-	+
8)	Proteins & Aminoacids	-	+	-	-	+
9)	Carbohydrates	+	-	-	+	+
10)	Gums & Mucilage	+	-	+	-	-

Table1 The result of the chemical test for extracts

D. Freeze drying

Freeze drying of prepared aqueous extracts were performed in a laboratory scale freeze dryer. The dried product was carefully ground into a fine powder using a pestle and mortar, and then kept in an airtight container in a desiccator until use.

Evaluation of Antifungal Potential

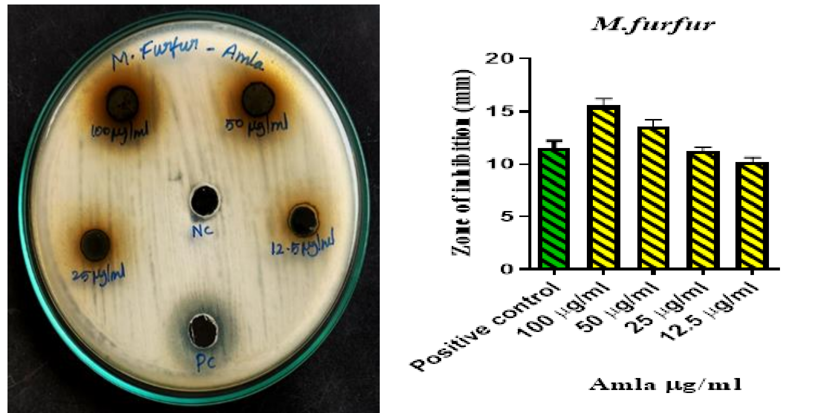


Fig : 3 Effect of sample Amla against *M. furfur*.

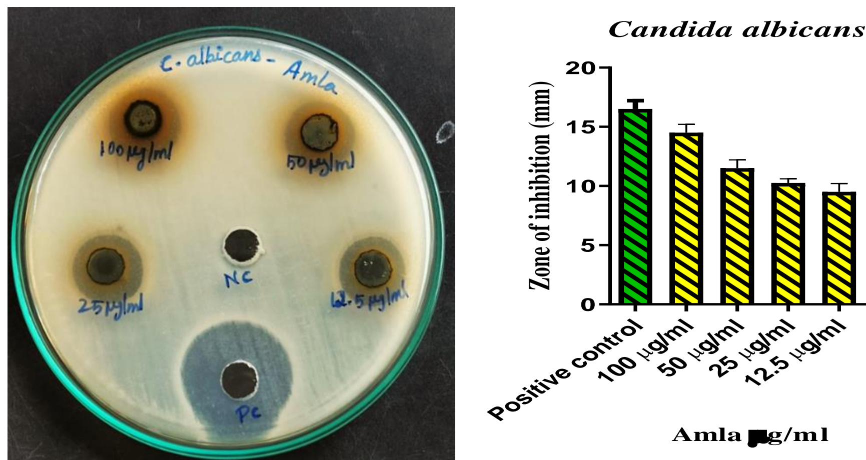


Fig : 4 Effect of sample Amla against *C. albicans*.

