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Study On Phytochemical Screening of *Datura Stramonium* Plant Extract and their Antimicrobial Activity

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ABSTRACT

The present study was carried out to evaluate Antimicrobial Activity of Hydro-alcoholic Extract of *Datura stramonium* leaf (HAESO). Herbal medicines have been widely utilized as effective remedies for the prevention and treatment of multiple health conditions for centuries by almost every known culture. The incidences of harmful nature of synthetic drugs, which were regarded as harmful to human beings and environment led to focus more on herbal medicine use for various treatments. Belonging to the family: Solanaceae, a widely available plant commonly called as Datura, Ethnomedical information revealed that it was used in various ailments for long time all over the world. In present study Ketoconazole is used as Standard, Dimethyl sulfoxide (DMSO) as Control and Hydro-alcoholic Extract of *Datura stramonium* leaf (HAESO) as Test. Pharmacological screening of hydroalcoholic extract of *Datura stramonium* leaf showed in vitro antidandruff activity which was evaluated by well diffusion method and hair strand test. The results revealed that hydroalcoholic extract of *Datura stramonium* leaf showed significant antidandruff activity for these two methods. The result exposed that isolated compound (apigenin) showed the zone of inhibition at 250mg/ml & 500mg/ml stating significant antidandruff activity. Hair strand test was found to be an interesting and reliable new test model for evaluation of the antifungal activity especially with regards to a possible depot effect where 250mg/ml & 500mg/ml proved to be effective. Most of the recent data supports a direct causal link between *Malassezia* fungi and dandruff. In present study, the obtained results showed *Datura stramonium* leaf exhibiting a significant atimicrobial activity by inhibiting the growth of *Malassezia furfur.* Future Studies are needed for the formulation and different methods to develop new techniques.

Keywords: *Datura stramonium*, Ketoconazole, Well diffusion method, Hair strand test, Hydro-alcoholic Extract of *Datura stramonium* leaf (HAESO).

1. INTRODUCTION

Herbal medicines have been widely utilized as effective remedies for the prevention and treatment of multiple health conditions for centuries by almost every known culture. The first documented records of herbal medicine use date back 5,000 years in China. Similarly, India's Ayurvedic medicine tradition is thought to be more than 5,000 years old and herbal medicines remain an essential component of its practice[1]. Today, the populations of certain countries still depend on herbal medicines to address their healthcare needs. In the U.S. the use of herbal medicines continues to grow since the first national study of complementary and alternative medicine use was conducted.

Additionally, as a general rule, older adult populations are more likely to use both conventional drug therapy and herbal medicines. This population is also more likely to have a higher incidence of chronic disease, which often does not require the use of increasingly complex conventional drug therapy. As such, the potential for herb-disease and herb-drug interactions increases with older adult populations. At present, there is an increase in research evaluating the use of herbal medicines, especially clinical trials, this, together with the ongoing development of new conventional drug therapies, further combining the number of unknown outcomes when using elements of these two treatment approaches together. In many countries, including the U.S., herbal medicines are not regulated as extensively as conventional drug therapy. Also, globalization has greatly increased accessibility of herbal medicines from all parts of the world to any single consumer. Clearly there is a great need for coordinated efforts to conduct the necessary clinical trials to study the efficacy and safety of herbal medicines, both alone and in conjunction with conventional drug therapies.[2]

Dandruff: Dandruff (also called as Pityriasis capitis) means scaliness of the scalp skin without signs of inflammation. Dandruff is so common that it can be considered

physiological. It represents desquamation of the skin surface, due to separation of layers of stratum corneum, which is a continuous process, in the form of scales.[4]

Malassezia

The microbial origin of dandruff centers on the causal role of yeasts of the genus *Malassezia*. The majority of recent data supports a direct causal link between *Malassezia* fungi and dandruff. First, effective treatment of the condition can occur with a wide range of material types, from zinc and selenium salts to highly specific azoles, with the only known functional link between these materials being antifungal activity.[5] The second supporting factor is that improvement in dandruff correlates considerably with reduction in scalp *Malassezia* level. While the absolute level of *Malassezia* correlates less well with dandruff, its reduction amongst those individuals that express the symptoms strongly supports its role. Originally named *Malassezia* by Malassezia in 1898,[4-6] this genus was renamed and referred to as *Pityrosporum* during the second half of the 20th century.[5-6] At one time, members of *Malassezia* were classified into two species: a lipid-dependent species, *M. furfur*, and a non-lipid-dependent species, *M. pachydermatis*. In the mid-1990s studies of the morphological, ultra structural, physiologic and genomic differences in *Malassezia* led to the identification of multiple lipid-dependent species (including *M.globosa, M.restricta, M.furfur, M.obtusa, M.slooffiae, M.sympodialis, M. japonica, M.nana, M. dermatis,* and *M. yamatoensis*), in addition to the nonlipid-dependent, primarily zoophilic species, *M. pachydermatis*. Use of molecular markers is generally required to correctly differentiate between the various lipid-dependent species.[6] *Malassezia globosa* reside on the surface of the scalp and in the follicular infundibulum.

