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Pharmacognostical standardization and in vitro pediculocidal activity of *Datura innoxia* and *Cyclea peltata*

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Abstract

Datura innoxia and *Cyclea peltata* are two medicinally important plants with multiple medicinal values. The folklore of India uses these two plants for pediculocidal activity. Hence we have initiated to standardize both the plant parts pharmacognostically and then evaluate its pediculocidal potency. The micro anatomical studies of seeds of *D.innoxia* and leaves of *C.peltata* were studied. The proximate analysis, fluorescence analysis, preliminary chemical tests were performed for the both plant parts. Fixed oils were isolated from seeds of *D.innoxia* and the GC-MS studies were performed. The selected crude extract of *C.peltata* and the fixed oil of *D.innoxia* were evaluated for pediculocidal activity by filter paper diffusion bioassay. Permethrin(1%) was used as the standard and water as control. Lignified sclerenchyma was identified as tissue of diagnostic importance for *D.innoxia* seed and lignified unicellular covering trichomes was found to be the most important tissue of diagnostic importance for the leaves of *C.peltata*. Both the plants showed the presence of alkaloids as one of the major chemical constituents. GC-MS report of fixed oils from *D.innoxia* revealed the presence of different types of fatty acids. Both the plants showed potent pediculocidal activity. Among the two plants *Datura innoxia* seed oil was found to be best. It showed almost same activity as that of standard drug used. Further investigations have to be carried out on the isolated compounds which can set a base for the development of lead compounds with potent pediculocidal activity.

Keywords: *Datura innoxia* ,*Cyclea peltata*, pediculocidal, nits, nymphs

INTRODUCTION

Human head lice infestation is a common condition worldwide, but more prevalent in children. The louse clings to the hair near the scalp and the multiple obligatory feeding each day of the host's blood results in pruritus reaction from the lice saliva.

They may have considerable impact on health of humans. Their bites, especially in cases of huge infestations, may lead to enormous pruritus, skin inflammation, urticaria, exudations, lymph node swellings, eczema and scars [1]. Lice are small, wingless insects that cannot live independently from their hosts. They are frequent parasites of birds and mammals, each host species having its own type of louse. Humans harbor three kinds of these ectoparasites: head lice, body lice and pubic lice. *Pediculus humanus capitis*, the so-called head louse, is anobligate ectoparasite which is found exclusively on humans. These lice have evolved with mankind and, thus,were distributed all over the world One of the reasons of the constant spreading of head lice is surely the increasing globalization, leading to a closer contact between many individuals. This is important since the transmission of head lice occurs practically exclusively by close hair-to-hair contact. The second reason for the recent constant spreading of lice is the reduction of the efficacy of available anti-louse compounds since many of them had been used for a very long time. This recent lack of efficacy is surely based on a variety of reasons among which are the incorrect use and development of resistances [1,2].

Datura innoxia is commonly called Angel's Trumpet and belongs to the family Solanaceae (Figure: 1). *Datura innoxia* is an annual shrubby plant that typically reaches a height of 0.6 to 1.5 metres. Its stems and leaves are covered with short and soft grayish hairs, giving the whole plant a grayish appearance. It has elliptic entire-edged leaves with pinnate venation. All parts of the plant emit a foul odor similar to rancid peanut butter when crushed or bruised, although most people find the fragrance of the flowers to be quite pleasant when they bloom at night. The flowers are white, trumpet-shaped, 12–19 cm (4.75-7.5) in long. It flowers from early summer until late fall.The fruit is an egg-shaped spiny capsule, about 5 cm in diameter. It splits open when ripe, dispersing the seeds The chief chemical constituents present in the seeds are Scopalamine , atropine ,resin and fixed oil.*Datura innoxia* plants are considered toxic. [3,4]

Figure: 1 *Datura innoxia*

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Cyclea peltata is commonly called raj patha belongs to the family *Menispermaceae*. It is a slender twining shrub, frequently climbing up on tall trees (Figure: 2).The leaves are simple, alternate, heart shaped, 2.5-10 cm long and 2.5-3.75 cm broad, stipule 5-10 cm long and nerves 7-11.The flowers unisexual, pale yellow, in axillary panicles. The fruits are ovoid drupes, brown or scarlet in color. The seeds are covered.The roots are tuberous, cylindrical, irregularly curved, with grayish brown surface. From the leaves cycleanine, (-) bebeerines, hayatinin, hayatidin, hayatin and (+) bebeerines and (+) querticol isolated The roots - External application of the paste of its roots and leaves is extremely beneficial, in infected wounds, sinuses, and skin diseases. The root juice is salutary in headache, as nasal drops. Patha is a valuable wound healer and antidermatosis herb.The leaf paste is used to cure head louse infestation [5,6,7].

