

COMPARATIVE STUDY OF ANTI-FUNGAL EFFICACY IN MEDICINAL PLANT LEAVES EXTRACT FROM *Tages erecta*, *Terminalia arjuna* , *Chrysopogon zizanoides* AGAINST *Trichosporon asahii*

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ABSTRACT

Trichosporon sp. is widespread in nature. *Trichosporon sp.* is a leading source of invasive infection in immunocompromised people, with *Trichosporon asahii* being the most commonly reported species. This present study focuses on isolated and characterised fungi from infected people, and growth optimisation was performed at different pH and temperatures. The susceptibility was tested against solvents methanol from *Tages erecta*, *Terminalia arjuna*, and *Chrysopogon zizanoides*, medicinal plants. The agar-well diffusion experiment was used to analyse the zone of inhibition. Cipla, Ketokem, and Salisia Kt served as positive controls. Phytochemical screening was conducted to identify phytochemicals in the plant. The methanol extract was shown to be the most effective. Phytochemicals have an important role in anti-*Trichosporon* action.

KEYWORDS: *Trichosporon asahii* ,medicinal plants, methanol extract, Phytochemical, anti-*Trichosporon* action

INTRODUCTION

Hair has historically been connected with grace and social distinction, and is a vital aspect of the general attractiveness of the human body. Numerous examples from all creative forms show the exceptional importance given to hair by people of every generation and culture (1). Human White Piedra can cause hair loss in the scalp, axilla, and in rural areas. White Piedra of the scalp is uncommon in tropical and subtropical climates (2). People of all ages are affected, and young women bear a disproportionate share of this burden. It has soft white nodules similar to nits; however, unlike nits, it can be easily plucked off. Nodules formed by compact fungal components may be white, pale green, or yellow. The hairs are not penetrated, although they may break if the fungi are present for a long time, as in our cases.

Intertrigo, a type of cutaneous trichosporosis, can occur when the adjacent skin becomes infected, particularly in regions such as the groyne(3). It is a rare, asymptomatic, superficial fungal infection of the hair that is characterized by the development of many distinct, soft, asymptomatic nodules loosely linked to infected hair shafts. They can appear on the scalp, eyebrows, eyelashes, beards, axillae, or groynes. White Piedra affects scalp hair less frequently than Black Piedra, which affects scalp hair less frequently and is more common in other hairy locations of the body. It is caused by *Trichosporon beigeli*, now known as *T. asahii*.

Shaving and applying 5% ammoniated mercury ointment, topical 2% miconazole, 2% ketoconazole, or 1% terbinafine four times a day for two weeks or until remissions emerge will help control problems (4). Shampoos and other hair care products are widely accessible and are used in the market. Shampoo is a cosmetic preparation package that handles hair and scalp. Its principal role is to remove accumulated sebum, scalp debris, oil, and other debris from hair. Shampoo has other functions such as lubrication, conditioning, hair building, static charge prevention, and medication (5). Plants and their derivatives are used worldwide for the prevention and treatment (10). Commercially available and widely used shampoos are synthetic and herbal shampoos (including plant-derived components). The demand for and knowledge about herbal cosmetics is growing because they are safe and free of adverse side effects. Plant products contain various antifungal chemicals such as alkaloids, flavonoids, tannins, and terpenoids (6). Marigold flower extracts have been shown to exhibit antibacterial activities.