***Datura stramonium* (datura)**

Datura is an herbaceous perennial plant from Solanaceae family, grown in temperate and tropical region of the globe. It has been used in traditional medicine to relieve pain, breathlessness, fevers, etc. It is a powerful deliriant and hallucinogen. However, as the alkaloids are responsible for both the medicinal and hallucinogenic properties, are toxic in higher amounts, and careless use often results in hospitalization and deaths. Considering this, the plant has been grouped under Schedule E-1 of Drugs and Cosmetics Act-1940.[7] Even being a poisonous plant, it is being used since the ancient times by Ayurveda physicians for various purposes.

The therapeutic activities are due to the presence of different active components and research revealed the presence of saponins, tannins, steroids, alkaloids, polyphenols and glycosides in this plant. The plant contains different functional groups such as saponins, tannins, steroids, alkaloids, flavonoids, phenols and glycosides. Atropine and scopolamine are competitive antagonists of muscarinic cholinergic receptors and are central nervous system depressants. All parts of the plant are toxic but the highest amount of alkaloids is contained in the ripe seeds.[8]

Traditionally, the Datura plant is acrid, narcotic, anodyne, antispasmodic, intoxicant, and emetic. It is useful in asthma, cough, fever, inflammations, edema, neuralgia, insanity, myalgia, hyperacidity, duodenal ulcer, renal colic, calculi, and dysmenorrhea. Roots are used for bites of rabid dogs. Seeds are aphrodisiac and used in toothache, earache, gastric disorders and are good to treat dandruff and lice.

2. METHODS AND MATERIALS

Quantitative estimation of phytoconstituents

Determination of gallic acid equivalent in (HAESO)

Principle: Total phenolic content of the various concentrations of HAESO was determined by Folin-ciocalteu reagent method. The hydroxyl group (OH) of phenolic compounds reduces the phosphomolybdic acid to molybdenum blue in the presence of alkaline medium (present in Folin reagent). The blue coloured complex was then spectrophotometrically measured at 760nm.

Instrument: UV visible spectrophotometer, (Shimadzu -Model 1800)

Reagents required: Folin-Ciocalteu Reagent (1N), Sodium carbonate solution (10%), Standard Gallic acid solution.

Procedure: About 1 mL (1mg/ml and 0.5 mg/mL) of Hydroalcoholic extract of *Datura stramonium* (Leaf) (HAESO), 0.5 mL of Folin-ciocalteu reagent (1N) were added and allowed to stand for 15 minutes. Then 1 mL of 10% sodium carbonate solution was added to the above solution. Finally, the mixtures were made up to 10 mL with distilled water and allowed to stand for 30 minutes at room temperature and total phenolic content was determined spectrophotometrically at 760nm wavelength.

Determination of rutin (flavonoid) equivalent in (HAESO)

