Summary
During late 19th and early 20th centuries, microbes were not considered as etiological agents of infections. In the later part of 20th century numerous studies have revealed the severity of infectious diseases caused by bacterial and fungal pathogens. Developing countries including India have suffered more due to these infectious diseases and still these countries stand apart in disease management compared to the developed nations. The dermatological pathogens such as *P. acnes* and *Malassezia* spp. are afflicting a significant proportion of human population. Although they are recognised as dermatological pathogens, they have proved their selves as serious pathogens with the potential to cause fatal diseases. Lack of knowledge, inaccurate diagnosis, abuse and overuse of drugs, self-medication and negligence are few reasons which paved the way for these infectious diseases as life threatening ones.

Though antimicrobials are available today to control and cure the infections caused by *P. acnes* and *Malassezia* spp., the need for novel drugs to combat drug resistance and recalcitrance in a long run is huge. In this era, the reports of antimicrobial treatment failures are emerging a lot. The biofilm formation, CSH and lipase production are the major predicaments that aid directly or indirectly in the transformation of an infection to a life threatening disease. Therefore the interference of aforesaid virulence phenotypes will be an attractive as well as handy strategy to control *P. acnes* and *Malassezia* spp. associated diseases. Hence the novel antimicrobial agents against which the pathogen is not yet exposed and antipathogenic agents that quench only virulence factors without the peril of resistance are highly needed. Medicinal plants and phytochemicals have been of interest for centuries as they are easily available, renewable, non-toxic and cost effective. The plant extracts and phytochemicals have also been reported as promising candidates for combinatorial drug therapy. They can be more efficacious as combinations and can be used to potentiate the existing approved drugs. With this backdrop, medicinal plants and phytochemicals were
explored for the identification of novel antimicrobial, antibiofilm and antipathogenic agents as potential alternatives for antimicrobials to combat infections caused by *P. acnes* and *Malassezia* spp.

The present study is focused on screening Indian medicinal plants with antibacterial activity against *P. acnes*. Upon screening 40 extracts from 10 medicinal plants, *C. aromatica* methanolic extract has shown profound activity at 200 µg mL⁻¹ (MIC). The column chromatography and thin layer chromatography were performed for partial purification of *C. aromatica* methanolic extract and resulted in *C. aromatica* oil with a MIC of 52 µg mL⁻¹. The GC-MS analysis revealed the chemical composition of *C. aromatica* oil, majorly a molecule called “Curcumene”, one of the signature phytochemicals of *Curcuma* family. In parallel, a total of 17 phytochemicals were tested with the aim of developing a bioactive combination. Upon screening, undecanoic acid was found with significant growth inhibitory potential having a MIC of 400 µg mL⁻¹. To determine the interaction between *C. aromatica* oil and undecanoic acid, checker board assay was performed. Out of 30 combinations, 12.5 µg mL⁻¹ of *C. aromatica* oil + 100 µg mL⁻¹ of undecanoic acid and 12.5 µg mL⁻¹ of *C. aromatica* oil + 50 µg mL⁻¹ of undecanoic acid were identified as synergistic combinations with FIC indices of 0.5 & 0.375 respectively. Additionally, the antibacterial potential of the synergistic combinations was validated using 18 *P. acnes* clinical isolates. Thus the antibacterial synergistic combinations comprising of *C. aromatica* oil and undecanoic acid are envisaged as promising therapeutic agent against *P. acnes* associated infections. In furtherance, it can be a prospective prophylactic and / or curative anti - acne skin care formulations such as Cream/ Lotion/ Gel/ Soap suitable for topical application to human skin.

*P. acnes* is a notorious opportunistic pathogen by its ability to form recalcitrant biofilm and to develop drug resistance. In the current study, it was also aimed to develop antibiofilm agent against clinical isolates of *P. acnes* under *in vitro* and *in vivo* conditions.
On that prospect, a total of 15 phytochemicals were tested individually and as combination with non antimicrobial concentration of tetracycline. Upon screening 15 phytochemicals and their combinations with tetracycline, the combination of ellagic acid and tetracycline [ETC (250 µg mL\(^{-1}\)+0.312 µg mL\(^{-1}\))] was identified with effective inhibition of \(P.\) \(acnes\) biofilm development (80-91%). This combination exhibited biofilm inhibition without affecting its growth and therefore it could potentially limit the possibility of the bacterium attaining resistance. Furthermore, ETC has also reduced EPS production of about 20-26% and thereby making \(P.\) \(acnes\) more susceptible to the host immune system and antibiotics. In addition, the antibiofilm potential of ETC was further substantiated under \textit{in vivo} condition using \(C.\) \textit{elegans}. This study reports a novel antibiofilm combination for the first time that could be developed as an ideal therapeutic agent with broad cosmeceutical and pharmaceutical applicability in the era of antibiotic resistance.