Figure: 2 *Cyclea peltata*

Pediculosis and dandruff are two infectious disease which affect school going children, hence a treatment with plant based remedy would be a better choice as plants are considered to be safe and efficient[8]. The present investigation is aimed to set the pharmacognostic standards as well as screen the pediculocidal activity of the two medicinal plants.

MATERIALS AND METHODS

Plant Materials

Datura innoxia seeds were collected from the fields of the Nalgonda region , identified and authenticated by Dr Bhadraiah, Department of Botany, Osmania university, Hyderabad and *Cyclea peltata* leaves was collected from Irity, Kerala, identified and authenticated by Mr. A. Laxma Reddy Retired Professor in Botany, Nagarjuna Government College, Nalgonda. A herbarium was prepared and deposited in the Department of Pharmacognosy under the voucher no. 0407(*Datura innoxia*) and 10-11/032(*Cyclea peltata*). Both the plant materials were collected from the months of October to January,2010-11.

Collection of head lice

Adults, nymphs, and nits of *P. humanus capitis* were collected from children by combing through sections of the scalp using a clean comb. After combing, the lice were carefully removed from the teeth of the comb into plastic boxes. All the subjects had not been treated with any anti-lice products for the preceding 3 months.

Transverse section [9].

Free hand sectioning was done for the water soaked seeds of *Datura innoxia* and fresh leaves of *Cyclea peltata* to obtain a thin section. The section was stained with phluroglucinol-hydrochloric acid (1:1) and iodine to identify the anatomy of the section.

Powder microscopy [9]

The dried seeds and leaves were powdered with the help of an electric grinder till a fine powder was obtained. This was then subjected to powder microscopy, as per standard procedures mentioned.

Measurement of cell structure and content[9,10]

The microscopic measurements for various parts were performed as per standard procedures.

Leaf constants [9]

The stomatal number, stomatal index, vein islet number and vein termination number were determined as per standard references.

Determination of physico chemical parameters [10,11]

The parameters like total ash, acid insoluble ash, water soluble ash, water soluble extractive and alcohol soluble extractive values were determined.

Fluorescence analysis [10,11,12,13]

Powdered seeds and leaves were subjected to analysis under ultra violet light after treatment with various reagents.

Extraction

The dried seeds and leaves of the plants were powdered and 100g of both the parts were extracted with hexane and chloroform for *Datura innoxia* seeds and benzene, chloroform, butanol and water for *Cyclea peltata* leaves. The extracts obtained were subjected to various chemical tests as per the procedure mentioned in the standard reference books.

Thin layer chromatographic analysis[14]

The TLC of *D.innoxia* seed oil was performed on the solvent system for alkaloids(Ethyl acetate : methanol (90:10).The extract was spotted on pre coated plates of silica gel F and the spots obtained were visualized in UV chamber and with the help of dragendroff reagent.

TLC of *C.peltata* extract was performed for the solvent systems like chloroform: methanol: water (70:30:4) for saponins . The extract was spotted on pre coated plates of silica gel F and the spots obtained were visualized in UV chamber.

GCMS Studies of D.innoxia seed oil

GC was performed to fatty acid methyl ester sample on an Agilent 6890 series gas chromatograph equipped with a DB-225 capillary column (30 m, 0.25 mm i.d. 0.5 mm). The column temperature was initially maintained at 160 ºC for 2 min, increased to 230 ºC at 5 ºC/min and hold for 20 min. The injector and detector temperatures were maintained at 230°C and 270ºC, respectively with a split ratio of 50:1. Nitrogen was used as carrier gas with flow rate of 1.0 ml/min. The injected oil was eluted in the DB-225 MS column and the eluted constituents were detected by FID and the GC chromatogram was recorded. Mass spectra were taken at 70ev (EI) with mass scan time 4 seconds. Identification of constituents was done on the basis of retention Indices. The mass spectra and relative retention indices were compared with those of commercial. Area percentages were obtained from the TIC (Total Ion Current) response without using an internal standard.

