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COMPARATIVE EFFECT OF *DATURA METEL* LEAF AND FLOWER EXTRACTS FOR HAIR REGROWTH

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Abstract:

Datura metel is a green leafy plant that has reportedly been employed for its medicinal and toxic properties. In this study, dried and pulverized *Datura metel* leaves and flowers were extracted using n-hexane and methanol, and then characterized. Methanolic leaf extract had the highest percentage recovery yield (23.5%). Phytochemical tests on both sets of extracts revealed the presence of Flavonoids, Tannins, Glycosides, Alkaloids, Phenols, Saponins and Reducing sugars. The methanolic portions contained Steroidal compounds in the flowers but not in the leaves while Terpenoids were absent in the flowers but present in the leaves. Animal treatments with methanol leaf extract initiated the fastest hair growth in 5 days, and the shaved areas were completely filled with hairs in 13 days. Hexane flower extracts produced the longest regrowth hairs (80.6%) after 30 days. Methanol leaf extracts also caused mass switching of 60% hair follicles from resting telogenic (T) to growing anagenic (A) phases with T/A ratio 0.45. The results plainly demonstrate that *D. metel* leaf and flower extracts have complementary effect in inducing actively growing cyclic phases, finer and denser hairs.

Keywords **— *Datura metel*, methanol, n-hexane**

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I. INTRODUCTION

Medicinal plants have varieties of biologically active chemical compounds that work synergistically together and which is a direct result of natural selection process [1]. This chemical diversity also includes many compounds that are highly beneficial to humans and animals as: vitamins, nutrients, antioxidants or anti-carcinogens with anti-inflammatory, antimicrobial, antidiabetic, antispermatogenic, immunomodulatory, and even reno-protection or hepato-protective effects**.** These bioactive compounds which include alkaloids, steroids, tannins, saponins, terpenoids, phenols, flavonoids, cardiac glycosides and anthraquinones among others, are secondary metabolites which are capable of producing definite physiological action on the human body [2, 3].

With newer discoveries in herbal medicine, the chemistry behind the action of plant extracts is becoming clearer and a number of drugs made entirely from plants extracts have been approved [4]. An implication is that the use of synthetic chemicals which have attendant side effects will become more unpopular and will eventually cease to be the standards for comparing the activity of future products. Presently, the treatment of skin conditions and a wide variety of dermatological disorders, including inflammation, psoriasis, atopic dermatitis and alopecia, by plants has attracted numerous research interests. Technological advances has also enhanced innovations in product

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development and testing, and regulatory bodies around the world have set rigorous safety standards for the endorsement of herbal drugs. Many products in various combinations of herbal formulations are now available in the market as hair tonic, hair growth promoters, hair conditioners, hair-cleansing agents, antidandruff agents, as well as for the treatment of other skin infections [5, 6, 7, 8]. Substantial improvement is still needed in their development because potential plant extracts must be relatively safe.

Datura metel (Gegemu) is a green leafy and important medicinal plant which thrives in rich and moist, or very alkaline soil and has the capacity to re-seed itself. In Nigeria, the two known varieties of the plant have white and purple flowers, and are found in the Southern parts of the country. The leaves and flowers are known to contain varying amounts of poisonous alkaloids, depending on the soil and weather conditions in which the plant grows [9, 10, 11]. In Southwest Nigeria, gegemu leaf extracts are mostly consumed to induce sleep and could be beneficial when applied in hair growth treatments. In this study, the effect of *Datura metel* leaves and flower extracts were investigated using n-hexane and methanol as extracting solvents.

II. MATERIALS AND METHOD

Sample Collection and Preparation

Fresh leaves and flowers of *Datura metel* collected from wastelands in Ibadan, Southwest Nigeria were washed and rinsed with clean water and then, air dried for 3 weeks. The dried samples were crushed to powder, using an Electric Blender and kept in a dry place. Methanol and n-Hexane, used in this study were of analytical grade.

Extraction of Samples

100 g of the ground flower sample was soxhlet extracted with n-hexane for 6 hours, then taken off and air-dried. The dry marc was extracted again with methanol until exhausted. The same process was repeated with the pulverized leaf samples. A rotary evaporator was used to recover both solvents and the extracted samples were kept for further analysis.

Phytochemical Screening

Identification of phytochemical constituents present in the flower and leaf extracts under study were carried out using standard procedures described by Uddin *et al.* 2012 [12] and Kumar *et al.* 2013 [13].

