Anti-Psoriatic Activity of Indigofera Tinctoria Leaves Extract on Staphylococcus Aureus Embedded Hacat Cells: A Systematic Approach

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Abstract: *The present study aimed to examine the efficacy of traditionally used herbal plant, Indigofera tinctoria in Kolli hills of Tamil Nadu against bacterial pathogen responsible for psoriasis. The plant was phytochemically screened and crude extract was prepared using solid – liquid extraction process. Then the crude extract was initially checked for in-vitro antibacterial activity at concentration of 50 mg/ml against Staphylococcus aureus by agar well diffusion method. The extract showed better activity (31 – 33 mm of zone of inhibition) compare to standard antibiotic, Tetracycline. In addition, anti-psoriatic study was carried out on Staphylococcus aureus embedded HaCat Cell line by performing the Sulphorhodamine B (SRB) assay. Based on the results, percentage activity of Indigofera tinctoria extract showed maximum inhibition of about 58.95 % at 150 µg/ml. The extracts showed potent anti-proliferant activity in HaCaT cell lines with IC_{50} value of 68.75±14.80 µg/ml in cytotoxicity method which is comparable to the standard drug. Medicinal plants traditionally used against psoriasis are therapeutically active against group of bacterial pathogens. Indigofera tinctoria found to be a potential candidate species for the development of novel psoriasis drugs with low cost and fewer side effects.*

Key words : *Indigofera tinctoria, psoriasis, HaCaT cells, Staphylococcus aureus*

1. INTRODUCTION

Psoriasis is a genetically determined chronic inflammatory skin disease characterized by red, scaly and raised patches that affects 2.3% of the population worldwide [1]. It affects mainly knees, elbows and scalp. It is learned that heredity, stress, environment and immune system also plays an important role in psoriasis [2,3]. Psoriasis is an immune-mediated disease where activation of T lymphocytes is central to the inflammation in the dermal microenvironment and the epidermal hyper proliferation is secondary to the inflammatory events that track a Th1 type of immune response [4].

The worldwide incidence of psoriasis is assessed to be approximately 2–3%. Even though the disease is recognized to have higher prevalence in the polar regions of the world, its burden in a tropical/subtropical country like India cannot be undervalued. In a diverse country such as India, the prevalence of psoriasis may vary from region to region due to variable genetic and environmental factors. A higher prevalence in males has been reported with a peak age between 30 to 40 of life. In Northern India, point prevalence of pediatric psoriasis was assessed to be 0.0002%. The peak age at onset among boys is in the 6–10 years age group compared to girls in 11–15 years age group. A positive family history may be provoked in 9.8-28% of the children.

The usual human skin flora colonized by huge numbers of bacteria to live harmlessly as commensals on its surface and within its follicles. At time when over growth occurs, some of these resident organisms may cause minor disease of the skin or its appendages. Organisms not normally

considered as resident members of the skin flora may occasionally colonize and became established in modest numbers for relatively long periods, proliferate, and produce disease. Bacteria of this intermediate category have been labelled temporary residents [5] some of the most commonly resident aerobic flora consists of Gram-positive *Staphylococcus aureus, Staph. epidermidis, Corynebacterium diphtheriae* and *Micrococcus spp.* The only significant Gram-negative residents are *Acinetobacter* spp. [6].

Affordability, accessibility, and side effects of prolonged use of allopathic drugs still persist a challenge and concern. Flavonoids and polyphenols are proving to be highly effective and are therefore gradually emerging as viable alternatives to conventional drugs for various diseases. A large number of flavonoids have been shown to be potential immunomodulators, acting as anti-inflammatory, antistress, anticancer agents and in various skin diseases [7]. The therapeutic potential of flavonoids and the necessity for scientific validation in popular medicine have encouraged more interest in the field. In the present study, an attempt has been made to evaluate the anti-psoriatic activity of flavonoids rich selective herbal plants against a group of gram positive and gram negative bacteria. In addition, cytotoxicity potential was revealed using HaCat Cells. The cell lines are previously infected with psoriatic causing proteins derived from predominant *Staphylococcus* sp. The psoriatic protein containing HaCat cell lines are treated individual herbal extracts at different concentrations and its cytotoxicity effects was evaluated.