In the recent era, the genus \textit{Malassezia} has been recognized as one of the prominent fungal opportunistic pathogens in medical microbiology and dermatology by causing recurrent and recalcitrant infection. As they are lipophilic in nature, it is obvious that they do possess high level of CSH. Upon screening twelve phytochemicals, L- Glutathione (GSH) was found to have maximum degree of anti-hydrophobicity activity against \(M.\) \textit{furfur}. GSH is a ubiquitous antioxidant which offers protection against microbial infections. Consequently, the present study was intended to scrutinize the effect of GSH on CSH of \textit{Malassezia} spp. MATH assay was performed to assess the AHA of GSH against four \textit{Malassezia} spp. The assay revealed that GSH at 400µg mL\(^{-1}\) concentration inhibited CSH, ranging from 84 to 95% in \(M.\) \textit{furfur}, \(M.\) \textit{globosa}, \(M.\) \textit{restricta}, and \(M.\) \textit{sympodialis} without killing the cells. The autoaggregation assay and zeta-potential measurement was also carried out to further substantiate the AHA of GSH, through which delayed cell aggregation was observed due to reduction in CSH level and not by modification of cell surface charge. In
deference to AHA of GSH, CFU assay was performed in which 62 – 93% of CSH reduction was observed in *Malassezia* spp. Furthermore, it has been demonstrated that GSH treatment has enhanced the sensitivity of *Malassezia* spp. towards human blood at the rate of 64 – 72%. Comparative FTIR spectrum analysis was also performed to attest the AHA of GSH. Thus, the present study portrays GSH as a prospective therapeutic alternative for *Malassezia* mediated infections.

As the genus *Malassezia* comprises of extremely lipophilic yeasts, they generally secrete lipases as a vital factor for its survival. In the battle field against *Malassezia*, combinatorial therapy is envisaged as a constructive strategy that paves a path to combat infectious diseases. In that prospect, totally 16 Indian medicinal plants were screened and a maximum degree of growth inhibition was ascertained in *E. ribes*. Subsequently, comparative antimicrobial assay and FTIR analysis have revealed “embelin”, a signature phytochemical of the genus *Embelia*, as a bioactive principle with a MIC of 400 µg mL⁻¹ exhibiting ~75% of growth inhibition. Further, a fungistatic activity based on anti-lipase potential (65 – 89%) of embelin has been clearly substantiated by XTT and lipase assay. In addition, a synergistic action has been arrived at FIC index of 0.5 for a combination of embelin and KTZ. Therefore, the combinations of embelin and KTZ represent a promising therapeutic regimen to treat *Malassezia* infections with subjugated clinical and environmental toxicity. This is the first report delineating the anti-lipase activity of embelin and *in vitro* synergistic interaction between embelin and KTZ against *Malassezia* spp.

However, this study on the whole highlights the potential of medicinal plants and phytochemicals as a potential source for antagonistic agents that are effective against the human dermatological pathogens. The active principles of the present study such as Undecanoic acid, ellagic acid and embelin are categorised as Generally Recognized As Safe (GRAS). They are found in common medicinal plants which are routinely used in traditional
medicines since time immemorial. Hence, it is established that these phytochemicals do not possess any toxicity towards human skin. Further, previous reports explicate the usage of these phytochemicals for topical application (Hart et al., 1999; Kandimalla et al., 1999; Bae et al., 2010; Kumar et al., 2011; Hseu et al., 2012; Deshmukh & Gupta 2013). This study also unveils the combinatorial effect of natural derivatives which could be further formulated for clinical and cosmetic applicability. Further studies in cell lines and animal models are also indispensable to prove their efficacy in higher eukaryotes. The proteomic, transcriptomic and computational analysis are also expected to delineate the molecular mechanism of these antagonistic agents.