Preparation of Maeyer's reagent

0.355 g of mercuric chloride was dissolved in 60 ml of distilled water. 5.0 g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water.

Preparation of Dragendorff's reagent:

Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of distilled water. Solution B: 16 g of potassium iodide was dissolved in 40 ml of distilled water. Both solutions (A and B) were mixed in 1:1 ratio.

Test for Flavonoids

0.5 g of various extract was shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in 20 ml of 80% ethanol and filtered. The filtrate was used for the following tests:
(a) 3 ml of the filtrate was mixed with 4 ml of 1% potassium hydroxide in a test tube. Formation of a dark yellow colour indicated the presence of flavonoids.
(b) 5 ml of the dilute ammonia solution was added to the portion of the aqueous filtrate of each plant extract followed by the addition of concentrated H_2SO_4 and then observed for the appearance of yellow colour.

Test for alkaloids

0.5 to 0.6 g of each extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with Maeyer's and Dragendorff's reagents, and observed for the development of a precipitate.

Test for Glycosides

Five ml each of various extract were hydrolysed separately with 5 ml each of conc. HCl and boiled for few hours on a water bath and hydrolysates were subjected to the following test: A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous 10% sodium hydroxide was added. (b) Each extract was hydrolyzed with HCl and neutralized with NaOH solution. A few drops of Fehling solution A and B were added. Formation of a yellow colour indicated the presence of glycosides

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Test for steroids
0.5 g of the various solvent extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The presence of steroids was observed when the mixture turned green.

Test for Phenols
To 1ml of various solvent extracts of sample, 2ml of distilled water followed by a few drops of 1% and 10% aqueous ferric chloride solution were added to check for the formation of green colour.

Test for Terpenoids (Salkowski test)
5 ml of various solvent extracts were mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated (H_2SO_4) and a layer of the reddish brown colouration formed at the interface indicated positive result for the presence of terpenoids.

Test for Saponins
0.5 g of various solvent extract was dissolved in boiling water in a test tube. Test cooling aqueous extracts were mixed vigorously to froth and the height of the froth was measured to determine the saponin contents in the sample. 2.0 g of the powdered plant material was boiled in distilled water in a test tube in boiling water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and was observed when shaken vigorously. The formation of stable persistent froth during concentration of samples on a rotatory evaporator and which when shaken vigorously gave an emulsion was indicative of saponins.

Test for Reducing Sugars
Each extract was shaken with distilled water and filtered. The filtrate was boiled with fews drops of Fehling solution A and B. An orange red precipitate on addition of Fehling's solution indicated the presence of sugars

Test for Tannins
0.25 g of various solvent extract was dissolved in 10 ml distilled water and filtered. 1% aqueous Iron (iii) chloride ($FeCl_3$) solution was added to the filtrate. An intense green coloration was taken as evidence for the presence of tannins

FT-IR Analysis
FTIR spectrophotometry as a research tool identifies functional groups and chemical bonds present in a sample mixture. The FT-IR data of the sample plant extracts were obtained using Fourier Transform Infrared Spectrophotometer (SHIMADZU FITR-8400S).

Ointment Preparation
1% weight of each crude extract was incorporated into pure petroleum jelly (as the application vehicle) and homogenized with continuous stirring at 60 ^{o}C for 2 – 3 minutes, and then cooled to solidify in a water bath.

Animal studies
The experimental protocol was approved in accordance with the ethical guidelines for investigations in laboratory animals. Five groups of male albino rats, each having 5 animals, were used for the ointment tests at the Experimental Animals Unit, Departmental of Veterinary Physiology and Biochemistry, University of Ibadan. The skin at the back of each animal was shaved using an electric shaver to expose the test areas and the animals were kept under visual observation for any irritation or erythema for 24 hours. Equal quantities of the test ointments were administered topically on the shaved areas for 21 days. Hair growth activity of extract was determined by observing the difference in hair growth initiation time, hair growth completion and hair lengths. A 20th, 25th and 30th-day hair length study was scheduled for so as not to cause injuries to the animals while plucking hairs strands in the regrowth regions.

$$\% \text{ hair regrowth} = \frac{Hair\ length\ on\ regrowth\ area}{Hair\ length\ of\ unshaved\ area}\ x\ 100$$

After 28 days, one animal from each group was sacrificed and samples were taken from the treated areas, preserved in 10% formalin and sent to the lab for skin biopsy tests.