2.MATERIALS AND METHODS

Plants collections

The fresh plants were collected from the herbal farm, Kolli hills, based on the recommendations in the review of literature, for psoriasis & dermitis treatments. Fresh plant leaves of the *Indigofera tinctoria*, were collected from across different medicinal farms in and around Kolli hills, Tamil Nadu.

Extraction of plant materials

The collected plant materials were shade dried and coarsely powdered. Measured amount of air-dried powdered plant materials was taken in an aspirator bottle and was soaked in hexane for 2nd days at room temperature. On 3rd day the extract was distilled off and residue subjected to further analysis. Chloroform and alcohol were also added subsequently in the order of increasing polarity and extracts were obtained after distilling off the solvents. Then the extracts obtained were filtered and evaporated using a vacuum rotary evaporator at 40° C. For activity, DMSO used as solvent.

Antimicrobial Assay against test pathogens

Antimicrobial assay of selected plant extract was performed by disc diffusion method in Mueller Hinton Agar (MHA) plates. The predominant gram-positive *Staphylococcus aureus* was inoculated in Nutrient broth and incubated overnight at 37° C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5×10^8 CFU/ml. MHA plates were lawn cultured with standardized microbial culture broth. Plant extract of 50 mg/ml concentration was prepared in Dimethyl Sulfoxide (DMSO). Sterile empty discs were obtained from Hi-media and extract at different concentration was poured in each disc and placed on the MHA culture plates. Tetracycline discs (30 mcg) were used as positive control. It was allowed to incubation for 18-24 hours at 37° C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm [8].

In Vitro Anti-Psoriatic Activity Using HaCaT Cell Inhibition Assay

In-vitro anti-psoriatic activity was carried out in *Staph. aureus* seeded HaCaT human keratinocyte cell line [9]. Human HaCaT keratinocytes were obtained from NCCS, Pune, India. The

cells were seeded at a concentration of 1.0×10^5 cells/ml in a 96 well microtitre plate and grown in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (BioWest). After 24 h, the supernatant was decanted and the monolayer was washed once. Then 100 µl of test substance in various concentrations (25-400 µg/ml) was added to the cells in microtitre plates. Test compounds were prepared in dimethyl sulphoxide (DMSO) and then diluted with DMEM; the final concentration of DMSO was 0.2% in the culture medium. Each sample concentration was tested in triplicates. Controls were performed with DMSO or medium alone. Asiaticoside (Sigma) was used as positive control. The plates were then incubated at 37º C for 3 days in 5% carbon dioxide atmosphere.

Antiproliferation activity was assessed by performing the Sulphorhodamine B (SRB) assay. SRB assay was carried out according to the method of Skehan *et al*., [10]. Cells were fixed by adding 25 µl of ice-cold 50% trichloro acetic acid on top of the growth medium and the plates were incubated at 4° C for 1 h, after which plates were washed to remove traces of medium, drug and serum. SRB stain (50 µl; 0.4% in 1% acetic acid) (Sigma) was added to each well and left in contact with the cells for 30 min after which they were washed with 1% acetic acid, rinsing 4 times until only dye adhering to the cells was left. The plates were then dried and 100 µl of 10 mM Tris buffer (Sigma) added to each well to solubilise the dye. The plates were shaken gently for 5 min and absorbance read at 550 nm using a micro plate reader (Biorad, USA). Data obtained at different concentrations were used for IC_{50} calculations.

3. RESULTS AND DISCUSSION

The use of antimicrobial agents is critical to the successful treatment of infectious diseases. Although there are numerous classes of drugs that are routinely used to treat infections in humans, pathogenic microorganisms are constantly developing resistance to these drugs because of indiscriminate use of antibiotics [11,12]. The use of higher plants and preparations made from them to treat infections is a longstanding practice in a large part of the population, especially in the developing countries, where there is dependence on traditional medicine for a variety of ailments [13]. Interest in plants with antimicrobial properties increased because of current problems associated with the antibiotics [14]. Recently, the antimicrobial effects of various plant extracts against certain pathogens have been reported by a number of researchers [15,16].