Furthermore, our previous study demonstrated that extracts made from marigold roots inhibited various common plant diseases (7). The chloroform/methanol extracts of *Tagetes lucida* prevented the radial expansion of *F. moniliforme* colonies by 89 expansion (8). *Trichoderma* spp. are efficient biocontrol agents against various diseases and specific isolates have been shown to induce systemic resistance in plants (9). *Terminalia arjuna* is a tree with a wide range of therapeutic properties. This herb has traditionally been used to treat various ailments. It contains triterpenoids with cardiovascular characteristics, tannins and flavonoids with anticancer qualities, and many more(11). Vetiver (*Chrysopogon zizanioides*, formerly *Vetiveria zizanioides*) is an Indian perennial grass(12). Vetiver fibers are natural fibers derived from leaves(13). Aromatic, antifungal, cooling, antiemetic, diaphoretic, haemostatic, expectorant, diuretic, stimulant, hysteria, insomnia, skin diseases, asthma, amentia, amenorrhoea, antispasmodic, kidney problems, gall stones, mosquito repellent, tonic, and antioxidant are some of the end uses of vetiver(14). As a result, the current study intends to assess the phytochemical compound, molecular characterization of isolated organisms and antifungal activity of *Tages erecta*, *Terminalia arjuna*, *Chrysopogon zizanoides*, antidandruff shampoos against *Trichosporan asahii* and The inhibitory activity was investigated using the agar well diffusion study being conducted.

MATERIALS AND METHODS

1.1 Isolation of morphology, physiology and 18S rRNA gene sequencing from collected sample

The organism was isolated from the scalp of individuals who had Dandruff Flakes or Scales and was obtained by dividing the hair with a sterile comb and scraping a one-inch region with a sterile blunt scalpel. The specimen was then placed on dark sample paper to avoid exposure to sunlight, and Sabouraud Dextrose Agar (SDA), which was made from 100 ml SDA media 6.14 g, agar (2 g), and chloramphenicol (1 mg/mL), was added to prevent bacterial contamination. The culture medium was sterilized in an autoclave at 121°C for 15 min. The plates were subsequently incubated at 30°C for five days with regular inspection, and microscopic examination was performed using the lactophenol cotton blue (LPCB) method. Genomic DNA was isolated as previously described method (15). The isolate was identified molecularly using the (16) approach, which involves sequencing the ITS1 and ITS2 segments of ribosomal DNA. The sequences produced were compared with existing data in the NCBI database using the Basic Local Alignment Search Tool (BLASTn)(17).

1.2 COLLECTION AND EXTRACT PREPARATION OF MEDICINAL PLANT LEAVES

Plants were collected, rinsed, and dried in the shade for one week. Subsequently, the dried plant material was ground into a powder and three distinct solvents were used for solvent extraction. Solvents including methanol, petroleum ether, and n-hexane were added to 20 g of powdered plant material and incubated in a shaker incubator for 24–48 h. After stirring, the mixture was passed through a Whatman No. 1 filter paper to separate the extract. The obtained substances were allowed to cool to ambient temperature and were subsequently scraped into powder form for further examination.

Table:1 represents the list of plant used for this study

S.NO	PLANTS	PARTS
1	<i>Tages erecta</i>	leaves
2	<i>Terminalia arjuna</i>	leaves
3	<i>Chrysopogen zizanoides</i>	leaves

1.3 ANALYSIS OF PHYTOCHEMICAL COMPOUNDS

Phytochemical analyses, including examinations for tannins, saponins, flavonoids, terpenoids, glycosides, alkaloids, phenols, steroids, carbohydrates, anthraquinone, and biuret, were performed using various tests, such as the Fehling test, Benedict's test, Molisch's test, and anthraquinone detection and biuret tests.

1.4 FOURIER TRANSFORM INFRA-RED SPECTROSCOPY (FT-IR)

Fourier Transform Infra-Red Spectroscopy (FT-IR) Elucidation of infrared spectra involves the association of fascination bands within the spectrum of an unidentified complex through the known incorporation frequencies for types of bonds. A significant method for identifying the basis of an absorptive band is intensity (weak, average, or tough), appearance (wider or sharper), and position (cm⁻¹) in the spectrum. FT-IR analysis of the methanol extract was performed to identify the practice groups in the bioactive mechanism based on its peak percentage and electron transition of compounds.

1.5 GROWTH OPTIMIZATION OF ISOLATED ORGANISM

1.5.1 EFFECT OF TEMPERATURE:

The inoculated substance was maintained at three different temperatures: 25°C, 35°C, and 55°C for 24 h of growth of the organism.