Statistical Analysis
Analyses of data was carried out by one way ANOVA comparing all test groups versus control using SPSS 20.0 for Windows (SPSS Inc, USA).

III. RESULTS AND DISCUSSION
Yields

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The percentage recovery yield of the leaf methanolic extract (23.5%) was the highest in all four extractions (see Table 1.1). From the results, the approximately 6% higher yield of methanol extract of the leaves compared to those of the flowers suggests that the leaves have greater repository of secondary metabolites, which in lay terms mean the leaves possess more medicinal property for various applications. For the n-hexane extracts, the slightly higher yield obtained for the flowers than the leaves suggest that the leaves possess less non-polar components required for the same application.

Table 1.1 Yields of *Datura metel* flower and leaf extracts

Plant part	Extracting solvent	Weight of sample (g)	Weight of extract (g)	Percent extracted (%)
Flowers	Hexane	100	6.5	6.5
	Methanol	90	16.04	17.8
Leaves	Hexane	700	41.2	5.8
	Methanol	350	82.3	23.5

Phytochemical Screening

The results in Table 1.2 show that n-hexane extracts of *Datura metel* flowers and leaves contain Saponins, Sterols and Glycosides. The leaf and flower methanol extracts both contain alkaloids, saponins, tannins, flavonoids, phenols, glycosides and reducing sugars. According to the tests, steroid compounds were present in the methanolic flower extracts but absent in those of the leaves while Terpenoids were absent in the flowers but present in the leaves. Trace amounts of steroids are present in the methanolic leaf extracts. The positive tests confirm that *Datura metel* has a rich arsenal of hair restoration phytochemicals. Steriods function as strengtheners while flavonoids and triterpenoids reportedly enhance hair growth promoting activity by strengthening the capillary wall of the smaller blood vessels supplying hair follicles and improving blood circulation to nourish the hair follicles, thereby promoting hair growth. The lipid components of hair, produced in the hair bulb, are formed from sterols, fatty acids and ceramides. This mixture of triglycerides, waxes and squalene form a film on the surface of the skin and lubricate the hair, thus preserving its suppleness and sheen [14, 15].

Table 1.2 Phytochemical results of *Datura metel* flowers

	Hexane extract		Methanol extract	
Phytochemicals	Leaves	Flowers	leaves	flowers
Alkaloids	–	–	+	+
Saponins	+	+	+	+
Flavonoids	–	–	+	+
Terpenoids	–	–	+	–
Tannins	–	–	+	+
Sterols	+	+	–	+
Phenols	–	–	+	+
Glycosides	+	+	+	+
Reducing sugars	–	–	+	+

+ = positive test; – = negative test

FT-IR Analysis

Fig. 1.3a & b shows analogous IR absorption patterns for *Datura metel hexane* flower and leaf extracts. The same is also seen in the methanol extracts (Fig. 1.3c & d). The n-hexane flower extract (HLE) has 20 peaks while the hexane leaf extract (HLE) has 18 peaks. However, their peak heights and widths are different, indicating the presence of more or less of the same bonds for a group of compounds. The absorption (doublet) for both HLE and HFE at 2935 and 2868cm^{-1} indicates C–H$_{stretch}$ for aldehydes, but the peak heights for HLE doublets (16.56 & 34.87) are higher than those of HFE (2.99 & 1.09) indicating that there are more C–H$_{stretch}$ bonds for saturated aliphatic aldehydes in the leaves. For both samples, there is the C=O$_{stretch}$ aldehydes at 1739 cm^{-1} (HFE, 10.8) and 1734 cm–1 (HLE, 57.5), O–H$_{stretch}$ intermolecular (hydrogen) bonded alcohols between 3550 and 3100 cm^{-1} and C–H$_{bending}$ alkanes 1461 cm^{-1} (HFE, 13.1) and (HLE, 62.3).

