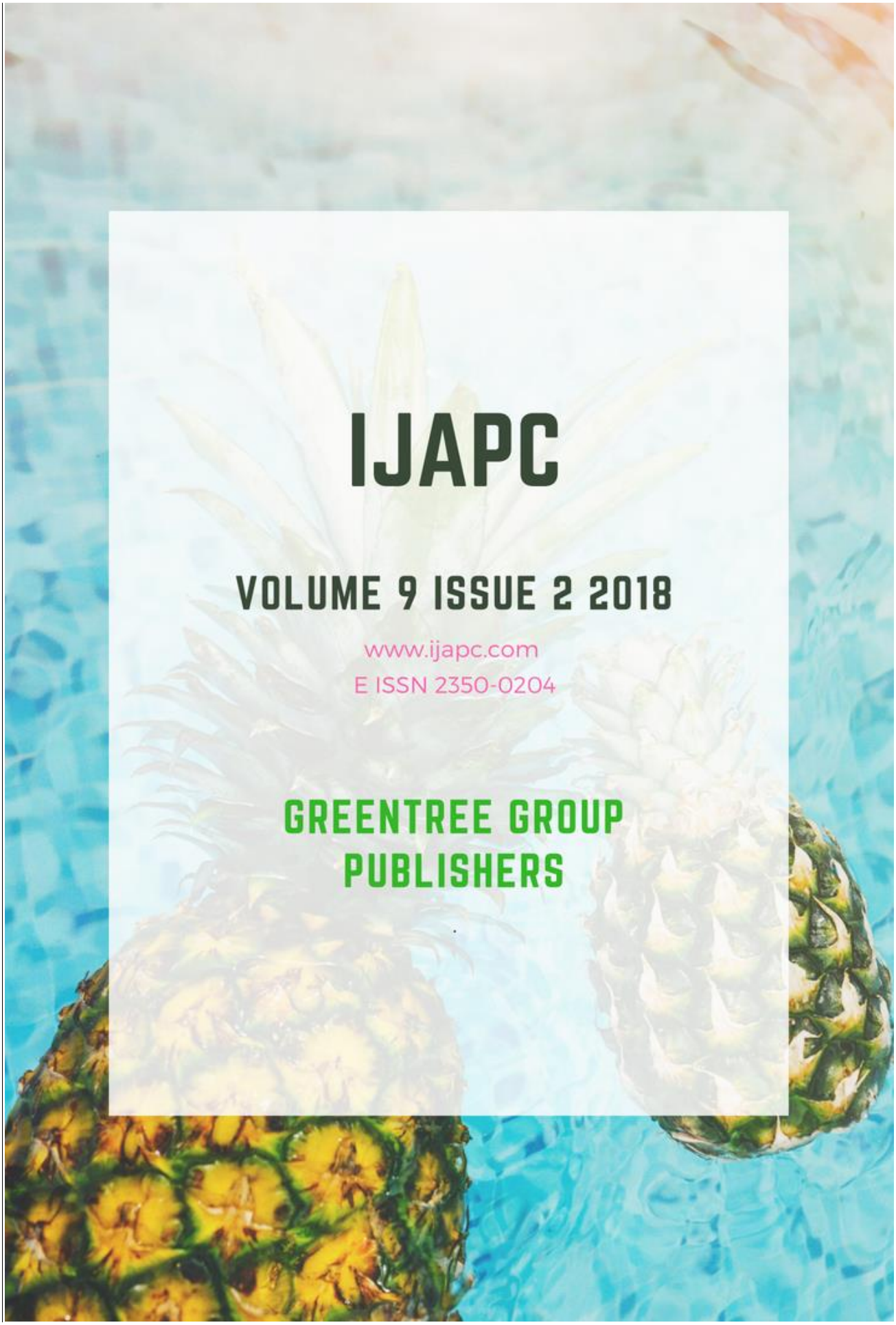

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A Remedy against Dandruff Causing *Malassezia furfur* using *Ixora coccinea*: A Cost Effective Herbal Approach