1.5.2 EFFECT OF pH:

The inoculated substance was kept at different pH in the range of 7,8,10 for 24 hours to monitor the growth of the isolated fungus.

1.6 PREPARATION OF INOCULUM AND DILUTION OF SHAMPOOS

The inoculum was created by inoculating 10⁶ cells/ml in 5 ml of Sabouraud's broth and incubating at 28 °C. Commercially available shampoos were utilized in antifungal testing after diluting with sterile distilled water to achieve 10-fold and 20-fold dilutions.

Table:2 denotes that different shampoos were used

S.NO	SHAMPOOS USED
1	CIPLA
2	KETOKEM
3	SALISIA KT

1.7 Antifungal assay

Agar well diffusion method

The antifungal activity of the different extracts against the isolated organism was examined by pouring agar into a petri dish and allowing it to cool before spreading the organism uniformly over the agar surface. The wells were punched aseptically using a cork borer along the plate margins (3 cm apart). Each well was carefully filled with 25,50,75,100 microlitres of the extracted solutions. The plates were allowed to diffuse for 30 min before incubation at 37 °C for 72 h. The diameter of the zones of inhibition serves as an approximate measure of the relative activity of different antimicrobial drugs in all cases. The zone of inhibition was measured using a meter rule after incubation.

RESULTS

1.1 Isolation of micro organism from collected sample



FIG:1- Morphology of isolated pure culture



FIG.:2- Microscopic morphology by LPCB

Characterization of molecular studies