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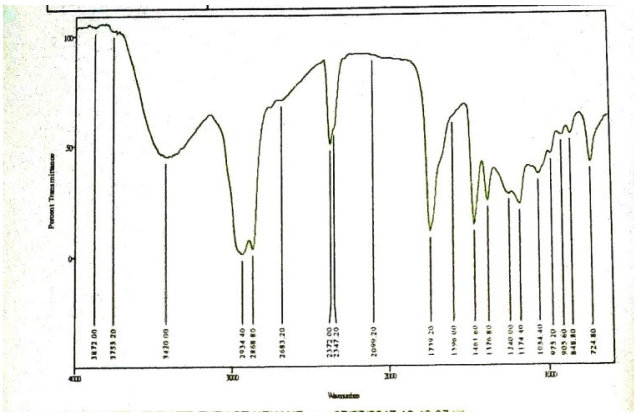

Fig. 1.3a n-hexane Flower extract (HFE)

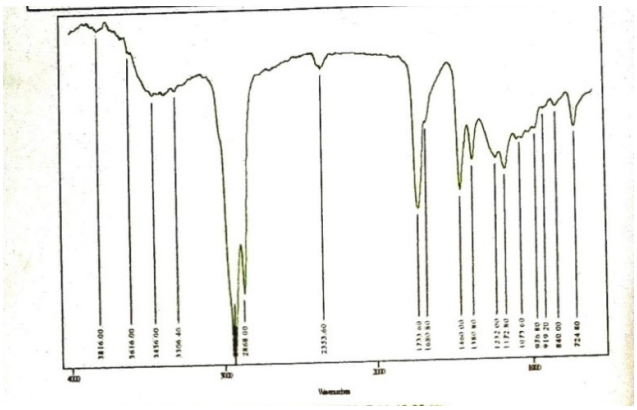

Fig 1.3b n-hexane Leaf extract (HLE)

For the methanolic extracts, there are 15 and 10 peaks for flowers (MFE) and leaves (MLE), respectively. Due to the broad bands in both extracts between 3700 – 2500 cm^{-1}, representing $O–H_{stretch}$ (for carboxylic acids, free alcohols and hydrogen bonded alcohols), other absorptions on the broad bands appear very weak to provide reasonable analytic information about more bonds present in the MFE and MLE extracts. Beyond the fingerprint region, both samples have strong absorptions at 2092 cm^{-1} representing $N=C=S_{stretch}$ isothiocynate (MFE, 37.14 & MLE, 59.0), medium $C=C_{stretch}$ alkenes at MLE 1638 cm^{-1} (8.66) & MFE 1640 cm^{-1} (9.46) and medium $S=O_{stretch}$ band – sulphates/sulphonyl chlorides at MLE 1404 cm^{-1} (11.26) & MFE 1399 cm^{-1} (7.33). Isothiocyanates have anti-nutritional or toxic effects. They are considered to be responsible for the protective, anticarcinogenic, antifungal, antimicrobial and insecticidal properties of plants [16, 17].

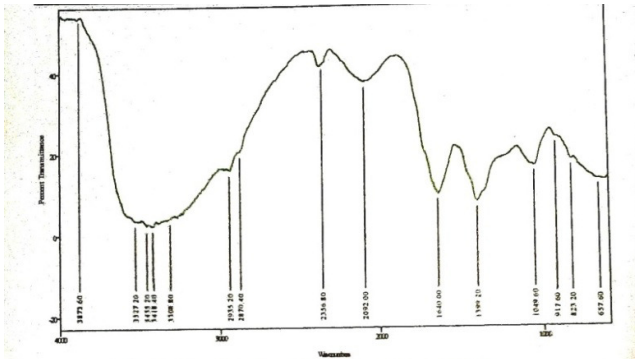

Fig. 1.3c methanol Flower extract (MFE)

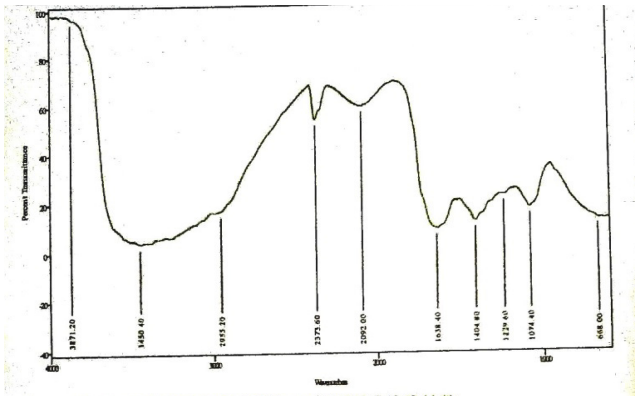

Fig. 1.3d methanol Leaf extract (MLE)