Disc diffusion method is the most widely used procedure for testing antimicrobial susceptibility [17]. The present study focused on the antibacterial activity of crude extract, which was extracted from *Indigofera tinctoria.* The results showed that the activity has been increasing under different concentrations of extracts tested. The zone of inhibition against *Staph. aureus* was recorded as 31 mm and 33 mm (50 and 100 µl of 50 mg/ml concentration) respectively. Similarly, the standard drug Tetracycline showed 18 mm of zone of inhibition in diameter **(Figure 1)**. The cytotoxic effect of methanol extract was evaluated using HaCaT cells, a rapidly multiplying human keratinocyte cell line, as a model of epidermal hyperproliferation in psoriasis (**Figure 2**). Among the tested *Indigofera tinctoria* showed appreciable anti-proliferant activity in HaCaT cell line. The results were validated using asiaticoside as positive control. On the Anti-psoriatic activity tested using HaCaT cell lines, *Indigofera tinctoria* had significant scavenging effects with increasing concentration in the range of 25–150 µg/ml. In this study, the Anti-psoriatic ability of methanolic extract of was compared with Asiaticoside; Asiaticoside shows more pronounced activity in a dose-dependent manner. The % activity of *Indigofera tinctoria* extract showed minimum inhibition of about 25.16 % at 25 µg/ml and the maximum inhibition of about 58.95 % at 150 µg/ml. The extracts showed potent anti-proliferant activity in HaCaT cell lines with IC_{50} value of 68.75±14.80 µg/ml in cytotoxicity method which is

comparable to the standard used (**Table 1 and Figure 3**). Here, the extracts showed anti-proliferant activity due to the presence of alkaloid and terpenoid compounds in the crude extract.

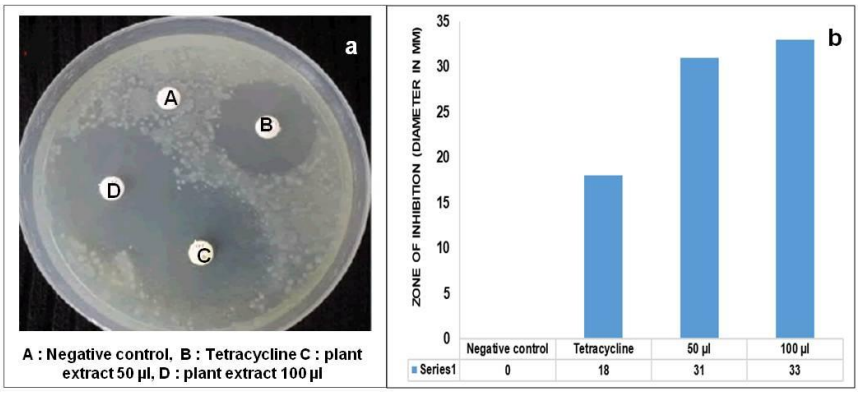

Figure 1. Antibacterial activity of *Indigofera tinctoria* crude extract on *Staphylococcus aureus* (a) zone of inhibition with negative control and standard antibiotic (tetracycline) b. Antibacterial activity of crude extracts at two different concentrations