Screening of In vitro pediculicidal activity[15]

The *in-vitro* tests were started within one hour after collection of the organism. A filter paper diffusion bioassay was made. After careful selection of lice under a dissecting microscope, filter paper

discs (Whatman No 1) matching with internal diameter of petri dishes were cut and placed in it. 0.5 ml of each test solution(100mg/2ml-300mg/2ml for *C.peltata* and 1%,2%,3% for *D.innoxia* seed oil) was spread over the lice and filter paper. Negative control lice were placed directly on the filter paper spread with only distil water. The 1 % permethrin cream was simultaneously run as a positive control .Each group consisted of 10 lice. Always separate dish was taken for every test solution, control and standard solution. Petri dishes were then covered with another lid. The lice were considered dead if there were no vital signs such as movements of minimal leg movements upon stimulation with or without a forceps. 20 adults and nymphs were taken in each group. The test was done in triplicate and average considered.

Ovicidal activity [15]

The ovicidal activity was tested by placing 20 brownish oval eggs with an unbroken operculum on the filter paper (Whatmann No. 1) placed in the bottom of each petri dish.Then, 0.5 ml of each test solution and control were applied on the nits. All the dishes were then incubated in a dark chamber at 26 ± 0.5°C for 14 days. To maintain the moisture, 0.1 ml of distilled water was added at 48 hr interval. Hatching of eggs was monitored under a microscope and the percentage of emergence, i.e., partially hatched nits, was observed, and the findings were recorded [13]. Each treatment was replicated 3 times.

RESULTS AND DISCUSSION

The description of T.S is as per the reference book[16].

Transverse section of Datura innoxia seeds:

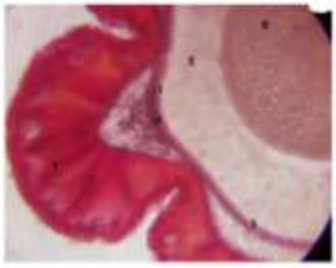

Figure: 3 Transverse section of *Datura innoxia* seeds
R- Radicle, E- Endosperm, I- Inner integument, O- Outer integument, T- Testa, S - Sclerenchymatous layer.

Testa was differentiated into outer and inner integuments. Outer integument consists of epidermis and sub epidermis (Figure: 3). Epidermis was well developed single layered polygonal cells. Sub epidermis consists of thick walled parenchyma cells. The inner integument consists of sclerenchymatous layer, pigment layer and endosperm. The sclerenchymatous layer consist of reddish brown single layered cells. Pigment layer is dark brown single layered thick walled cells. The cells of endosperm were colourless polyhedral parenchymatous with abundant aleurone grains and oil globules.

Transverse section of Cyclea peltata leaf : T.S of the leaf (Figure: 4) showed a dorsiventral nature. The section was divided into two regions, lamina and the midrib region.

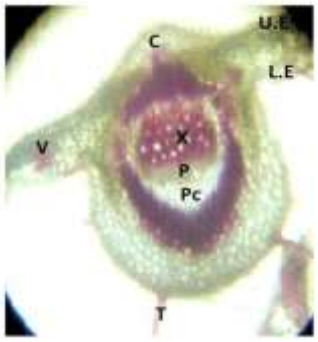

Figure: 4 Transeverse section of *Cyclea peltata*
U.E-Upper Epidermis, L.E- Lower Epidermis, C- Collenchyma, X- Xylem, P- Phloem,Pc- Pericycle, V- Vascular Elements, T- Trichome

Lamina:Upper epidermis was observed as continuous single layered polygonal cells covered on the outer side by a thin layer of cuticle. Lower epidermis was found similar to upper epidermis but number of stomata observed was more in number. Anisocytic type of stomata was observed. Mesophyll was observed as the ground tissue present between upper and lower epidermal layers. It was differentiated into an upper palisade cells and lower spongy parenchyma. Palisade cells were seen only on the upper epidermal region. The spongy parenchyma was seen below the palisade cells and was made up of loosely arranged parenchyma cells. Vascular elements were seen in the lamina region.