1.2 COLLECTION AND EXTRACT PREPARATION OF PLANT LEAVES



FIG:3- *Tages erecta*



FIG:4- *Terminalia arjuna*



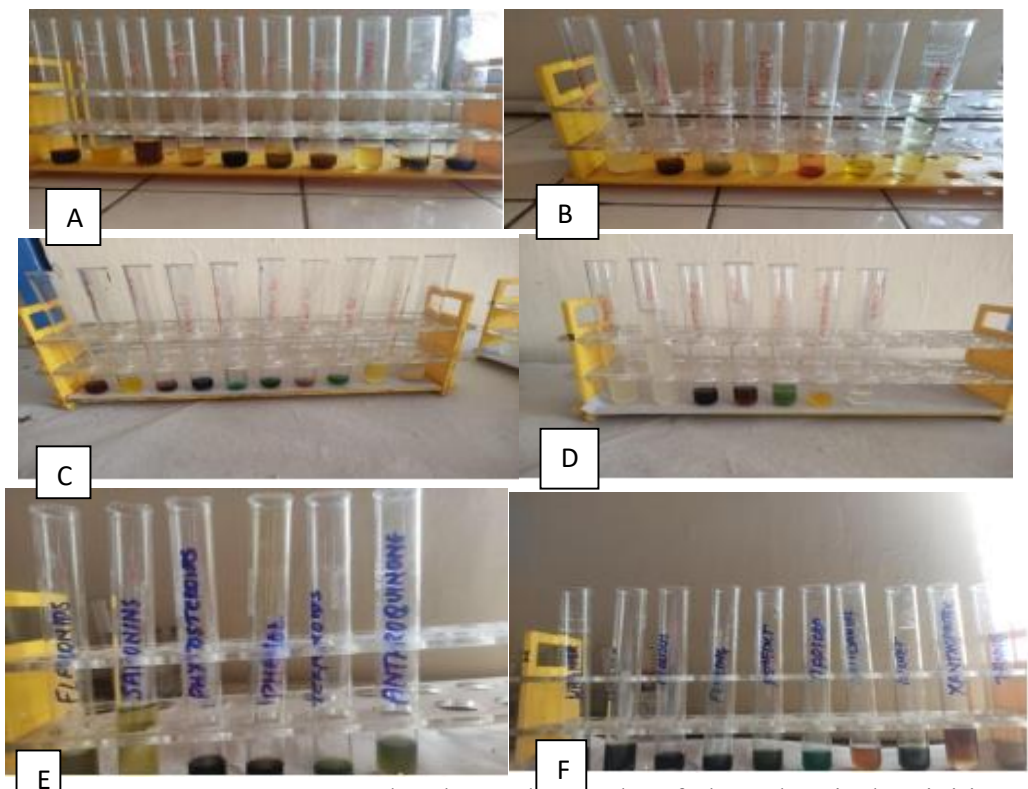
FIG:5- *Chrysopogen zizanoides*

FIG:6 - *Tages erecta*, *Terminalia arjuna* and *Chrysopogen zizanoides* leaves of methanol solvent extract



1.3 ANALYSIS OF PHYTOCHEMICAL COMPOUNDS

Figure A and B represents *Tages erecta*, C and D -*Terminalia arjuna* , E and F *Chrysopogon zizanoides* shows the tannins, saponins, flavonoids, terpinoids, glycosides, alkaloids, phenols, steroids, carbohydrates- fehling test, benedict's test , molisch's test, detection of anthraquinone, biuret results of phytochemicals compounds.



Figures 8 -A, B, C, D, E, and F shows the results of phytochemical activities.

QUALITATIVE PHYTOCHEMICAL ANALYSIS:

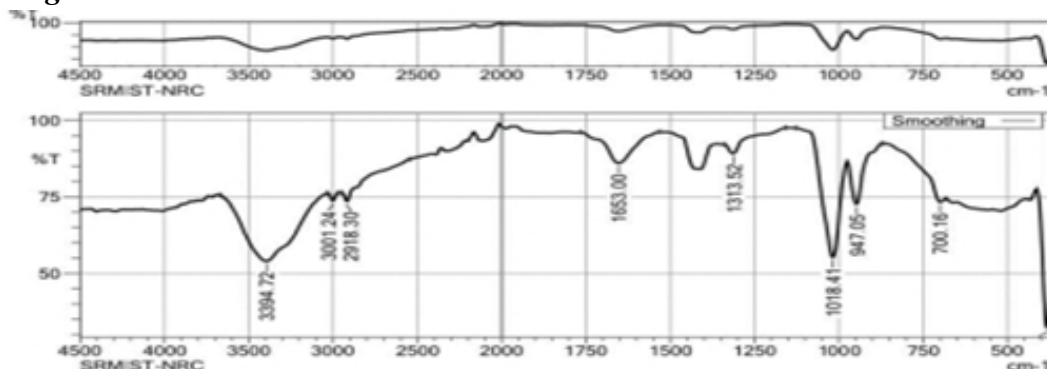
Table-3 represents the presence and absence of phytochemical compounds

PHYTOCHEMICAL TEST	<i>Tages erecta</i>	<i>Terminalia arjuna</i>	<i>Chrysopogonzizanioides</i>
Alkaloids	+	+	+
Flavonoids	+	+	+
Phenols	+	+	+
Tannins	-	+	+
Glycosides	+	+	+
Saponins	+	+	+
Reducing sugars	+	+	-
Proteins	+	+	-
Anthroquinone	+	+	+
Terpenoids	+	+	+

1.4 FOURIER TRANSFORM INFRA-RED SPECTROSCOPY (FT-IR)

The graph and table represents the value of Fourier Transform Infra-Red Spectroscopy for *Tages erecta* in the peak range 2997 the functional group of N-H stretching, 516.92- C-H bending, 700.16- C-H bending, 948.98- C=C bending, 1026.13- C-H bending, 1313.52- C-N stretching, 1996.32- C=C=C stretching, 2914.44- N-H stretching.