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ABSTRACT

Ixora coccinea is a shrub grown throughout India. It belongs to genus *Ixora* and is used for a variety of ailments as per Indian traditional Ayurveda system of medicine. Dandruff is a common scalp disorder with high prevalence in population. Our main aim was to check whether this plant have antidandruff activity or not. Our plant had showed that unique antidandruff property. The research work carried outlines isolation and identification of *Malassezia furfur* from dandruff sample, extraction of *Ixora coccinea* plant, synthesis of green silver nanoparticle from *Ixora coccinea* plant extract, comparison of plant extract and green silver nanoparticle by well diffusion technique, formulation of herbal shampoo and finally comparison of herbal shampoo and commercially available antidandruff shampoo. The antidandruff activity shown by Silver Nanoparticles of *Ixora coccinea* plant extract was significant. This suggests its potential activity against infections caused by pathogens. The green silver nanoparticle is developed as an antidandruff agent. Thus, formulated herbal shampoo was eco-friendly and effective, which showed equally good results as that of commercially available shampoo.

KEYWORDS

Ixora coccinea, *Malassezia furfur*, Green silver nanoparticle, Antifungal assay, Herbal shampoo

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INTRODUCTION

Ixora coccinea:

Fig 1 *Ixora coccinea* plant

Ixora is a genus of flowering plants in the Rubiaceae family as shown in Photo plate No. 1. *Ixora coccinea* flowers are used in Hindu worship and in Indian folk medicine[1]. *Ixora coccinea* is native to tropical South-East Asia, including Southern India and Sri Lanka, Thailand, South Florida etc. The common names of *Ixora coccinea* are West Indian Jasmine, Rangan, Kheme, Ponna, Techi, Jungle flame, Jungle geranium and many more. *Ixora* plants generally prefer acidic soil for their growth. The fruits when ripped fully are used as a dietary source. The flower of *Ixora* coccinea plant is traditionally used as anti-inflammatory, aromatic, antipyretic drug and useful in extensive thirst and fatigue[4]. *Ixora coccinea* is used for diverse pharmacological properties including anti-inflammatory and antimitotic activities[1].

Phyto-chemical screening of Ixora coccinea

The major phytoconstituents such as alkaloids, glycoside, flavonoids, steroids, triterpenoids, tannins, saponins, resins etc. are present in the *Ixora coccinea* have been reported[1]. From the essential oil of *Ixora coccinea* flower, 54 components have been identified representing 99.97% of the total no. of components[2]. The oil is composed mainly of triterpenes 62.60%, monoterpenes 31.73%, sesquiterpenes 3.35% and an ester 2.29%[1]. The anticancer activity of the leaves of *Ixora* coccinea was found to be principally due to the known alkaloid, camptothecin[1]. The average content of Camptothecin both in mature and young leaves was 2.8% and it paves way for new findings[2]. The chemical investigation of the roots which leads to the isolation of six phyto-constituents namely: 9, 12-octadecadienoic acid, di-n-octyl phthalate, β-amyrin, kaempferol-7-oglucoside, kaempferitrin and quercetin[2].

Pharmacological Actions:

Pharmacological studies of the plant suggest that this plant possesses antioxidative, antibacterial, gastroprotective, hapatoprotective, antidiarrhocal, antinociceptive, antimutagenic, antineoplastic and chemopreventive effects[1].

Dandruff

Dermatophilic infections is one of the major crises prevalent all over the world. Dermatophytes feed majorly on the secretions of the sebaceous glands present below the skin. Dandruff has worldwide occurrence. Its frequency is variable and depends on different climatic, occupational and socio-economic conditions. It is reported that approximately 30% of dermatophilic infections are due to the lipophilic yeasts[3-5]. Hereditary factors also play a major role in transmission of the disease. It was observed that the main symptom of dandruff infection was dark reddish-tan in colour. The most common sites were found to be the back, head, underarms, upper arms, chest and neck as shown in Figure: 2. Large lesions were multihued and had relatively sharp irregular margins and smaller lesions were circular or oval.

Fig 2 Range of visible flakes along dandruff/ seborrheic dermatitis disease sectrum

a. Mild dandruff, b. Moderate dandruff, c. severe dandruff.

Several micro-organisms are responsible for dandruff problem. One of the most important organism which play a vital role in dandruff infection is *Malassezia* species[6]. According to Shuster, dandruff is medically described as *Pityriasis capitis* is caused by *Malassezia* species like *M. furfur*. *M. globose, M. restricta, M. furfur* is the most common species which is actively causing infection as compared to other species.