Midrib: The upper and lower epidermis was found to be continuous in the midrib region. But the palisade cells were discontinued in the upper epidermal region. 2-3 layers of collenchyma cells were observed below the upper epidermis and above the lower epidermis. A prominent arc shaped collateral vascular bundle occupying the central portion of the midrib was observed. Pericyclic bundle sheath was observed surrounding the vascular bundle region. Calcium oxalate crystals and starch grains were scattered throughout the section. Abundant unicellular lignified covering trichomes were observed in the upper and lower epidermal region.

Powder microscopy of Datura innoxia seeds

The powder microscopy revealed the presence of the following characters:

Calcium oxalate crystals in the form of rectangular shape were identified in the powder (Figure: 3).

Lignified Sclerenchymatous cells were observed (Figure: 4).

Starch grains with compound nature were observed and were found to be dark blue in colour when stained with iodine solution (Figure: 5).

Testa was seen as reddish brown coloured polygonal cells distributed through out the powder. This was identified as the tissue of diagnostic importance (Figure: 6).

Figure: 3 Calcium oxalate

Figure: 4 lignified

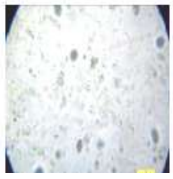

Figure: 5 starch grains Crystals Sclerenchymatous cells

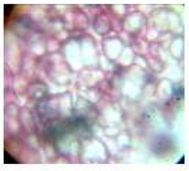

Figure: 6 Testa

Powder microscopy of Cyclea peltata leaf

Trichomes were found to be unicellular, slender, long and lignified. This was identified as the main tissue of diagnostic importance (Figure: 7).

Calcium oxalate crystals were observed(Figure: 8).

Lignified xylem vessel was observed (Figure: 9).

Stomata were observed in the powder (Figure: 10).

Starch grains are observed (Figure: 11).

Phloem fibres were seen as lignified (Figure: 12).

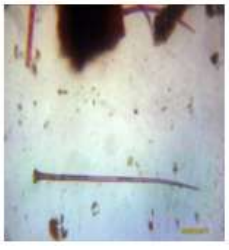

Figure: 7 Lignified trichome

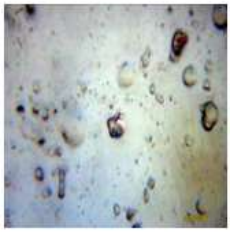

Figure: 8 calcium oxalate Crystals

Figure: 9 lignified xylem vessel

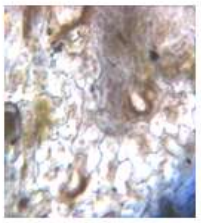

Figure: 10 stomata

Figure:11 starch grains

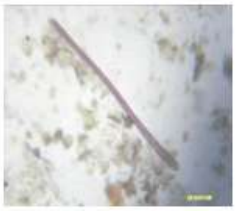

Figure: 12Lignified fibres

Measurement of cell structures and contents

The measurements of different cell structures and contents help to differentiate the species and adulterants. The measurements performed would help in fixing the standards and limits there by identification and authentication of the species. Measurement of the length of the trichomes of *Cyclea peltata*: 292.6µ-362.4µ-932.2µ and Diameter of starch grains in *Datura innoxia* was found to be *:* 13.25µ

Determination of Leaf Constants

Measurements set a limit and range for identification of authenticity and leaf constants (proximate analysis) determine the limits of extraneous matter that can be present in the plant specimen. Leaf constants aid to determine the adulteration and substitution of the drug, because these parameters are fixed to the particular plant. The leaf constants obtained for *Cyclea peltata* is tabulated in table:1

Table: 1 Leaf constants

Leaf constants	*Cyclea peltata*
Stomatal number	
Upper epidermis	-
Lower epidermis	15
Stomatal index	
Upper epidermis	-
Lower epidermis	20
Vein islet number	15
Veinlet termination number	11

Proximate analysis

Extractive values play a vital role for the evaluation of the crude. Alcohol and water soluble extractive values indicate the presence of the adulterants, faulty processing and poor quality of the drug. Ash values are used to detect the presence of any siliceous contamination and presence of any water soluble salts and incorrect preparation. It also denotes the concentration of inorganic salts present whereas the acid insoluble ash denotes the amount of dirt and sand present in the plant powder sample. The ash values and extractive values obtained for both the plant parts are denoted in table: 2&3.