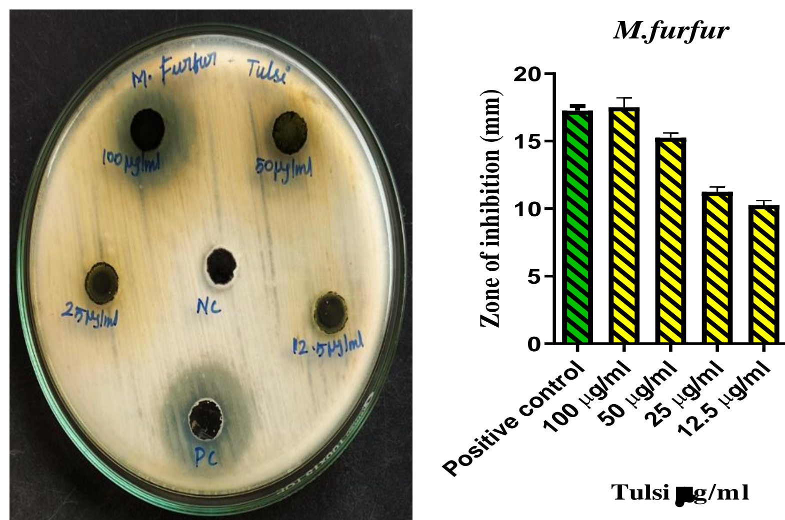
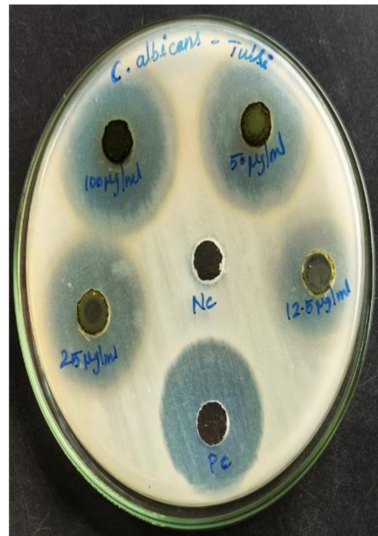


Fig : 5 Effect of sample Tulsi against *M. furfur*.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(1), 1303-1315 | ISSN:2251-6727



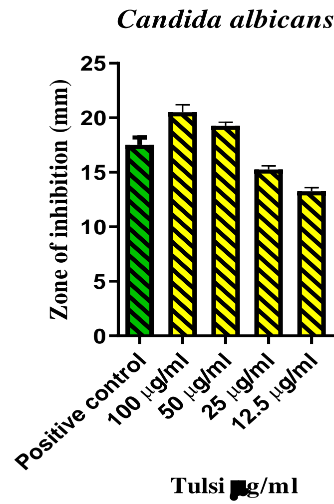
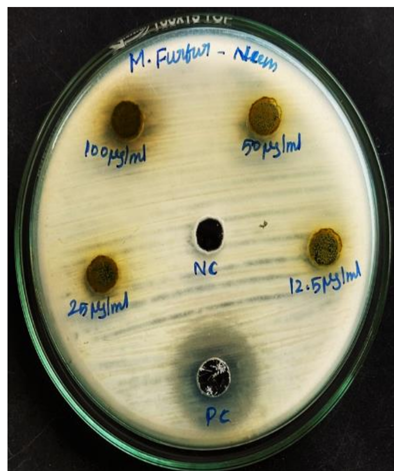


Fig : 6 Effect of sample Tulsi against *C. albicans*.



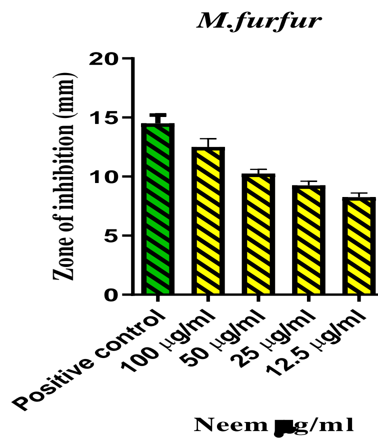
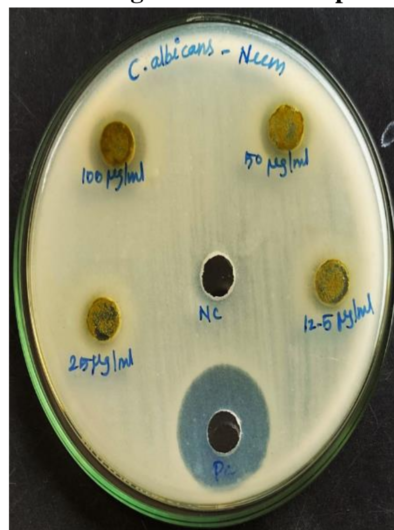


Fig : 7 Effect of sample Neem against *M. furfur*.



