5. DISCUSSION

Dandruff is a dermatological condition / disease which don’t have serious threat to life but can lead to embarrassing situations and negative social image. Tendency to relapse & chronic character of this condition relapse makes difficult to cope situation. Dandruff seems grayish white flakes visible on skin or hair and shoulders. Severity varies from person to person. Study highlights on testing of aqueous solvents rather than organic against the M.furfur thus promoting green pharmacy. our aim of treatment was to reduce level of M.furfur, and goal was to minimize the side effect caused by marketed anti dandruff shampoo with synthetic base. At the same time It cannot be denied that synthetic shampoo give instant shine, luture some time helps to remove dandruff after first wash also but the control and clinical cure are not completely addressed and reoccurrence is noticed, and hair becomes dry after prolong use together with many other scalp problems. The objective of present study was to prepare pure herbal formulation by screening the drugs on the basis of their antifungal and antibacterial activities from frequently sold herbal antidandruff products through market survey and literature review.

Many solutions are presently available but most of them offer strong chemical agents like ketoconazole, selenium sulphide, Tar, Tar etc, not only concern chemicals but also the excipients like preservatives within products have carcinogenic effects and other serious issues. The main causative agent responsible for dandruff is M.furfur which feeds on sebum and it causes tissue damage & dermal infections, with the release of free fatty acids. To get rid of dandruff several solutions are available in the market in different forms like creams, shampoo, oil. Synthetic solutions give instant results or worsen the problems due to serious side effects associated with chemicals.

Twenty six drugs were identified on the basis of their repeated use in different herbal formulations. They are Glycerrhiza glabra, Psoralea corylifolia, Terminalia bellirica, Curcuma longa Pterocarpus santalinus, Azadirachta indica, Rosa indica, Centella asiatica, Curcuma amada, Emblica officinalis, Withania somnifera, Spinindius trifolatus, Acacia concinna, Indigofera
Discussion

The microorganism selected for the study were S.aureus, E.coli, S.aureus MRSA, K.pneumonia and C. albicans and M.furfur. The aqueous and methanolic extracts were tested for anti-microbial activity against selected skin pathogens by Agar well method. The results indicated that out of 26 extracts 11 extracts possess significant antibacterial and antifungal activity. Following extracts were active against S.aureus Psoralea corylifolia , Terminalia bellirica ,Withania somnifera , Pterocarpus santalinus , Acacia concina ,Neel, Citrus acidica, Terminalia chebula , Azadirachta indica. In case of E.coli & K.pneumonia zone of inhibition was observed in Pterocarpus santalinus , Acacia concina, Terminalia chebula , Azadirachta indica. Maximum extracts exhibited antibacterial activity against S.aureus MRSA, which itself is antibiotic resistant. Positive antifungal activity was seen in strains of M.furfur and C.albicans by Psoralea corylifolia, Terminalia bellirica , Pterocarpus santalinus , Spindius trifolatus, Acacia concina , Hibiscus rosa-sinensis, Lawsonia innermis, Terminalia chebula , Citrus acidica .Lawsonia innermis & Spindius trifolatus posses no activity in C.albicans. Positive extracts were lesser than standard used. No inhibition was observed in DMSO and dextrin.

The sample collected through scrapping was identified and compared with standard ATCC strains of Malassezia by morphologically study; M.furfur cells are oval or spherical with budding with narrow neck with thick cell walls. Malassezia furfur was confirmed by the presence of short, blue-staining hyphen and spherical spores with CBS stain and KOH with direct microscopy. It grows rapidly, matures within 5 days at temperature between 30 -37 °C, budding leaves a prominent scar on mother cell through enteroblastic asexual reproduction. The M.furfur on CHROM agar media produce characteristically large pale pink and wrinkled colonies was easily distinguishable. They also have distinct physiological property in that lipid can be utilized as source of carbon. Positive results with evolution of bubbles were found in catalaze chain reaction. Darkening of the agar media by Splitting of esculin was observed.
which again confirms the presence of M.furfur. It is purely lipid dependent and
does not grow well on SGA. Maximum growth was seen on SDA with
compared butter with different lipid supplement like coconut oil, olive oil and,
til oil. Pigment induction and tween assimilation and scheme for identification
followed also confirms the presence of M.furfur. In the screening of media
Potato Dextrose Medium, CHROMagar medium, SDA and Dixon agar showed
excellent growth. Sub culturing was done on Leeming and Notmans agar &
Dixon agar. Antifungal study was carried on Candida albicans and Malassezia
furfur by agar well method. Psoralea corylifolia, Terminalia bellerica,
Pterocarpus santalinus, Spindius trifolatus, Hibiscus rosa-sinensis, Lawsonia
innermis, Terminalia bellirica were potent against C.albicans also Psoralea
corylifolia, Terminalia bellerica, Pterocarpus santalinus, Spindius trifolatus
Acacia concinna, Hibiscus rosa-sinensis, Lawsonia innermis, Terminalia
chebula, Citrus acidic inhibited the M.furfur colonies. The highest zone in
bacterial and fungal strains was observed in Pterocarpus santalinus,
Terminalia bellirica and Terminalia chebula. The extracts with promising
antimicrobial activity (with zone of inhibition > 10 mm) was subjected for the
minimum inhibitory concentration assay to find out the lowest concentration of
extract that inhibits the growth of the test organisms. MIC assay was
performed using the crude extracts with concentration of 10 to 500 μl/ml. After
incubation at 37°C for 48 hrs, the dilution showing highest microbial growth
inhibition was recorded as Minimum Inhibitory Concentration (MIC)
respectively for each pathogen. Standard used were Amikacin 30 mg,
Gentamicin 10 mg for antibacterial activity and zone size observed was more
than 24 mm. Ketoconazole 10 mg was used as standard for antifungal activity
and zone size observed was more than 28 mm.

Formulation was done using factorial design i.e Optimization Design (23
Factorial Design). Response Surface Plot Analysis (3D) on all the test species
was observed. Less variation was observed between predicted and observed
batch percentage biased was calculated Desirability study responses was
studied and was less than one. Medicinal plants used in the formulation of
herbal antidandruff hair pack were found as folk used as antifungal and
antibacterial preparation. The plants used were Terminalia chebula,
Terminalia bellerica, Pterocarpus santalinus wood and Sapindus trifolatus (as base).

Photochemical analysis confirm the presence of alkaloids, glycosides, tannins, phenols, carbohydrates, saponins and flavonoids. The satisfactory result of present investigation were fetched out of number of physical and chemical parameters. The product prepared is in dry form having wonderful wetting capacity and being dry is very good for the storage. The evaluation parameters like Organoleptic evaluation, General powder Characters, Physicochemical Evaluation, Cleaning action, foaming, Dirt dispersion, Wetting agent, Nature of hair after wash was carried out and was found to be within the standard range An excellent Metheylene blue result was observed with dead cells staining. The results of TLC, HPLC and HPTLC revealed the presence of Gallic acid in the prepared formulation. Pesticide residue and heavy metals were absent in the Prepared formulation except Cadmium which was present in traces and was in limit. Efficacy of the formulation was evaluated with agar well and disc diffusion method. Better zones were observed in agar well method. Prepared formulation was compared with synthetic and herbal antidandruff marketed preparations comparable zone with herbal preparation was observed. Synthetic shampoo zone of inhibition containing ketoconazole was highest. No eye irritation was absent with no chemicals. Stability studies at 40°C ± 2% / 75% RH ± 5% found Satisfactory with no change in the formulation. All the parameters evaluated and prepared formulation was particularly effective against dandruff at low concentration.