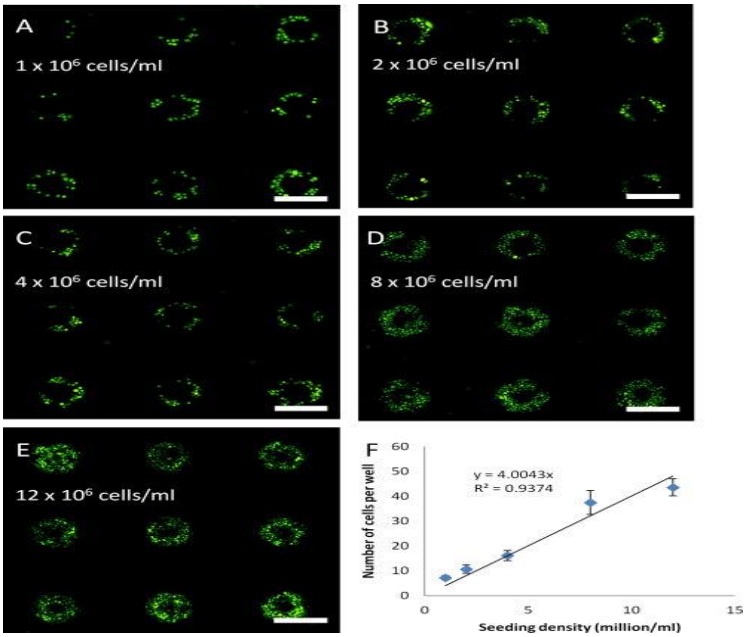

Figure 2: Various densities of HaCaT cells seeded. A–E: Representative images of HaCaT cells stained with calcein-AM fluorescent dye in the microwells with different cell seeding densities. F: The average number of cells per well increased with increasing initial cell concentration (n = 3). Scale bars represent 200 µm

Table 1. Evaluation of cell cytotoxicity effects of *Indigofera tinctoria* herbal extracts on HaCaT cell lines

Name of test sample	Test Concentration (µg/ml)	% Cytotoxicity	IC_{50} (µg/ml)
Indigofera tinctoria	25	30.06±2.0	68.75±14.80
	50	32.01±1.9	
	75	36.25±0.4	
	100	44.25±1.9	
	150	58.95±1.1	

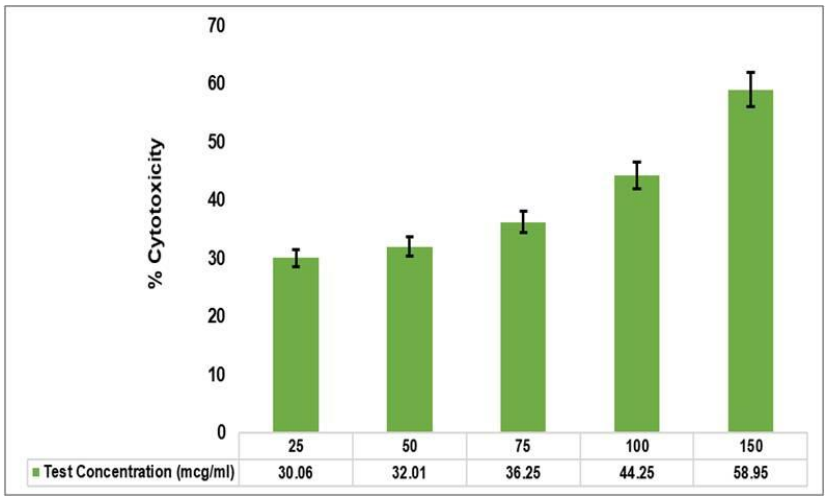

Figure 3. Cytotoxicity effects of *Indigofera tinctoria* herbal extracts on HaCaT cell lines

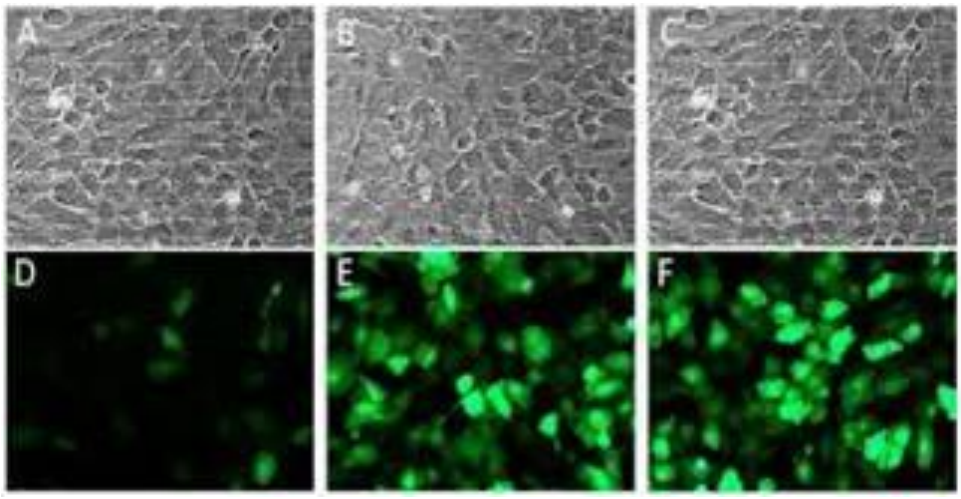

Figure 4. A & D) Control cells, B & E) Cells adherence on 2nd day, C & F) Cells adherence on 3rd day