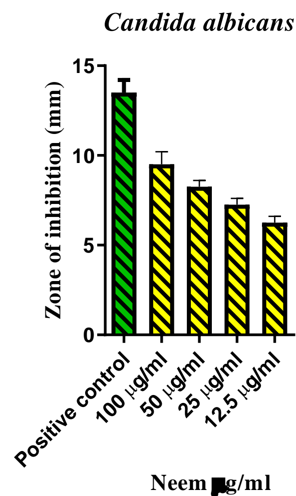


Fig : 8 The impact of Neem extract on C. albicans samples.

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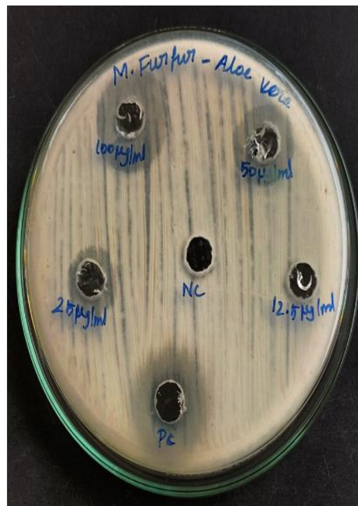


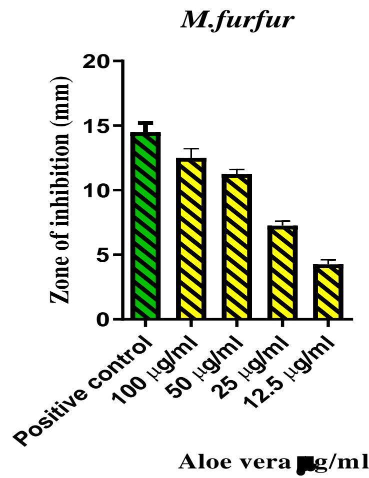
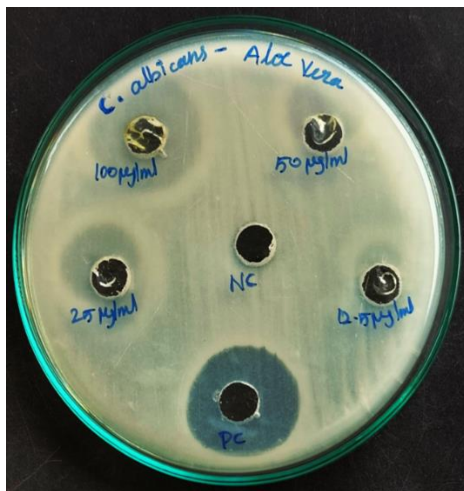


Fig : 9 Effect of sample Aloe vera against *M. furfur.*



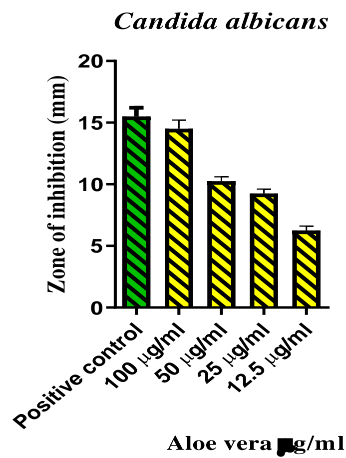
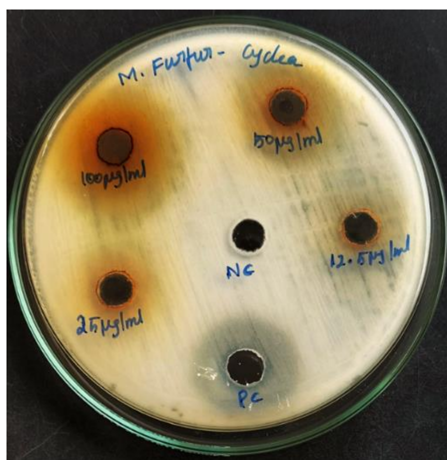