Tages erecta

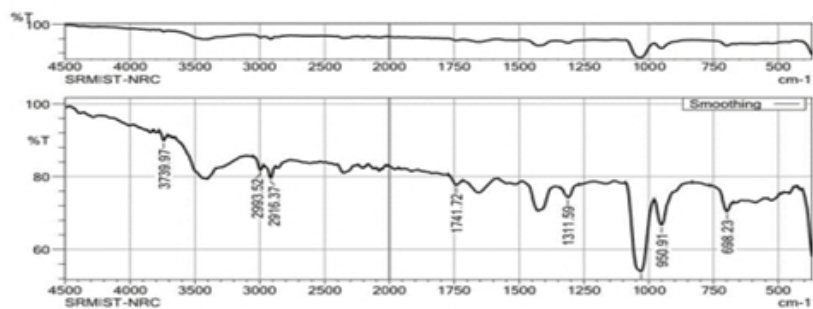


S.NO	PEAK VALUE	FUNCTIONAL GROUP
1	2997	N-H stretching
2	516.92	C-H bending
3	700.16	C-H bending
4	948.98	C=C bending
5	1026.13	C-H bending
6	1313,52	C-N stretching
7	1996.32	C=C=C stretching
8	2914.44	N-H stretching

Table-4 shows the peak value of functional group for *Tages erecta*

Terminalia arjuna

The graph and table indicate the values of Fourier Transform Infrared Spectroscopy for *Terminalia arjuna* in the peak range 698.23 and functional group C-I stretching, 950.91 - C=C bending, 10299.99- C-O stretching, 1311.59- C-O stretching, 1741.71- C=O stretching, 2993.52- N-H stretching and 3739.97- O-H stretching.

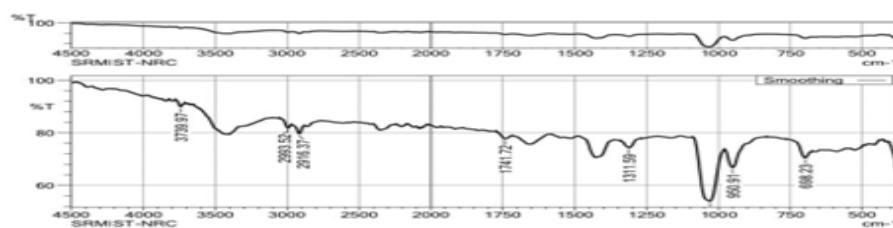


SNO	PEAK VALUE	FUNCTIONAL GROUP
1	698.23	C-I stretching
2	950.91	C=C bending
3	10299.99	C-O stretching
4	1311.59	C-O stretching
5	1741.71	C=O stretching
6	2993.52	N-H stretching
7	3739.97	O-H stretching

Table -5 represents the value of Fourier Transform Infra-Red Spectroscopy for *Terminalia arjuna*.

Chrysopogon zizanoides

Both the graph and table indicate the value of the Fourier Transform Infrared Spectroscopy for *Tages erecta* in the peak value and functional group of the plant 381.91-C-H bending, 516.92-C-H bending, 948.98- C=C bending, 1026.13-C-O stretching, 1313.52- C-N stretching, 1996.32-C=C=C stretching and 2914.44-N-H stretching.



S.NO	PEAK VALUE	FUNCTIONAL GROUP
1	381.91	C-H bending
2	516.92	C-H bending
3	948.98	C=C bending
4	1026.13	C-O stretching
5	1313.52	C-N stretching
6	1996.32	C=C=C stretching
7	2914.44	N-H stretching

Table -6 represents the value of Fourier Transform Infra-Red Spectroscopy for *Vetriveria zizanoides*.