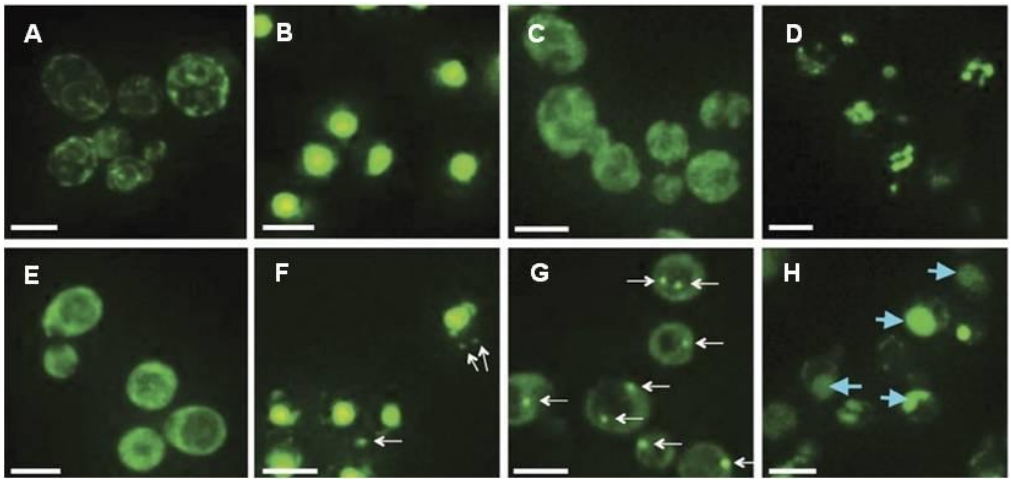

Figure 5. A) Control B) *Staph.* Cells C) Both cell line and *Staph.* cells D) Maturation of *Staph.* cells E) Treating the matured cells with *Indigofera* F) Depletion od *Staph.* cells starts day by day G) Depletion of *Staph.* cells increases H) Vast depletion of *Staph.* Cells

The cell lines treated with Alkaloids & Terpenoids of *Indigofera tinctoria* were used for treating over *Staphylococcus* causing psoriasis keratinocytes. The adherence of *Staphylococcus* to the keratinocytes was monitored frequently for three days. After treatment, the slight blobbing & disruptions of the cell plasma membrane indicated that the alkaloid & terpenoids treated over infected cell lines acted well & disrupted at 80 to 90% of psoriasis causing *Staphylococcus* cell membranes (**Figure 4 & 5**). While cross checking with the preliminary docking the disruption rates were proven in the *In-vitro* cytotoxic cell T Cell receptor activity (CTLs).

The probable mechanism in causing the cell cytotoxicity and cell death is by interacting with the cell membrane proteins and making the cell leak its cellular constituents and finally leading death or maybe it is able to interact with the DNA or cell signalling pathways and manipulating the cellular pathways leading or triggering the cell death pathways. The exact mechanism of action has to be studied in details, so that we could understand the exact mechanism of action, as this is could be better source of treatment in treating or controlling the Psoriasis disease or skin related diseases.

