Summary and Conclusion
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Dandruff of the scalp is a very common problem in aesthetic dermatology. Although most commonly it is a problem of just aesthetic nature but, chronic character of this condition and tendency to relapse makes it troublesome and difficult to cope for people suffering from this condition. The yeast *Malassezia furfur* is the main causative organism of dandruff and due to its lipase activity it releases proinflammatory free fatty acids, causing dermal inflammation and tissue damage. Other secondary causes of dandruff are increased oil production, oily skin, increased skin secretions, and increased numbers of normal skin yeasts. Dandruff may also be triggered by poor hygiene, and infrequent shampooing and washing. The immune system may play a part in dandruff. Although mild dandruff is a very common condition in many people with a normal immune system, severe dandruff is generally more common in people with chronic illnesses or a compromised immune system like advanced HIV/AIDS and Parkinson’s disease. Common triggers of dandruff include increased oil production, oily scalp, sweating, poor hygiene, weather, infrequent washing or shampooing, fungus, poor immune, chronic illness and emotional or mental stress. Two types of dandruff can occur Dry (common) and Oily dandruff. Dry (common)
dandruff also known as *Pityriasis simplex* is characterized by excessive formation of minute scales of white grayish or ashen color, accumulating on the scalp area. The other type of dandruff is called oily dandruff or *Pityriasis steatoides*. It arises on the scalp skin with varied intensity of sebum production. It appears most often in young men following puberty (aged between 18 and 24). Inflammation of varied intensity develops on the scalp skin along with the appearance of oily scales of dirty yellow colour that can form lesions.

Different types of formulations like shampoos, creams, lotions, emulsions, hair oils and other cosmetic formulations are available in the markets that are used to control dandruff. Among all, antidandruff shampoos are most popular.

A shampoo may be described as a cosmetic preparation meant for washing hair and scalp, packed in a form convenient for use. Its primary function is of cleansing the hair of accumulated sebum, scalp debris and residues of hair grooming preparations. The added functions of shampoo include lubrication, conditioning, bodybuilding, prevention of static charge build up, medication and so on. Finally, the complete shampoo formulation must be medically safe for long-term usage. Now a days washing hair and scalp with shampoo has become a nearly universal practice.

Antidandruff shampoos are probably the most widely used hair products today to get rid of dandruff. These antidandruff shampoo formulations include therapeutic use of antidandruff agents that are classified into three groups according to their mechanism of action.

**Fungicidal Substances** – For example, zinc pyrithione, ketoconazole, flutrimazole, econazole, bifonazole, clotrimazole etc.

**Cytostatic Substances** – For example, selenium sulphide, tar, piroctolamine, octopirox, cyclopiroxolamine etc.

**Keratolytic Substances** – For eg. salicylic acid, urea and sulphur derivatives.

Antidandruff products containing these agents work only symptomatically and often recurrence of dandruff after stopping treatment is observed, which is the most common problem. Moreover, side effects associated with them cannot be neglected. Such complications have prompted the search for noble and effective antifungal agents of natural origin. Natural remedies have been used for centuries for treating skin and hair
conditions and a wide variety of dermatological disorders including inflammation, phototoxicity, psoriasis, atopic dermatitis, dandruff and alopecia areata. In the management of dandruff plant derived drugs and their formulations are viable alternative to synthetic drugs. In traditional Indian System of Medicine many plants and herbal formulations are reported to possess antidandruff property. Some of the plants which possess antidandruff property are *Tridax procumbens*, *Azadirachta indica*, *Trigonella foneum graecum*, *Melaleuca alternifolia*, *Lawsonia inermis*, *Quillaja saponaria*, *Rosemarinus officinalis*, *Citrus limon*, *Sapindus indica* etc.

The potential of essential oils as antimicrobial agents is well established. Essential oils are mixtures of volatile secondary metabolites isolated from different parts of aromatic plants. Based on these facts, the present study emphasized the importance of plants essential oils as an alternative antimalassezia agent against pathogenic fungi causing dandruff. The aim of this study was to evaluate the essential oil of cinnamon, cajeput, kapur tulsi, eucalyptus and a fixed oil obtained from karanj seeds, against a pathogenic fungi *Malassezia furfur* and develop a new antidandruff shampoo formulation based on validated antimicrobial activity of selected oils alone or in combination and evaluation of the developed shampoo formulation by methods prescribed by Bureau of Indian standards and also by various official and non official methods.

For the present study plants were selected on the basis of their ethno-medicinal importance and literature survey. The essential oils for the study were extracted from leaves of *Melaleuca leucadendron* (cajeput oil), *Eucalyptus globulus* (eucalyptus oil) and *Ocimum kilimandscharicum* (kapur tulsi oil), bark of *Cinnamomum zeylanicum* (cinnamon oil) by hydrodistillation process using Clevenger apparatus. And a fixed oil from seeds of *Pongamia glabra* (karanj oil) was isolated by expression method.