1.5 GROWTH OPTIMIZATION OF ISOLATED ORGANISM

1.5.1 EFFECT OF TEMPERATURE:

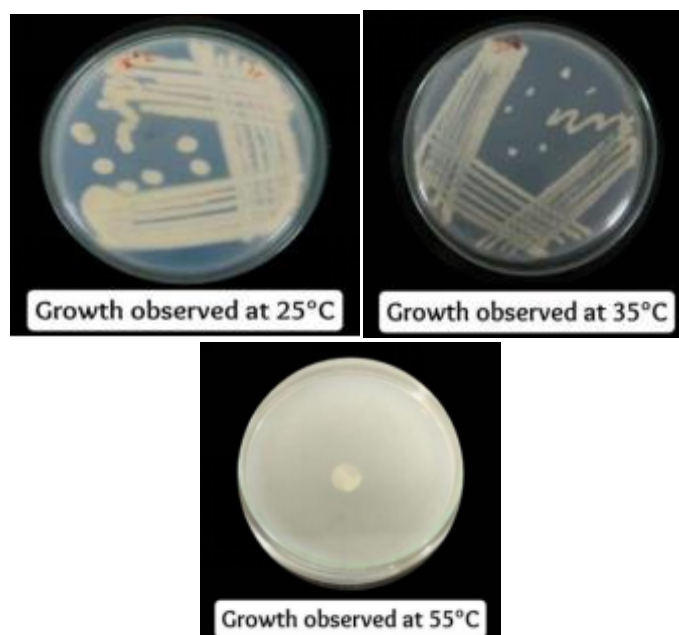


Figure 9- shows the optimization of temperature for isolates organism

- a) Maximum growth was observed at 25°C
- b) Poor growth at 35°C
- c) No growth at 55°C

1.5.2 EFFECT OF PH: (Maximum growth observed at pH 7)

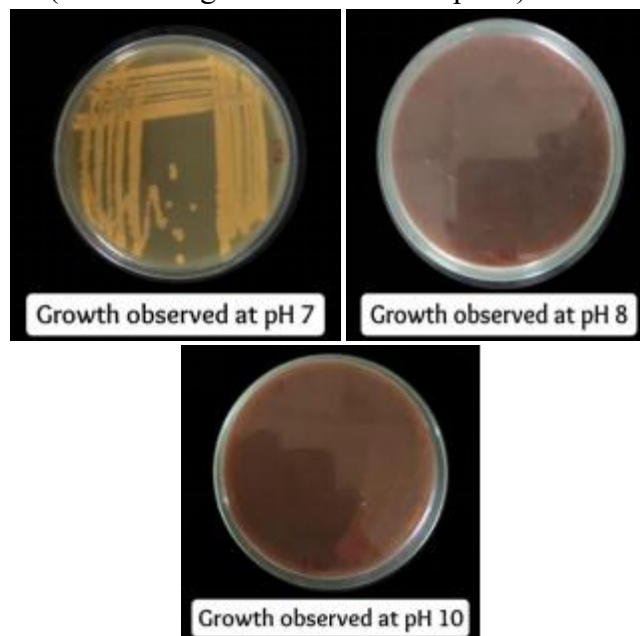


Figure 9- shows the optimization of pH for isolates organism

- a) Maximum growth was observed at pH of 7
- b) No growth at pH of 8
- c) No growth at pH of 10

1.7 Antifungal assay

Evaluation of antifungal potential of the selected extracts

prepared in DMSO from the resultant extract to determine their antifungal activity. Experimental controls were carried out using DMSO at concentrations that corresponded to those utilized for testing the extract. Isolates from *Trichosporon* were inoculated by swabbing the surface of gelled MHA plates. Wells of 8mm in diameter were performed in the MHA media, and each well was filled with various concentrations of extract ranging from (25, 50, 75, 100). The plates were kept under laminar air flow for 30 min for proper diffusion of the extract and then incubated at 37°C for 3-5 days. The radius for the zone of inhibition was in millimetres and recorded against the corresponding concentration.