Nano particle synthesis: Gold nanoparticles were synthesized in aqueous medium using flower extracts of *Ixora Coccinea* as reducing and stabilizing agent. On treating chloroauric acid with the extract obtained from the *Ixora coccinea* plant it leads to rapid reduction of chloroaurate ions results in the formation of highly stable gold nanoparticles in the solution. The study also showed that the gold Nano particles with antibiotics showed more inhibitory zones as compared to the standard antibiotics[7].

Dandruff is a common scalp disorder and a major cosmetic problem as it causes hair fall, it has been investigated and reported that there is no complete cure for this disease. This disease is of global prevalence and needs an effective therapeutic remedy. Commercially available chemical shampoos show high rate of side effects. Now a day's people prefer to use eco-friendly natural shampoos. In our work

Ixora coccinea plant parts were collected and extracted by different methods. Antidandruff activity of plant extract were checked, green silver nanoparticle were synthesized and compared.

MATERIALS AND METHODS

1. Isolation, Growth and Identification

In the clinical study the dandruff causing organism was isolated from scalp of person suffering from dandruff and maintained on Sabouraud's media slants and stored in refrigerator at 4°C. The isolate was screened by plating the scalp swab on Sabouraud's media enriched with 2 % lipid source like olive oil. The organism was identified based on cultural, microscopic and biochemical methods

2. Preparation of McFarland standard:

Mcfarland standard was prepared as per the guidelines described in the National Committee for Clinical laboratory Standards.

3. Extraction of *Ixrora coccinea* plant:

3.1 Collection of the plant material:

The leaves, stem and flower part of the *Ixora coccinea* was collected from HAL Ojhar T/S, Nashik and HPT Arts & RYK Science College, Nashik, India

3.2 Preparation of the plant extract:

The plant parts were washed thoroughly with distilled water and Mercuric Chloride to remove the earthly matters and freed from debris. They were shade dried, powdered (80% coarse: 20% fine) and subjected to successive (90% ethanol, 90 % methanol and distilled water) extraction by four methods: Soxhlet apparatus method, Overnight extraction method, Fresh aqueous paste extraction method and Boiling extraction method as shown in Photo plate No. 6. Leaf, stem and flower part of the *Ixora coccinea* plat were extracted in 1: 10 ratio. Extracts were concentrated, dried and stored for further analysis.

4. Synthesis of the green silver nanoparticle from the *Ixora coccinea* plant.

The silver nanoparticles were biosynthesized by using aqueous extract of *Ixora coccinea* plant. The 100ml of fresh leaf extract was added into the aqueous solution of 1mM Silver nitrate in 4:2 ratio. After addition, tubes were incubated in a dark condition for 24 hours. Green silver Nano particles synthesis was indicated by change in colour and confirmed by UV spectrophotometery with λ max = 434 nm. To get much smaller particles, above solution was centrifuged at a rate of 25000 rpm for 15 minutes and air dried under hot air oven. The dried green silver nanoparticles were re-dissolved in sterile distilled water and further used for

antifungal assay and formulation of herbal shampoo.

5. Formulation of herbal shampoo.

Herbal shampoo was formulated by adding 1.12 ml of green silver Nano particles,0.8 ml of sodium lauryl sulphate, 0.04ml of citric acid, 0.04ml of sodium chloride, 0.6ml of hibiscus juice, 0.2ml of honey, 0.004ml of essential oil, 1ml of vitamin A and diluted the mixture by adding distilled water up to the total volume of 4ml. Herbal shampoo was formulated and then compared it with commercially available shampoo by antidandruff activity against *Malassezia furfur* by well diffusion method as shown in Figure: 11.

6. Antifungal activity of the plant extracts, green silver nanoparticle and formulated herbal shampoo against *Malassezia furfur* by well diffusion method.

Antifungal activity of the plant extracts, green silver nanoparticle and formulated herbal shampoo against *Malassezia furfur* was performed by well diffusion method. The extracts, green silver nanoparticle and formulated herbal shampoo at the concentration of 50µl were loaded on the well of SDA with 2% olive oil media. Plates were incubated for 3-5 days and after incubation zone of inhibition was measured and compared with positive and negative control.