The antifungal activity of cinnamon oil, eucalyptus oil, kapur tulsi oil, cajeput oil and karanj oil was evaluated against *Malassezia furfur* by Disc diffusion method and compared with the antifungal activity of standard antifungal agents tea tree oil and ketoconazole. Among the five selected oils only three essential oils exhibited antifungal activity against the fungus with MIC values ranging from 32 to 128 µg/ml. The bark oil of cinnamon showed the highest activity with 14.0 mm ZOI and 32 µg/ml MIC against the fungus and
other oils showing good inhibition were the leaf oil of cajeput with 12.0 mm ZOI and 64 μg/ml MIC followed by leaf oil of kapur tulsi with 8.0 mm ZOI and 128 μg/ml MIC. Although cinnamon oil was found to possess best antifungal activity among the test oils but, it was lesser than the activity shown by the standard tea tree oil with 17.5 mm ZOI and 32 μg/ml MIC and ketoconazole with 22.0 mm ZOI and 16 μg/ml MIC.

Some studies have concluded that combinations of essential oils have greater antimicrobial activity than their individual components and studies also suggested that the components of essential oils are responsible for providing synergistic or potentiating effects probably. Hence cinnamon oil in combination with cajeput oil and kapur tulsi oil in ratio 1:1 was evaluated against *M. furfur*. In an investigation of different combinations, it was noted that both cajeput oil as well as kapur tulsi oil were showing good synergism against the *M. furfur* when combined with the cinnamon oil. But most of the antimicrobial activity appeared to be associated with the combination of cinnamon oil and kapur tulsi oil with 23.3 mm ZOI and 4 μg/ml MIC. These two oils in combination were more effective against the fungus *M. furfur* than when they were used separately and the combination was showing better activity than the activity shown by standard tea tree oil and ketoconazole alone. The MIC of the combinations got significantly reduced to 4 µg/ml from 32-128 µg/ml of individual oils.

The antimicrobial activity of cinnamon oil and kapur tulsi oil could be associated with cinnamaldehyde and camphor as these are the main components of respective oils. These components have been shown to have antimicrobial activities against common laboratory target strains in other studies. Their mechanism of action has most often been attributed to the disturbance of microbial membranes, disrupting the proton motive force, electron flow, and active transport and resulting in the coagulation of intracellular contents.

TLC fingerprint profile of cinnamon oil and kapur tulsi oil was studied under UV at 254 nm and 366 nm. Rf values of different components (bands developed on TLC plate) were determined.

The cinnamon oil and kapur tulsi oil were further subjected to Gas chromatography Mass spectroscopy (GC-MS) analysis to study their chemical composition. Total eight components of cinnamon oil and eight components of kapur tulsi oil were identified. The
The major component of cinnamon oil was found to be cinnamaldehyde (91.82%). Minor compounds were found to be 1-8 cineole, α-murolene, selinene, geraniol, E-cinnamic acid, vinyl trans-cinnamate. The major component of kapur tulsi oil was found to be camphor (46.14%). Other components were found to be eugenol, 1-8 cineole, limonene, α-pinene, camphene, beta-myrecene and α-terpineol.

On basis of the antifungal studies of five different oils against *Malassezia furfur*, the combination of cinnamon oil and kapur tulsi oil, being most potent was selected as main active ingredient for the formulation of an antidandruff shampoo. Appropriate base for the formulation of an antidandruff shampoo was selected. Three different types of shampoo bases – liquid cream base (B1), clear liquid base (B2) and coconut oil base (B3) were formulated and evaluated for stability. Base B2 was found to be most stable as compare to B1 and B3 in terms of physical stability like colour, odour, viscosity, phase separation, foam height/foam stability. Hence B2 was selected as base for the formulation of an antidandruff shampoo.

An antidandruff shampoo formulation was formulated by incorporating selected active ingredients in selected base by adopting proper procedure. The shampoo formulation so prepared was coded as TS.

Selecting the evaluation parameter of a shampoo was a challenging task, simply because of the multitude of both subjective and instrumental test methodologies available for this purpose. The present study was focussed on the evaluation of herbal shampoo using the simple, rapid and reproducible parameters. The formulated antidandruff shampoo was evaluated by methods prescribed by Bureau of Indian Standards for surfactant based shampoos, other official and unofficial methods. The formulated antidandruff shampoo was also evaluated for ideal properties that a shampoo must possess. The performance of formulated shampoo was compared with popular commercial shampoos. The results of these tests gave an idea about what standards should be met by a shampoo formulated in the laboratory as well as for commercial purpose.