Several lines of studies indicated that flavonoids such as quercetin possess antioxidant and free radical scavenging potential, anti-inflammatory activity and inhibit the growth of various cancer cell lines *in-vitro* [18]. The phytochemical data showed the presence of increased amount of flavonoid, alkaloid and terpenoid content and it is suggested that the presence of flavonoids, alkaloids and terpenoids might be responsible for the anti-psoriatic activity, similarly anti-radical, anti-proliferative, and anti-inflammatory properties. However, the prospective studies to elucidate the exact mechanisms underlying the protective role of, *Indigofera tinctoria against* psoriasis are highly warranted. The cell lines treated with *Indigofera tinctoria* having a rich source of alkaloids and terpenoids showed 60% cell inhibition. Above results also corporate with docking analysis indicated that these components were proven to be better drug candidates for the treatment of psoriasis.

Vijayalakshmi *et al*., (2014) [19] reported *Givotiarottleri formis* (White Catamaran Tree) has been used in the indigenous systems of medicine for the treatment of inflammatory diseases like rheumatism and psoriasis. In order to evaluate this information, anti-psoriatic activity of three flavonoids isolated from the ethanol extract of the bark of *Givotia rottleriformis* were investigated using HaCaT cell lines, a rapidly multiplying human keratinocyte cell line, as a model of epidermal hyperproliferation in psoriasis. Among the tested flavonoids, II and III showed appreciable anti-proliferant activity in HaCaT cell line. The results were validated using asiaticoside as positive control. Flavonoid III was found to have more potent anti-proliferant activity (56.50 ± 12.84 µg/ml) which was followed by flavonoid II (76.50 ± 8.60 µg/ml), flavonoid I (180.70 ± 15.60 µg/ml) and ethanol extract (220.30 ± 7.40 µg/ml). Except flavonoid I, II and III showed appreciable anti-proliferant activity in HaCaT cell line. Asiaticoside showed a potent activity with IC_{50} value of 31.50 µg/ml.

In another work, *in vitro* anti-psoriatic activity and cytotoxicity of ethanolic extract of Nigella sativa seeds (black cumin) was carried out by SRB Assay using HaCaT human keratinocyte cell lines. The ethanolic extract of *Nigella sativa* seeds extract produced a significant epidermal differentiation, from its degree of orthokeratosis (71.36±2.64 %) when compared to the negative control (17.30±4.09 %). The 95% ethanolic extract of *Nigella sativa* shown IC_{50} as 239 µg/ml, with good anti-proliferant activity compared to Asiaticoside as positive control which showed potent activity with IC50 value of 20.13 µg/ml [20].

4. CONCLUSION

In the present study, phytochemical data showed the presence of increased amount of flavonoid, alkaloid and terpenoid content and it is suggested that the presence of flavonoids, alkaloids and terpenoids might be the reason for anti-psoriatic activity, similarly the anti-inflammatory, anti-radical and antiproliferative properties. However, the prospective studies to elucidate the exact mechanisms underlying the protective role of *Indigofera tinctoria against* psoriasis are highly warranted. The cell lines treated with *Indigofera tinctoria* having a rich source of Isatin and Tryptanthrin are alkaloids and terpenoids showed 60% cell inhibition. Above results also corporate with docking analysis indicated that these components were proven to be better drug candidates for the treatment of psoriasis.

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5. REFERENCES

[1] *R.S. Azfar, and J.M. Gelfand, "Psoriasis and metabolic disease: epidemiology and pathophysiology". Curr. Opin. Rheumatol., vol. 20, no. 4 (2008), pp. 416-22.*

[2] *J.E. Gudjonsson, A.M. Thorarinsson, B. Sigurgeirsson, K.G. Kristinsson, H. Valdimarsson, "Streptococcal throat infections and exacerbation of chronic plaque psoriasis: a prospective study". Br. J. Dermatol., vol.149, no. 3(2003), pp.530-534.*

[3] *C. Hwerta, E. Rivero, A. Luis, "Incidence and risk factors for psoriasis in the general population". Arch. of Dermatology, vol. 143, no. 12, (2007), pp. 1559-1565.*

[4] *S. Gelfant, "On the existence of non-cycling germinative cells in human epidermis in vivo and cell cycle aspects of psoriasis". Cell tissue Kinet. Vol. 15, no. 4, (1982), pp.393-397.*