Table 7 indicates the zone of inhibition of three different plants extract with different concentration against *Trichosporon asahii*

PLANT EXTRACTS (concentration)	25µl	50µl	75µl	100µl
<i>Tages erecta</i> (Zone of inhibition) (mm)	17mm	20mm	25mm	27mm
<i>Terminalia arjuna</i> (Zone of inhibition) (mm)	13mm	17mm	18mm	20mm
<i>Chrysopogen zizanoides</i> (Zone of inhibition) (mm)	14mm	16mm	17mm	18mm

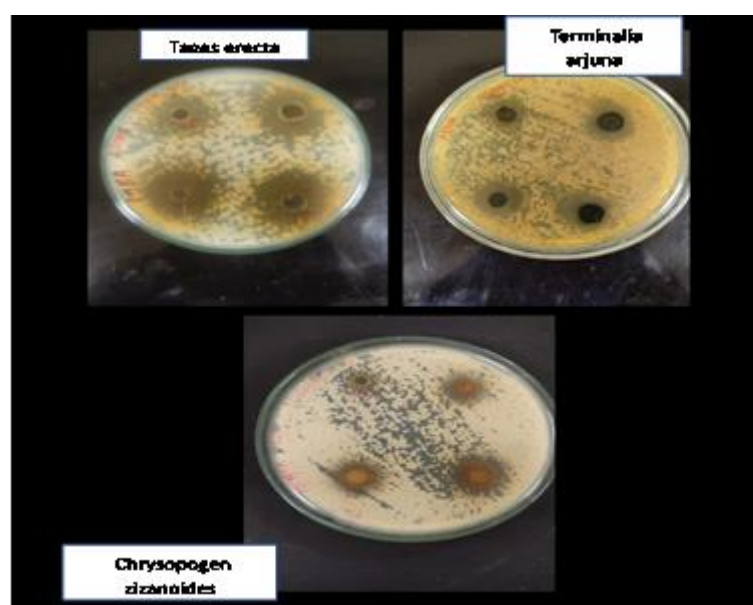


Figure 10- indicates the results for agar well diffusion for three different medicinal plant extract against *Trichosporon asahii*

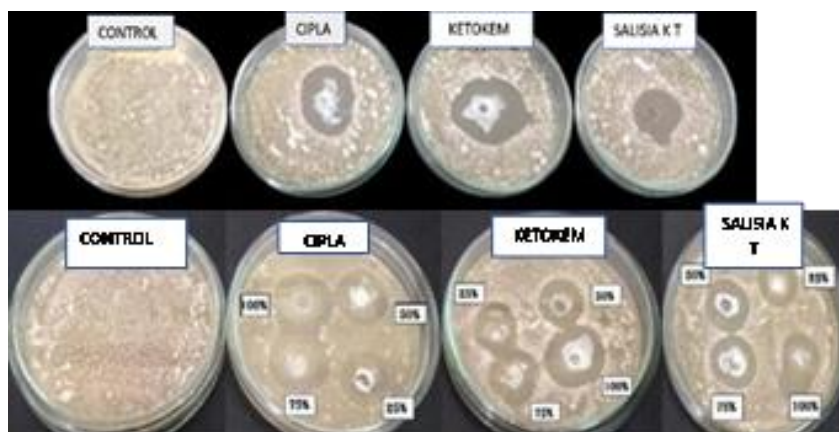


Figure 10- indicates the results for agar well diffusion for three different commercial against *Trichosporon asahii*

NAME OF THE SHAMPOOS	ZONE OF INHIBITION (mm)
CIPLA	18mm
KETOEM	18mm
SALISIA KT	19mm

Table 8 indicates the zone of inhibition of three different commercial antifungal shampoos against *Trichosporon asahii*

Conclusion

The study suggests that *Tages erecta*, *Terminalia arjuna* and *Chrysopogon zizanoides* plant extract can treat Invasive Trichosporonosis in patients suffering from the disease. The study also suggests the use of plants in treating Trichosporon infections in India, where no reports of medicinal plants against Trichosporonosis have been published. *ages erecta*, *Terminalia arjuna* and *Chrysopogon zizanoides* is a medicinal plant previously used to treat various infections and can now be used to treat Trichosporonosis.

Acknowledgments

The authors express their gratitude to the authorities of Department of Microbiology, SRM Arts and Science College, Kattakulathur, Tamil Nadu for valuble suggesstion and guidance during this research work.

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