Animal Studies

Methanolic leaf extract (1% MLE) initiated hair growth on the fifth day and it took 13 days for hairs to completely fill the shaved areas, making the sample the fastest hair regrowth initiator on the albino rat model (Table 1.4). Methanolic flower extract (1% MFE) treated group was slower (12 days) than the control (10 days) and other groups in initiating hair growth. The slow hair growth initiation, on one hand, can be attributed to the absence of terpenoids in *Datura metel* flowers. On the other hand, the steroids present in the flower extracts tend to slowly strengthen the hair strands so that on regrowth the hairs seemed to grow much longer than the extracts that had faster hair regrowth initiation time. The results in Fig. 1.4 agree with the second case, where the hairs of

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animals treated with *Datura metel* flower extracts grew longer than those of the leaves, after 30 days.

Table 1.5a shows that the percentage regrowth hairs of n-hexane and methanol flower extracts, 1% HFE (80.6%) and 1% MFE (75.4%) were higher than the methanol leaf extracts, 1% HLE (72.7%) and 1% MLE (71.4%). The higher hair regrowth lengths by the hexane extracts supports the fact that natural oils are good strengthening components for the hair. Table 1.5b shows the correlation between weight of the treated and their average hair lengths. All samples showed finer, hard and denser hairs in the regrowth regions after 30 days. Increase or decrease in weight of the animals in some groups had no direct effect on the rate of hair growth, i.e. the relationship between weight gain and hair regrowth is not linear. An evidence of this from the research is that animals treated with 1% methanolic flower extract (Group F) which had slight reduction in their mean body weight from 132 g to 128 g, still had their mean hair lengths increased from 0.46 cm to 0.78 cm after a 5-day interval (between the 20th and 25th day). Therefore, weight gain more directly has to do with the health status of the body, that is, the healthier the body, the more the tendency for the growth of finer hairs.

Table 1.4 Effect of ointment formulations on hair growth of albino rat model

Group	Treatments	Hair growth initiation (days)	Hair growth completion (days)
A	Control (vehicle only)	10±0.51	17±0.75
C	1% HLE	6±0.37*	16±0.37
D	1% MLE	5±0.51	13±0.55
E	1% HFE	9±0.37*	15±0.51
F	1% MFE	12±0.32*	19±0.86

*Data are presented as mean ± SEM, n = 5 *P < 0.01 significance vs control*

Table 1.5a Percentage regrowth hairs

Group	Treatments	Regrowth hairs after 30 days (%)
A	Control	73.0
C	1% HLE	72.7
D	1% MLE	71.4
E	1% HFE	80.6
F	1% MFE	75.4

Table 1.5b Mean weight (MW) and hair lengths (HL) of treated animal groups (G) after 20, 25 and 30 days

G	After 20 days		After 25 days		After 30 days	
	MW (g)	HL (cm)	MW (g)	HL (cm)	MW (g)	HL (cm)
A	101.5	0.52±0.058	114.0	0.83±0.073	130.3	0.92±0.058
C	149.0	0.92±0.49	147.0	1.00±0.037	158.5	1.12±0.066
D	88.3	0.50±0.633	93.0	0.76±0.087	105.5	0.80±0.054
E	122.0	0.78±0.663	123.5	1.02±0.049	139.5	1.16±0.051
F	132.4	0.46±0.812	128.4	0.78±0.111	136.4	1.04±0.121

*Values for mean length are reported as Mean ± SEM, n = 5, *p < 0.05 (considered significant).*
SEM – Standard error in the mean