7. Comparison of formulated *Ixora coccinea* shampoos with commercially available antidandruff shampoo.

Formulated *Ixora coccinea* shampoo was compared with the commercially available antidandruff shampoo by observing the diameter of zone of inhibition by well diffusion method.

Results:

1. **Isolation and Identification of *Malassezia* from dandruff**

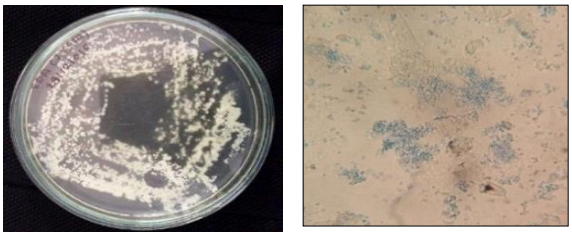


Figure 3: Isolation & Microscopic image of *Malassezia* spp.

From dandruff sample *Malassezia furfur* grew well in Sabouraud's dextrose media enriched with 2% olive oil. *Malassezia furfur* grew as white to tan cream colored colonies smooth pasty consistency on Sabouraud's media and the cells appeared bottling shaped when observed microscopically as shown in Figure 3.

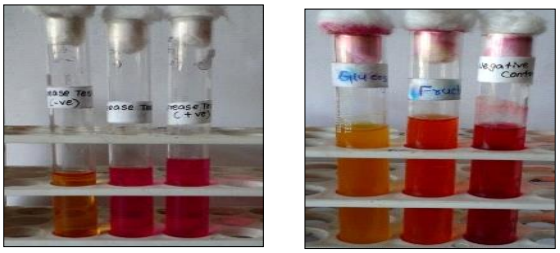


Fig 4 Biochemical test – Urease test & Sugar utilization and fermentation test

The biochemical studies indicated that glucose and fructose were utilized and

fermented by producing acid but not gas Urea in the media was degraded by urease enzyme which indicates that urease test is positive as shown in Figure: 4.

a) Extraction of Ixora coccinea plant:

Fig 5 leaf, stem and flower powder of *Ixora coccinea* plant

b) Extraction by soxhlet apparatus

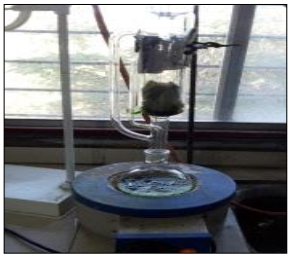
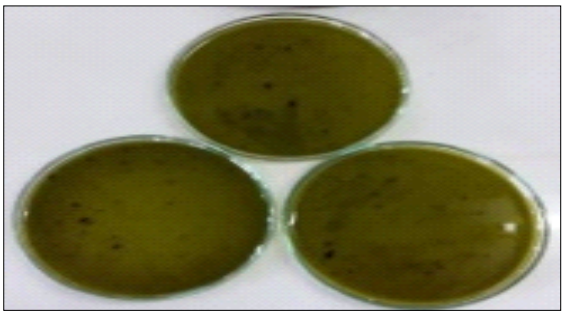


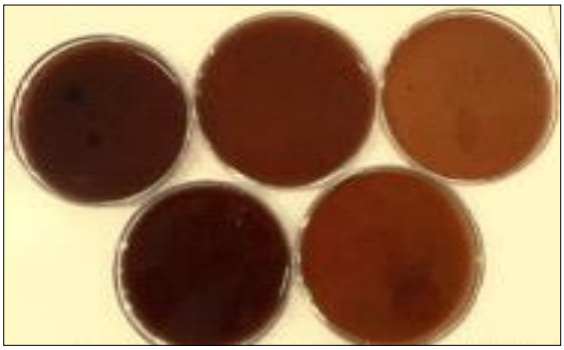
Fig 6 Extraction apparatus.

c) Extraction by overnight method:

Fig 7 Extraction of flower, leaf and stem by overnight method

d) Ixora coccinea leaf, flower and stem extracts:





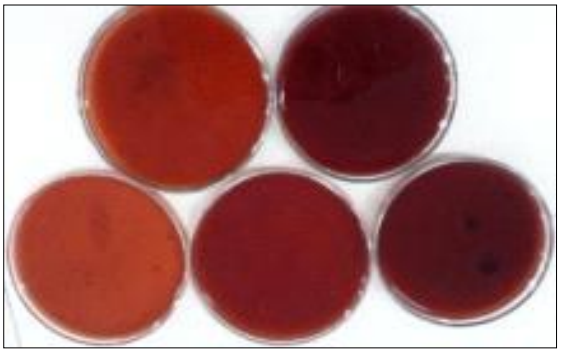


Fig 8 Ethanolic extract of *Ixora coccinea* leaf, stem and flower

4. Synthesis of the silver nanoparticle from the *Ixora coccinea* plant extract.

Fig 9 Synthesis of the silver nanoparticle from the *Ixora coccinea* plant extract.

The formation of silver nanoparticle by the aqueous extract of *Ixora coccinea* plant in the solution of 1mM silver nitrate was indicated by change in colour as shown in Figure: 9.

2. Antidandruff Assay of green silver Nano particle and plant extract by well diffusion method:

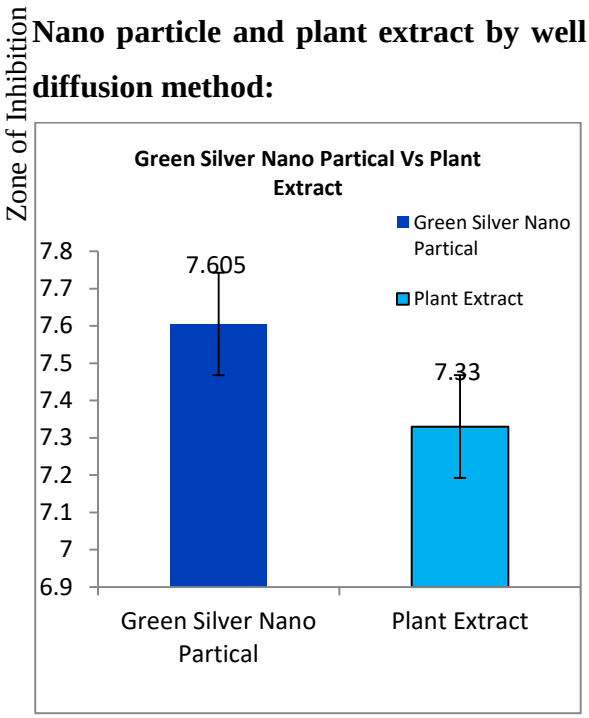


Fig 10 Comparison of green silver Nano particles and plant extract

Zone of inhibition of Green silver Nano particles shows good results as compared to zone of inhibition of plant extract as shown in figure: 10.

3. Formulation of herbal shampoo:

Fig 11 Formulated herbal shampoo.

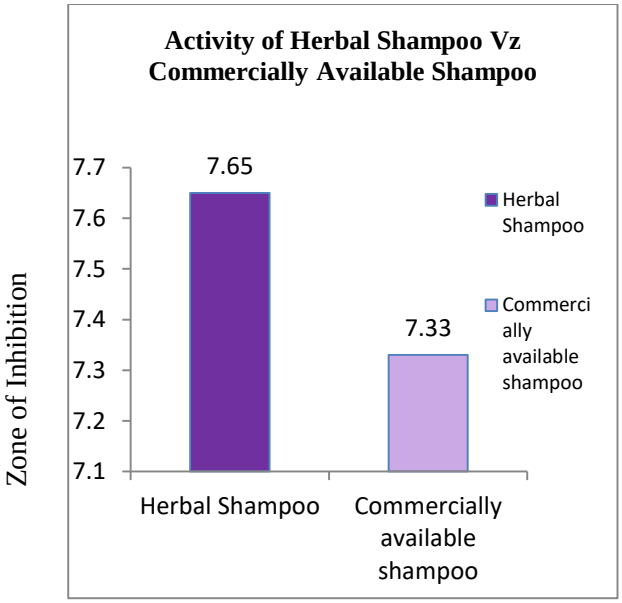


Fig 12 Comparison of formulated herbal shampoo and commercially available s2hampoo

Zone of inhibition of formulated herbal shampoo shows good results as compared to zone of inhibition of commercially available shampoo as shown in figure: 12.