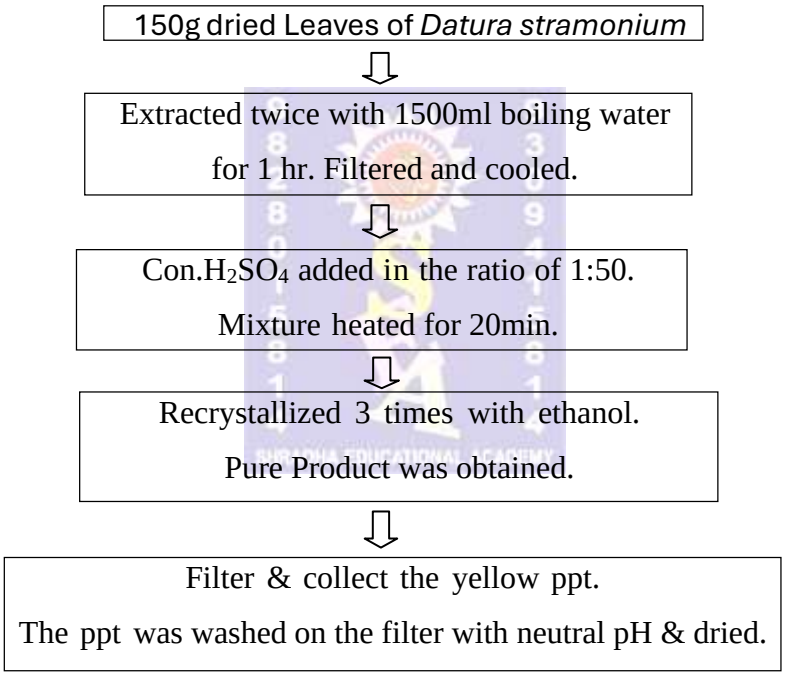
Principle: Flavonoids present in the extract form, a charge transfer complex with several heavy metals to give a characteristic colour. In this reaction, the high electron positive nature of aluminium attracts the atomic nuclei of the aromatic rings in the flavonoids. Then it will react with potassium acetate in alkaline medium to form a pink-coloured complex that is measured spectrophotometrically at 415 nm.

Instrument: UV Visible spectrophotometer, Shimadzu (Model 1800).

Reagents required: 10% aluminium chloride, 1M potassium acetate, Standard rutin.

Procedure: 1mL of hydroalcoholic extract of *Datura stramonium* (Leaf), 0.1 mL of aluminium chloride solution, 0.1 mL of potassium acetate solution and 2.8 mL of ethanol were added, and the final volume was then made up to 5 mL with distilled water. After 20 min the absorbance was measured at 415 nm.

Isolation of apigenin from *Datura stramonium*:



Pharmacological studies:

Pharmacological screening procedures are important and necessary in order to estimate the harmful or therapeutic potential of useful drug. Molecular procedures are used nowadays to screen the herbal compounds and extracts. The classical method of pharmacological screening involves sequential testing of any new chemical compounds or extracts from herbal sources by in vitro and in vivo experiments. Most of the extracts or drugs used in the therapy have been found and evaluated with these methods.

In vitro anti-dandruff activity:

Collection and maintance of the culture:

Pure culture of *Malassezia furfur* (MTCC: 1374) was obtained from Institute of microbial type of culture collection, Pune, India. The culture was maintained in SDA (Sabouraud dextrose agar) medium.

Inoculum preparation: The peptone was added to the liquid SDA medium in the concentration of 5, 10, 15 and 20 g/l. Pure culture of M. furfur grown in liquid medium was inoculated and incubated at 30±2ºC for 7 days.

Preparation of the medium: 2g of SDA medium and 1g of Agar was dissolved in 50ml of

distilled water than it is boiled to dissolve the medium completely. Further sterilized by autoclaving at 15Ibs pressure (121ºC) for 15min, pH is adjusted to (5.6±2ºC). The medium was poured into the sterile petridishes to get a thickness of 5-6mm. The medium was allowed to solidify and petridish was inverted and were dried at 37ºC just before inoculation.

In vitro Anti-dandruff activity:

Well diffusion method

The broth culture of *M. furfur* was swabbed over the SDA (sabouraud dextrose agar) by using sterile cotton buds. Sterile 6mm diameters well were punched and added in plant extracts and Ketoconazole (Standard drug 10µg/disc) and control DMSO well, which were placed equidistantly (3cm apart) round the margin of the plate (plate 3 & 4) at 30±2ºC and zone of inhibition was observed after 3days.

Hair strand test

Malassezia Species

Hair specimens were taken from ten volunteer so different hair color (six female, four Male: mean[2] 8.2 years, 5-53 years), who did not use antidandruff preparations or hair dyes. By means of scissors, hair strands were cut near the scalp surface (hair roots were not included in the sample). Two different concentrations were used.

Structure of the trial

Sterile glass Petri dishes (3 cm in diameter) were filled with 4 ml of selective agar for Pathogenic fungi (SDA). Cold sterile olive oil was inoculated with different *Malassezia* strains, which were cultured for four days on SPF (Specific Pathogen Free) overlaid earlier with olive oil and adjusted to an inoculation density of 5×103 CFU/µl using Neubauer Chamber. From each volunteer, hair strand approximately 5cm in length were incubated with one of the five test substances at 30ºC for 5min in sterile petri dishes. The hairs were then transferred to a sieve with filter paper, rinsed for 1min in running water (30ºC), and dried at room temperature. By means of sterile scissors, 1-cm pieces were cut from the dried hair and distributed in the center of the different test dishes to approximate natural scalp conditions, 200 hairs/cm^2 were inoculated.