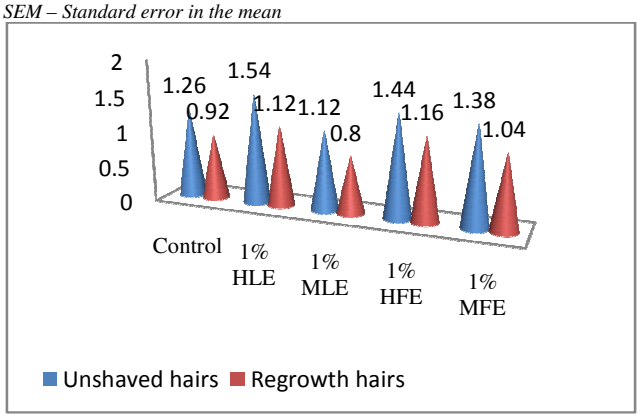

Fig. 1.4 Average hair lengths of treated animals after 30 days

Fig. 2 shows histological pictures of the skin of treated animals. In the control group, more resting telogenic phases were observed with Telogen/Anagen (T/A) ratio of 1.18. From both set of extracts, the leafy portions showed slightly higher response with more actively growing anagenic phases than those of the flowers. The T/A ratios for animals treated with methanolic extracts (D & F) were 0.45 & 0.48 while those treated with hexane extracts (C & E) were 0.60 & 0.61. Based on the results, treatment with methanolic leaf extracts caused mass switching of (on average) 60.59±1.0% hair follicles from the telogenic to the anagenic phases while hexane leaf extracts had 48.73±1.0% of the same influence. The hair follicle switching caused by the same extract (say methanol) are not significantly different and simply informs us that the methanol extracts had more dynamic effect in stimulating cyclic phases on the hair follicles than their hexane counterparts.

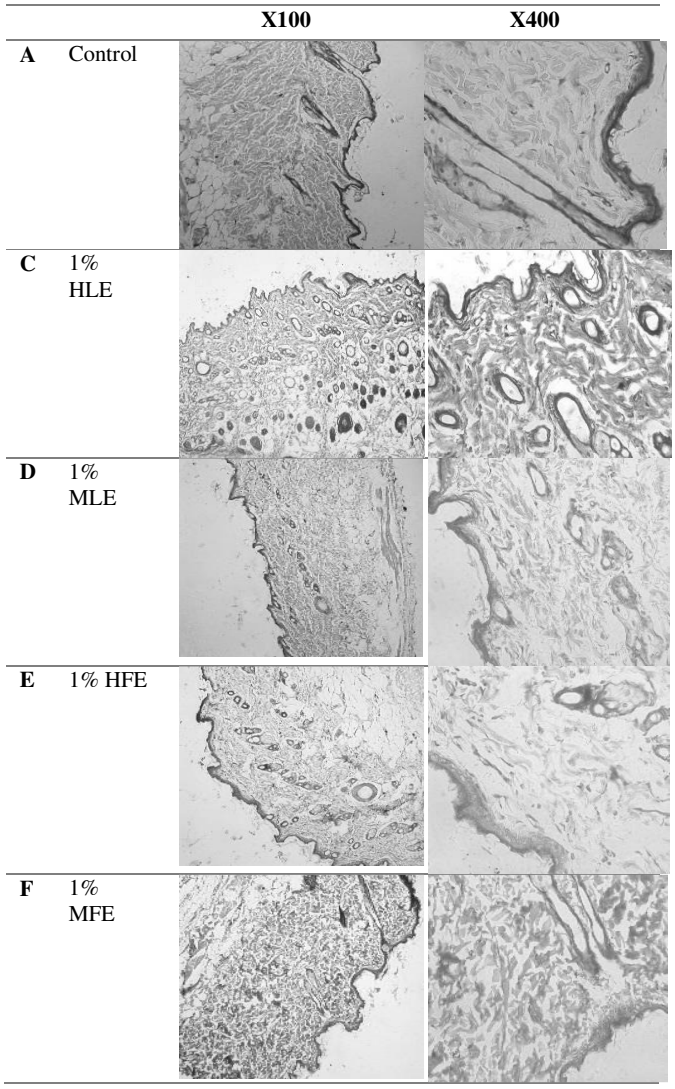

Fig. 2 Histological pictures of skin of treated animals

IV. CONCLUSIONS

The medicinal tendencies of *Datura metel* leaves and flowers towards wide-range applications; treatment of diseases, skin care and other health related problems have been far-reaching, due to their large store of active secondary metabolites. The FTIR spectra, using the same type of extracting solvent, have shown that the distribution of bioactive components between the leaves and the flowers varies slightly, with the leaves seen to contain more of the same bond types. It is clear that methanolic leaf extract is suitable for initiating faster hair regrowth while hexane flower extract prompts the growth of longer and denser hairs. The extracts showed complementary effects in inducing hair regrowth and did not cause any irritation on the skins of the albino rat model. It remains to be seen how much *D. metel* leaves and flower extracts can be optimized in a single mixture for hair formulations. Ethanol can also be a useful extracting solvent to affect the growth of dense, coarse and hard hairs without adverse effects.

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