Fig : 10 Effect of sample Aloe vera against *C. albicans.*



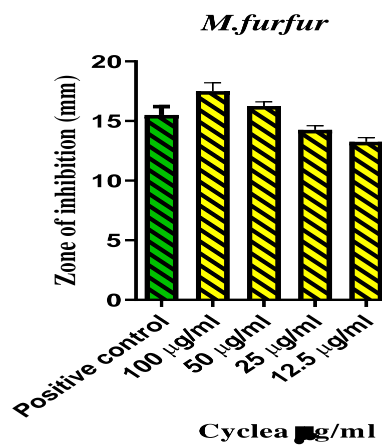


Fig : 11 Effect of sample Cyclea against *M. furfur.*

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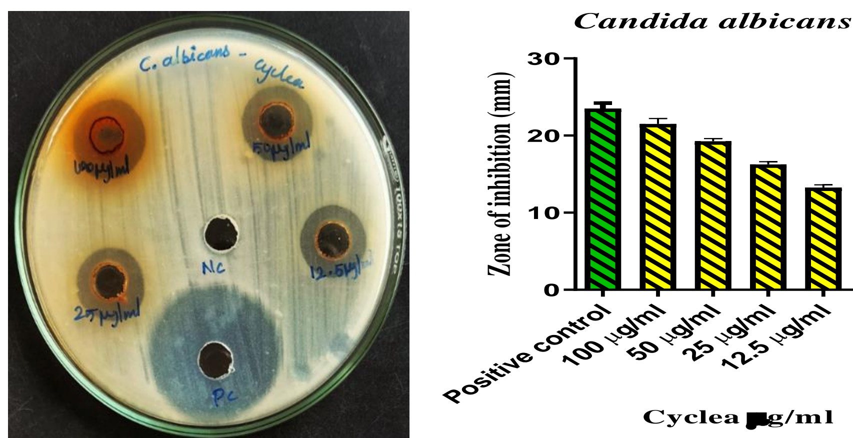

Fig : 12 Effect of sample Cyclea against *C. albicans.*

S. Num-ber	Tested organism names	Tested samples names	Inhibition zone (mm) SD(Standard Deviation)± Average				
			100 µg/ml	**50 µg/ml**	**25 µg/ml**	**12.5 µg/ml**	**PC**
1. .	*M. furfur*	Amla	15.5±0.70	13.5±0.707	11.25±0.35	10.25±0.3	11.5±0.70 7
2. .	*C. albicans*		14.5±0.7	12.5±0.70	11.5±0.707	10.25±0.35	9.5±0.707 1
3.	*M. furfur*	Tulsi	17.5±0.707 1	15.25±0.35	11.25±0.3	10.25±0.3	17.25±0.3
4.	*C. albicans*		20.5±0.7	19.25±0.35	15.25±0.35	13.25±0.3	17.5±0.70 71
5.	*M. furfur*	Neem	12.5±0.70	10.25±0.35	9.25±0.3	8.25±0.3	14.5±0.70
6.	*C. albicans*		9.5±0.7071	8.25±0.35	7.25±0.3	6.25±0.3	13.5±0.70
7.	*M. furfur*	Aloe vera	12.5±0.707 1	11.25±0.3	7.25±0.3	4.25±0.35	14.5±0.70 7
8.	*C. albicans*		14.5±0.707	10.25±0.35	9.25±0.35	6.25±0.3	15.5±0.70 71
9.	*M. furfur*	Cylcea	17.5±0.7	16.25±0.35	14.25±0.35	13.25±0.3	15.5±0.70 71

10.	*C. albicans*		21.5±0.707 1	19.25±0.35	16.25±0.3	13.25±0.35	23.5±0.70 7

n = 3, mean ± S.D

Table 2: representing SD±(Standard Deviation)The zone of inhibition resulting from samples of Tulsi, Neem,Amla,Aloe vera and Cyclea against *M.furfur* and *C.albican*