DISCUSSION

The main objective of this study was to check the ability of *Ixora coccinea* to produce antidandruff activity against *Malassezia furfur*. Our study has shown that *Ixora coccinea* plant had an antifungal activity against *Malassezia furfur*. *Ixora coccinea* species against bacteria and yeast cell, plant extracts showed antimicrobial activity against the entire test organisms including *E.coli*, *P.aeruginosa*, *S.aureus and B. subtilis*. Plants used in traditional medicine are assumed to be safe due to the long term use by traditional healers. Information about the safety and effective

use of medicinal plants is difficult to find due to lack of rigorous clinical studies and limited toxicological data available. Preliminary qualitative phytochemical screening gives a clue for the medicinal aptitude of the herb. An important part of natural products from plants, biomolecule and secondary metabolites usually exhibits some kind of biological activities. *Ixora coccinea* plant is widely used in the human therapy, veterinary, agricultures and scientific research and in countless other areas.

UV-Visible spectral analysis: The bio reduction of Ag+ in the aqueous extract was monitored by sampling the reaction mixture by using UV-vis spectroscopy. Addition of the aqueous leaf extract of *Ixora coccinea* to 1mM solution of silver nitrate led to the appearance of yellow colour as result of formation of silver nanoparticles in the solution. Before reaction, the silver containing solution is colourless but changes to a brownish colour on completion of the reaction. The brown colour of the medium could be due to the excitation of surface Plasmon vibrations, typical of the silver nanoparticles. The UV-Vis spectra recorded from the aqueous silver nitrate *Ixora coccinea* leaf broth. A strong characteristic absorbance peak at around 434 nm was observed. The UV-Vis absorption spectrum recorded for the solution shows the characteristic surface Plasmon resonance band for silver nanoparticles in the range of 400-440 nm. Antifungal activity of silver Nano particles: The antifungal activity was carried out. Zone of inhibition in the plate showed that silver nanoparticles synthesized using aqueous leaf extract of *Ixora coccinea* have the antifungal activity against *Malassezia furfur* through the inhibition zone formation. Zone of inhibition was measured and compared with control silver nitrate solution. On comparison with the silver nitrate and plant extracts silver nanoparticles outperformed in the antifungal effect. The results of the investigation showed that silver nanoparticles synthesized from *Ixora coccinea* leaf and flower extracts possess discrete antifungal activity against clinically isolated pathogenic *Malassezia furfur*. In our study, the formulated herbal shampoo of *Ixora coccinea* plant extract had shown an effective and good antifungal activity as compared to the commercially available antidandruff shampoo against Dandruff causing *Malassezia furfur*. India is rich heritage for cultivation and production of herbal medicines due to its diversified climatic conditions. Indian traditional literature and ethanopharmacological studies presents a number of plants/ formulations with proven

efficacy as hair formulations. Dandruff is a common disease caused by *Malassezia* species especially *Malassezia fufur*. The lipolytic activity of these organism induces hydrolysis of human sebum tri-glycerides into a free fatty acids that cause both hair loss and scalp. *Ixora coccinea* plant extracts were showing good antifungal activity almost equivalent to that of commercially available shampoos. Leaf and flower extract of the *Ixora coccinea* plant had more antifungal activity and this could be because of their active compound like flavonoids. The present study gives significant information about the higher antifungal activity of *Ixora coccinea* at low concentration which can be exploited for polyherbal preparation. Use of natural product is not only cost effective but also negligible side effects. The present work has shown that formulated herbal shampoo of *Ixora coccinea* was a potentially good source of antifungal agent.

CONCLUSION

This research is focused on the antidandruff activity of *Ixora coccinea* plant. Green silver nanoparticles were synthesized and confirmed by spectrophotometry, a strong characteristic absorbance peak at around 434 nm was observed. Effective antidandruff activity was shown by plant extract as well as the synthesized green silver nanoparticles and formulated herbal shampoo. We had formulated an eco-friendly, cost effective shampoo which showed good results as that of commercially available shampoo. Hence we had proved that *Ixora coccinea* plant possess antidandruff activity.

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