The formulated shampoo was found to possess all the ideal properties that any good shampoo must possess. The shampoo was viscous enough to stay in the hand before application to the hair and scalp, yet during application the shampoo spreaded easily and
dispersed quickly over the head and hair. The shampoo developed a dense and luxurious lather. The shampoo was found to remove dust, soil, and sebum from the hair. It was rinsed out easily and did not leave a residual tackiness and stickiness. After rinsing, the hair combed through easily without tangling. There was no flyaway or frizziness observed in hair when combed dry. The hair were left in a lustrous condition, did not limp or over conditioned and shampoo left a clean and natural residual scent of kapur tulsi oil on the hair. The shampoo formulation was as simple as possible, it was not “over formulated”. Only those ingredients, which were necessary to accomplish the desired goal, were incorporated in the formulation.

Bureau of Indian Standards has given the permissible limit of non-volatile alcohol soluble matter in shampoo between the range 10 to 20 %. It was found that TS contain 16.32% of non-volatile alcohol soluble matter in it, which was in the permissible limit laid by BIS. In other marketed shampoos also the non-volatile alcohol soluble matter was found to be in the permissible limits.

The pH of TS and marketed shampoos was determined. The pH of TS and marketed shampoos MS1, MS2, MS3, MS4 and MS6 was found to be on slightly acidic side. It was desirable also as mild acidity prevents swelling and promotes tightening of the scales, thereby inducing sheen. Only shampoo MS5 showed a higher pH, where as all other shampoos were found to be acid balanced.

The TS and marketed shampoos were evaluated for determination of foam height and foam stability in distilled water, hard water and in distilled water with artificial sebum. It was found that the TS showed good foaming characteristics in distilled water as well as hard water and in the presence of artificial sebum. Among marketed shampoos, MS1, MS2, MS3 and MS6 foamed well in distilled water, hard water and in presence of sebum. However, MS4 and MS5 did not foam well in presence of sebum. But their performance was good in distilled water and hard water.

The results of detergency evaluation showed that shampoos TS, MS5 and MS6 possessed maximum detergency, so they were regarded as active cleansers, suitable for oily hair. The shampoos MS2 and MS4 showed moderate detergency, so they were regarded as good
cleanser suitable for oily as well as normal hair whereas MS1 and MS3 showed minimum detergency, so they were regarded as mild cleanser suitable for normal hair.

The wetting time of TS was evaluated and compared with the wetting time of marketed shampoos. From the results, it was observed that shampoos TS, MS5 and MS6 showed minimum wetting time and possessed maximum detergency. By contrast, MS1 showed minimum detergency and maximum wetting time, as literature revealed that wetting time of shampoo is inversely proportional to the detergency.

TS and marketed shampoos were also evaluated for reduction in surface tension of water. The shampoo TS and all the marketed shampoos showed reduction in surface tension of water with in prescribed limits, indicating good detergent action. TS reduced the surface tension of water from 72.8 dynes cm\(^{-1}\) to 33.4 dynes cm\(^{-1}\) (within prescribed limits).

Viscosity profile of TS and marketed shampoos was studied. The results indicated that the shampoos were showing pseudoplastic behaviour, which is a desirable attribute in a shampoo formulation. At a low rpm, TS and all the marketed shampoos showed high viscosity. On increasing the shear rate, the viscosity dropped, which would allow ease of spreading onto the hair.

The conditioning effect of the TS was evaluated by Scanning Electron Microscopy. The photomicrograph of hair treated with TS indicated that, the conditioning agent used in the shampoo formulation performed well. The scales were seemed to lay flat one over the other and no cuticle uplift was observed. Shampoo MS6 was also found to possess good conditioning effects while MS1 was proved as shampoo with mild conditioning effects.

The results of these evaluation parameters gave us the range of values for each test within which a shampoo formulation should fall, in order to compete with the already established shampoo brands in the market.

Shampoos are now used extensively all over the world. Safety in toiletries and cosmetics is of prime importance. It is highly important that a shampoo formulation must be non-irritant to eyes and skin while using. So the safety evaluation of the shampoo formulation was done by methods prescribed by Bureau of Indian Standards in IS 4011:1997. Safety evaluation was done by two methods: Draize eye irritation test and Draize skin irritation test on rabbits.
Draize eye irritation test was performed on a set of 12 New Zealand white rabbits, divided into two groups (G1 and G2) with six rabbits in each group. 0.1 ml of 1% antidandruff shampoo formulation in distilled water was instilled into the left eye of each animal. The treated eye of animals of G1 group were washed with 20 ml of lukewarm water, 2 seconds after instillation of the antidandruff shampoo formulation and for animals of G2 group, treated eye of each animal was washed 4 seconds after instillation of the antidandruff shampoo formulation. The right eye of each animal of both the groups remained untreated and served as reference control. The animals were observed for clinical signs of toxicity and mortality. The treated eye of each animal was examined and eye irritation scores were given according to the Draize numerical scoring system. Body weights were recorded on day 1 and on day 7. There were no treatment related adverse effects observed in the body weights of all the animals. And the animals did not reveal any lesions up to 7th day. Hence the experiment was terminated on 8th day of observation. Based on the results obtained from the present study the antidandruff shampoo formulation TS was considered as non-irritant to the eyes and safe for use.