E. Antioxidant Potential

The determination of the overall anti-oxidant capabilities was carried out for the freeze-dried extracts by scavenging activity of free radicals of DPPH where the extent of discoloration of the DPPH solution is an indication of the extent of antioxidant property of the compound. Clear DPPH solution is taken as blank and for reference standard ascorbic acid was considered. The anti-oxidant activity in terms of percentage scavenging activity is presented in Table 5.2 and graphically represented in Figure 5.11.

Sl. No	Freeze dried Herbal Extract	Concentration (µg/ml)	**% Scavenging activity**
1	Aloe vera	12.5	12.03 ± 0.40
		25	19.19 ± 0.20
		50	22.60 ± 0.20
		100	26.29 ± 0.12
2	Neem	12.5	05.31 ± 0.28
		25	08.89 ± 0.58
		50	15.67 ± 0.67
		100	28.69 ± 1.12
3	Patha	12.5	11.54 ± 0.72
		25	15.20 ± 0. 14
		50	36.58 ± 1.55
		100	51.49 ± 1.95
4	Tulsi	12.5	07.45 ± 0.92
		25	11.36 ± 1.01
		50	16.52 ± 0.53
		100	35.21 ± 2.32
5	Amla	12.5	8.36 ± 1.56
		25	22.51 ± 2.07
		50	31.25 ± 2.56
		100	43.23 ± 1.58
6	Standard (Ascorbic acid)	12.5	15.06 ± 1.64
		25	24.73 ± 2.14
		50	49.05 ± 1.55
		100	97.06 ± 1.32

Table 3 Antioxidant activity of freeze-dried herbal juices in terms of percentage scavenging activity

F. Evaluation of Anti-inflammatory Activity

In vitro anti-inflammatory study was carried out on the freeze-dried extracts by test for stability of Human Red Blood Cell (HRBC) membrane utilizing human blood samples taken from the blood bank of Dr. Moopen's Medical College, Wayanad.

Sl. No	Freeze dried Herbal Extract	Concentration (µg/ml)	% Protection
1	Aloe vera	12.5	12.03 ± 0.40
		25	19.19 ± 0.20
		50	22.60 ± 0.20
		100	26.29 ± 0.12
2	Neem	12.5	05.31 ± 0.28
		25	08.89 ± 0.58
		50	15.67 ± 0.67
		100	28.69 ± 1.12
3	Patha	12.5	11.54 ± 0.72
		25	15.20 ± 0. 14
		50	36.58 ± 1.55
		100	51.49 ± 1.95
4	Tulsi	12.5	07.45 ± 0.92
		25	11.36 ± 1.01
		50	16.52 ± 0.53
		100	35.21 ± 2.32
5	Amla	12.5	8.36 ± 1.56
		25	22.51 ± 2.07
		50	31.25 ± 2.56
		100	43.23 ± 1.58
6	Standard (Diclofenac Sodium)	50	71. 62 ± 1.32

Table 4 Effect of different freeze dried herbal extracts on cell membrane protection

4. Conclusion

Phyllanthus emblica L., Azadirachta indica A., Cyclea peltate, Ocimum sanctum, and Aloe barbadensis miller constitute a crucial medicinal plant. The methanolic leaf extract contains all the phytochemicals except for fixed oil and fat. The chloroform extract displays the presence of alkaloids, flavonoids, terpenoids, steroids, proteins, amino acids, fixed oil, and fat. Terpenoids, steroids, fixed oil, and fat are found in the petroleum ether extract.Consequently, an effort has been made to evaluate the effectiveness of these plants, traditionally utilized by the tribes of the Wayanad district, against dandruff and its associated effects. Moreover, the demonstrated antioxidant and anti-inflammatory properties of these extracts may enhance their efficacy against dandruff and alleviate associated inflammation.

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