3. RESULTS AND DISCUSSION

Determination of physical parameters of (HAESO)

The physical parameters of hydro alcoholic extract of *Datura stramonium* (Leaf) such as refractive index, weight per ml, consistency and colour was determined. It was found to be refractive index (1.370 ± 0.003), weight per mL (0.897 ± 0.007), and Dark green in colour with liquid consistency.

Quantitavie phytochemical studies

Determination of gallic acid equivalent in (HAESO)

Quantitative estimation of biological compounds showed that *Datura stramonium* has more of flavonoids, phenols and carotenoids. This could be used as diagnosis of the nature and amount of phytoconstituents.

Determination of flavonoid content

The presence of biologically active compounds like terpene, glycoside, flavanoids and phenols are attributed to antibacterial and antifungal, anti-oxidant, anti-inflammatory, antitumor and also used in treatment of respiratory complications.

Thin layer chromatography

Separation of phytoconstituents in hydroalcholic extract of *Datura stramonium* leaf carried out by thin layer chromatography. Thin layer chromatography (TLC) of the hydroalcoholic extract of *Datura stramonium* (leaf) showed the R_f value of 0.23,0.31,0.34 which may indicate the presence of rutin, quercetin, apigenin in the solvent system used, Ethylacetate: formic acid: glacial acetic acid: water (100:11:11:26) and Rf value of 0.26,0.34,0.37 may indicate the presence of rutin, quercetin, apigenin in the solvent system used as Toluene:ethyl acetate: formic acid: Methanol (3:6:1.6:0.4).

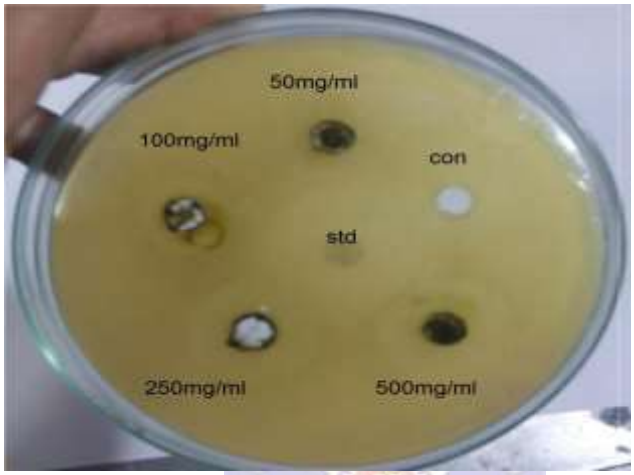
Pharmacological activity

Antidandruff activity of hydroalcoholic extract of *Datura stramonium* against *Malassezia furfur* (MTCC1374) by using well diffusion method

The hydroalcoholic extract of *Datura stramonium* was tested for their efficacy against dandruff causing agent *Malassezia furfur* by well diffusion method. The zone of inhibition was clearly visible, and the diameter of the zone was measured and shown (PLATE-3). *Malassezia furfur* was sensitive to all concentrations tested in hydroalcoholic extract of *Datura stramonium* and showed the inhibition of 12±0.236mm in 100mg/ml, 12.8±0.272mm in 250mg/ml and 14.2±0.286mm in 500mg/ml respectively.
From the above finding the HAESO was significantly inhibiting of the growth of *M. furfur* and when compared with standard Ketoconazole, 16.2±0.313mm for 30mg/disc, an significant growth inhibition was recorded.

PLATE-3 (Figure-1)

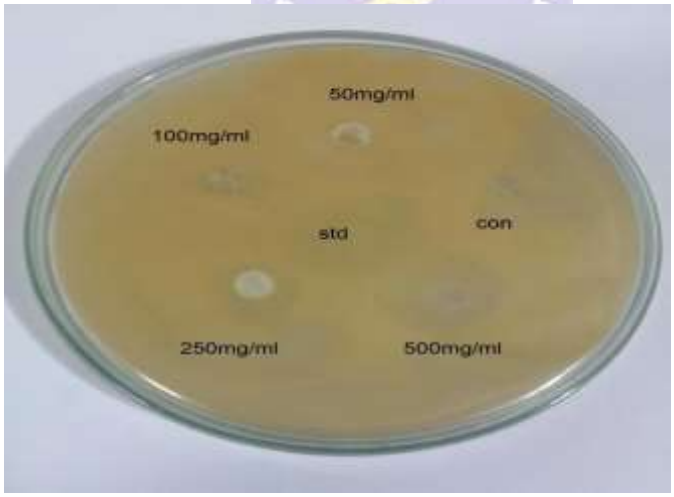
Anti-dandruff activity of HAESO against M. *Furfur* (MTCC 1374)



Con = control Std = standard
Control = 70% hydro-alcoholic Std= ketoconazole 30mg/disc.