Draize skin irritation test was performed in set of six New Zealand white rabbits. Approximately 24 hours before the treatment initiation, hair was carefully removed from the dorsal area of the trunk of all the test animals by clipping without abrading the skin. On test day, two areas of the back, spaced approximately 2-3 cm apart were designated for the position of the patches. 0.5 ml of 10% antidandruff shampoo formulation in distilled water was applied at posterior patch and 20% sodium lauryl sulphate (positive control) was applied at anterior patch simultaneously to each rabbit. The test patch were held in their respective positions with non-irritating adhesive tape and wrapping the whole trunk with a cotton bandage. The patch of shampoo formulation and positive control were kept in contact with skin for 4 hours. At the end of contact period, the protective covering and patch were removed and applied area was wiped using distilled water, dried with tissue paper and local skin reactions were recorded. Body weights were recorded on day 1 and day 3. There were no treatment related adverse effects noted on the body weights of all the animals. The animals were observed for clinical signs of toxicity and mortality. The test patches were removed after 4 hr of application of the test patch and the skin sites were scored at post removal (0 hr) of the test patch and at 48 hr later, using Draize dermal toxicity scoring scale. The animals did not reveal any treatment related lesions with 10%
antidandruff shampoo formulation up to 3\textsuperscript{rd} day. Hence the experiment was terminated on 3\textsuperscript{rd} day of the observation period and the shampoo formulation was considered as safe for skin.

The objective of the Draize eye irritation test and Draize skin irritation test in rabbits was to assess the possible eye irritation and skin irritation effects likely to arise from exposure of the skin to the shampoo formulation. This study had provided a rational basis of risk assessment in man.

Desired stability during shelf life is one of the most important steps in shampoo formulation. The stability of antidandruff shampoo formulation was checked after six months of its formulation. The formulation was inspected for physical and chemical stability. To check the physical stability of shampoo formulation, it was evaluated for organoleptic properties, foam production, detergency, pH and viscosity, after six months of its formulation and compared with the initial values of the same. The shampoo formulation was found to be stable during its storage period. To check the chemical stability, TLC profile of fresh shampoo formulation was compared with the TLC profile of after six months of its formulation. The TLC profile of both was found to be same. Further, TLC densitometric analysis of the shampoo formulation before and after six months confirmed its chemical stability.

Finally, the formulated antidandruff shampoo (TS) was evaluated for the antifungal activity against \textit{M. furfur} by Disc diffusion method. The Minimum Inhibitory Concentration (MIC) of TS was also determined. The zone of inhibition and MIC shown by TS were compared with the antifungal activity shown by marketed antidandruff shampoo MS\textsubscript{1} containing ketoconazole and marketed herbal antidandruff shampoo MS\textsubscript{2} containing essential oil of \textit{Melaleuca alternifolia} and \textit{Rosmarinus officinalis}. The formulated antidandruff shampoo showed ZOI less than marketed shampoo MS\textsubscript{1} with ketoconazole but better than the marketed shampoo MS\textsubscript{2} containing herbal ingredients. The difference in antifungal activity shown by TS and MS\textsubscript{1} was not significant. The TS was found to be effective against the fungus \textit{M. furfur}.

\textbf{CONCLUSION}
Dandruff induced by *Malassezia furfur* represents a fastidious cutaneous problem because of aesthetic problems. Its treatment is not a simple issue. Regardless of the existence and widespread use of numerous therapeutic substances, disease relapses are common. The prolonged chemical based treatments and the high rate of recurrence suggest the opportunity of alternative treatment. So, in this study selected essential oils were screened for antifungal activity against *M. furfur*. In conclusion, the present investigation demonstrated that binary mixture of cinnamon oil and kapur tulsi oil showed excellent antifungal activity. An antidandruff shampoo was formulated by incorporating cinnamon oil and kapur tulsi oil as main antidandruff ingredients. As additives play an important role in defining the performance, stability and aesthetic appeal of any formulation, this point was kept in mind while selecting the additives for the shampoo formulation. Finally the antidandruff shampoo was formulated and evaluated. As seen from the results, it is possible to formulate a herbal antidandruff shampoo that is not only equivalent in its performance to the synthetic ones but also have better safety, efficacy and purity.