[5] *W.C. Noble, "Microbiology of human skin". London, Lloydluke, (1981).*

[6] *R.J.Hay, B.M. Adriaans, "Rooks Text Book of Dermatology", Edited Burns, T.;Breathnach, S.; Cox, N. and Griffiths, C. Black Well publishing. Vol. 2, (2004), pp: 271.*

[7] *A.Menter, A. Gottlieb, S.R. Feldman, A.S.Van Voorhees, C.L. Leonardi, K.B. Gordon, M. Lebwohl, J.Y. Koo, C.A. Elmets, N.J. Korman, K.R. Beutner, R. Bhushan. "Guidelines of care for the management of psoriasis and psoriatic arthritis: Section 1. Overview of psoriasis and guidelines of care for the treatment of psoriasis with biologics". J. Am. Acad. Dermatol. Vol. 58, no.5, (2008), pp.826-50.*

[8] *S.Manandhar, S. Luitel, R.K. Dahal. "In Vitro Antimicrobial activity of some medicinal plants against human pathogenic bacteria". J. Trop. Med. (2019):1895340.*

[9] *P.Boukamp, R.T.Petrussevska, D. Breitkreutz, J.Hornung, A. Markham, N.E. Fusenig. "Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line". J Cell Biol. Vol.106, no. 3, (1988),pp.761-71.*

[10] *P.Skehan, R.Storeng, D.Scudiero, A.Monks, J.McMahon, D.Vistica, J.T.Warren, H.Bokesch, S.Kenney, M.R.Boyd. "New colorimetric cytotoxicity assay for anticancer-drug screening". J. Natl. Cancer Inst. Vol.82, no. 13, (1990), pp.1107-12.*

[11] *S.Gibbons. "Beat Psoriasis: The Natural Way". Thorsons Health Series. (1992), pp. 208.*

[12] *P.Rahman, R.D. Inman, W.P.Maksymowych, J.P.Reeve, L.Peddle, D.D.Gladman. "Association of interleukin 23 receptor variants with psoriatic arthritis". J. Rheumatol. Vol.36, (2009), pp.137-40.*

[13] *I.Ahmad, Z.Mehmood, F.Mohammad. "Screening of some Indian medicinal plants for their antimicrobial properties". J. Ethnopharmacol. Vol.62, no.2, (1998), pp.183-93.*

[14] *T.G. Emori, and R.P. Gaynes. "An Overview of Nosocomial Infections, Including the Role of the Microbiology Laboratory". Clinical Microbiology Reviews. vol.6, (1993), pp. 428-442.*

[15] *I. Ahmad, and A.Z. Beg. "Antimicrobial and Phytochemical Studies on 45 Indian Medicinal Plants against Multi-Drug and Resistant Human Pathogens". J. of Ethnopharmacol. vol.74, (2001), pp.113-123.*

[16] *P. Erasto, P.O.Adebola, D.S. Grierson, A.J. Afolayan. "An ethnobotanical study of the plants used for the treatment of diabetes in the Eastern Cape Province, South Africa". Afr. J. Biotech. Vol. 4, (2005), pp.458–1460.*

[17] *R. Sambath Kumar, T.Sivakumar, R.S. Sundaram, P. Sivakumar, R. Nethaji, M. Gupa, U.K. Mazumdar. "Antimicrobial and antioxidant activities of Careya arborea Roxb". Iran. J. Pharmacol. Ther., vol.5, (2006), pp.35–41.*

[18] *W.Bors, W. Heller, C. Michel, M. Saran. "Flavonoids as antioxidants: Determination of radical scavenging efficiencies". Methods Enzymol. Vol. 186, (1990), pp.343-55.*

[19] *A.Vijayalakshmi, M. Geetha, V.Ravichandiran, "Anti-Psoriatic Activity of Flavonoids from the Bark of Givotiarottleriforims Griff. EX Wight". Iran. J. Pharmace. Sci. vol.10, no. 3, (2014), pp.81-94.*

[20] *P.D.Lalitha, P. Dhanabal, N. Muruganantham, P.S. Raghu. "Antipsoriatic activity and cytotoxicity of ethanolic extract of Nigella sativa seeds". Pharmacogn. Mag. Vol.8, no.32, (2012), pp.268-272.*