PLATE-4 (Figure-2)

Anti-dandruff activity of isolated compound against *M. Furfur (MTCC 1374)*



Con = control (DMSO) Std = standard
Standard = Ketoconazole 30mg/disc

Anti-dandruff activity of isolated compound from *Datura stramonium* against *Malassezia furfur* (MTCC 1374)

The isolated compound from *Datura stramonium* was tested for their efficacy against dandruff causing agent *Malassezia furfur* by well diffusion method. The zone of inhibition was clearly visible, and the diameter of the zone was measured and shown (Plate-4). *Malassezia furfur* was sensitive to two concentrations tested in isolated compound from *Datura stramonium* and showed the inhibition of 12.7±0.144mm in 250mg/ml, 13.8±0.170mm in 500mg/ml. From the above finding the isolated compound of apigenin was significantly inhibiting the growth of *M. furfur* and when compared with standard 15.8±0.235 for 30mg/disc, an significant growth inhibition was recorded.

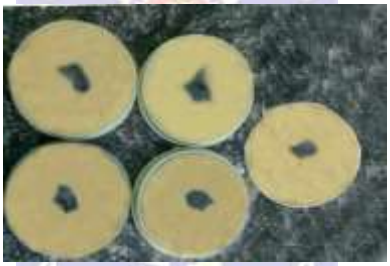
Hair strand test for Female volunteers

***Growth of Malassezia furfur* PLATE-5 (Figure-3)**

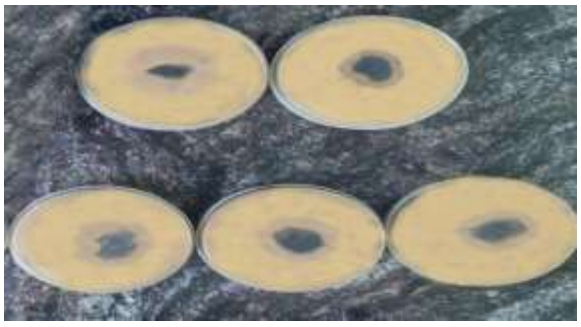
Inhibition of *Malassezia furfur* growth by HAESO (500mg/ml) (Figure-4)

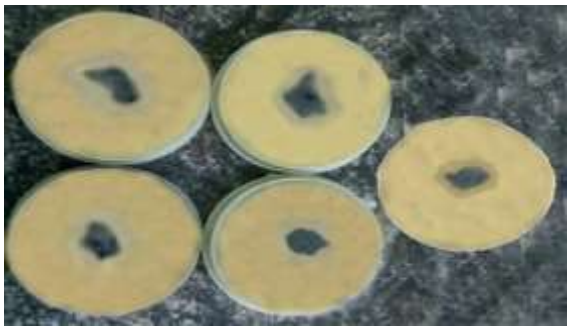
Hair strand test for Female volunteers

Growth of *Malassezia furfur* PLATE-6 (Figure-5)

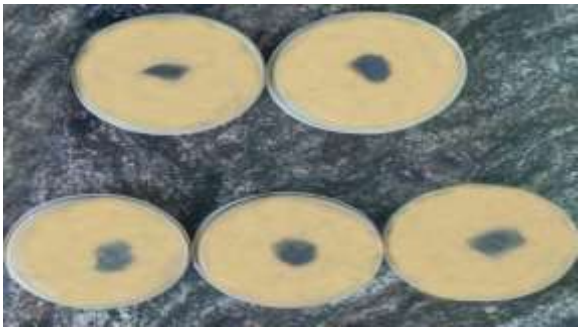


Inhibition of *Malassezia furfur* by HAESO (250mg/ml) (Figure-6)





Hair strand test for Male volunteers



Growth of Malassezia furfur PLATE-7 (Figure-7)

Inhibition of Malassezia furfur by HAESO (500mg/ml) (Figure-8)

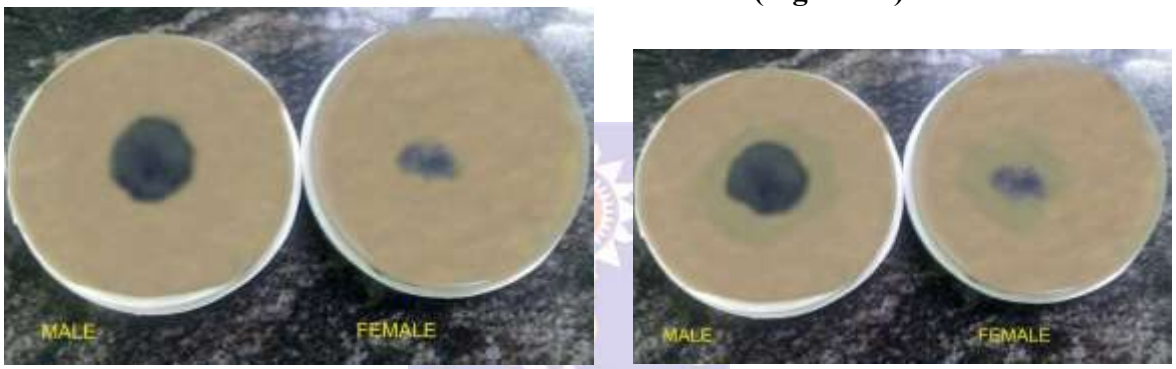
Hair strand test for Male volunteers

Growth of Malassezia furfur PLATE-8 (Figure-9)

Inhibition of *Malassezia furfur* by HAESO (250mg/ml) (Figure-10)

Hair strand test for both Male and Female volunteers

Growth of Malassezia furfur PLATE-9 (Figure-11)



Inhibition of Malassezia furfur by KETOCONAZOLE (30mg/ml) (Figure-12)

Hair strand test

Result of Hair strand test

The antidandruff efficacy of *Datura stramonium* was tested against *M. furfur*revealed that the inhibition of HAESO 250mg/ml & 500mg/ml and standard drug ketoconazole 30mg/ml in all ten hair specimens (male and female) that had been treated with growth of *M. furfur* after four days. Growth was only observed in the direct contact with inoculated hairs. There was no direct contact with the marginal region increasing incubation time, however, homogenous growth was observed. Results of the hair strand test for *Malassezia furfur* after 18 days and the (plate-5 to 9) was observed with *M. furfur*. The hair strand test of HAESO 250mg/ml & 500mg/ml had a significant growth-inhibiting effect respectively. Similarly standard antifungal drug ketoconazole 30mg/ml was recorded the growth–inhibiting effects.

Result of Hair strand test for zone of inhibition:

TABLE-1

Hair From volunteer	**HAESO** Female		**HAESO** Male		**KETOCONAZOLE** 30mg/ml	
	250mg/ml	500mg/ml	250mg/ml	500mg/ml	Male	Female
1	12.7±0.148	14.2±0.389	12.9±0.047	14.4±0.07	15.4±0.6	15.7±0.9
2	12.6±0.211	14.7±0.215	12.5±0.166	13.8±0.424	15.8±0.2	16.1±0.1
3	12.9±0.089	14.1±0.156	12.1±0.272	14.7±0.29	15.7±0.0	15.9±0.4
4	13.1±0.176	13.9±0.316	12.7±0.341	14.6±0.367	15.9±0.3	15.4±0.7
5	12.9±0.246	14.5±0.277	12.3±0.109	13.9±0.131	15.7±0.8	15.5±0.3

4. CONCLUSION

Pharmacological screening of hydroalcoholic extract of *Datura stramonium* showed *in vitro*

antidandruff activity which was evaluated by well diffusion method and hair strand test. The results revealed that hydroalcoholic extract of *Datura stramonium* leaf showed significant antidandruff activity for two methods. It was evident from the phytochemical studies of the plant, that essential amount of flavonoids and phenolic contents were present in these extracts which exhibited significant *in vitro* antidandruff activity. Pharmacological screening of isolated compound from *Datura stramonium* leaf showed antidandruff activity, which was evaluated by well diffusion methods. The result exposed that isolated compound (apigenin) showed the zone of inhibition at 250mg/ml & 500mg/ml stating significant antidandruff activity. Hair strand test was found to be an interesting and reliable new test model for evaluation of the antifungal activity especially with regards to a possible depot effect where 250mg/ml & 500mg/ml proved to be effective. Similarly standard antifungal drug ketoconazole zone of inhibition range 15.9mm for 30mg/ml showed significant microbial inhibition as estimated from experimental records.

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