Table: 2 Ash values

Parameter	*Cyclea peltata*	*Datura innoxia*
Total ash (%w/w)	8.4	7.2
Acid insoluble ash(%w/w)	1.32	1.14
Water soluble ash(%w/w)	2.13	0.98

Table: 3 Extractive values

Parameter	*Cyclea peltata*(%w/w)	*Datura innoxia*(%w/w)
Water soluble	27.6	65.1
Alcohol soluble	22.8	43.3

Fluorescence analysis

Fluorescence analysis of the powdered drugs were performed and tabulated in table: 4&5 which helps to detect the adulteration, because phyto constituents exhibits characteristic fluorescence under ultraviolet light when they got mixed with the reagents. The fluorescence exhibited by the mixture was attributed to the chemical constituents present in the crude drug.

Table: 4 Fluorescence analysis of *Cyclea peltata*

REAGENTS	DAY LIGHT	SHORT UV	LONG UV
Powder + 50%H_2SO_4	Pale Brown	Pale Green	Black
Powder +50%HNO_3	Green	Green	Black
Powder +5%NaOH	Pale Green	Green	Black
Powder+1NMethanolic NaOH	Pale Brown	Pale Green	Greenish Black
Powder +1N KOH	Pale Green	Pale Green	Greenish Black
Powder +5%KOH	Green	Green	Black
Powder +5% $FeCl_3$	Pale Green	Pale Green	Greenish Black
Powder +Methanol	Pale Green	Pale Green	Greenish Black
Powder +Conc. HCl	Pale Green	Greenish Brown	Greenish Black
Powder+Conc. H_2SO_4	Pale Green	Pale Green	Greenish Black
Powder+Ammonia	Pale Brown	Pale Green	Greenish Black
Powder +Conc .HNO_3	Pale Green	Pale Green	Greenish Black

Table: 5 Fluorescence analysis of Datura innoxia

REAGENTS	DAY LIGHT	SHORT UV	LONG UV
Powder + 50%H_2SO_4	Pale green	Green	Black
Powder +50%HNO_3	Brown	Green	Black
Powder +5%NaOH	Pale green	Palegreen	Black
Powder+1NMethanolic NaOH	Pale brown	Pale green	Light Black
Powder +1N KOH	Color less	Colorless	Light black
Powder +5%KOH	Green	Green	Black
Powder +5% $FeCl_3$	Pale green	Pale green	Greenish black
Powder +Methanol	Pale green	Pale green	Light black
Powder +Conc. HCl	Pale green	Pale green	Light black
Powder+Conc. H_2SO_4	Pale brown	Pale brown	Black
Powder+Ammonia	Pale green	Green	Black
Powder +Conc .HNO_3	Pale green	Pale green	Greenish Black

Preliminary phytochemical screening

The preliminary phytochemical screening is used to identify the presence of various chemical constituents present in the plant extracts. The presence of various primary and secondary metabolites can be confirmed by Thin Layer Chromatography (TLC).Phyto chemical evaluations like preliminary phytochemical screening was performed according to the standard procedures. The results obtained for the preliminary chemical test is given in table 6&7. These findings were further confirmed by TLC for the selected extracts namely aqueous extract of *Cyclea peltata* and chloroform extract of *Datura innoxia*(Table: 8) .

Table: 6 Preliminary phytochemical screening of *Datura innoxia*

Chemical constituents	Hexane	Chloroform
Proteins	-	-
Aminoacids	-	-
Carbohydrates	-	-
Alkaloids	+	+
Glycosides	-	-
Steroids	-	-
fixed oils	-	+
Tannins	-	-
Saponins	-	-
Flavonoids	-	-

Table: 7 Preliminary phytochemical screening of *Cyclea peltata*

Chemical constituents	Benzene	Chloroform	Butanol	Water
Proteins	-	-	-	-
Aminoacids	-	-	-	-
Carbohydrates	-	-	-	-
Alkaloids	+	+	+	-
Glycosides	-	-	-	-
Steroids	+	-	-	-
Fixed oils	-	-	-	-
Tannins	-	-	-	-
Saponins	-	-	-	+
Flavonoids	-	-	-	-

Table: 8 Thin Layer Chromatographic analysis

Chemical Constituents	*Cyclea peltata* (Rf value)	*Datura innoxia*(Rf value)
Saponins	0.8	-
Alkaloids	-	0.8

GC- MS studies: the GC-MS studies on the fixed oil isolated from the chloroform fraction of *D.innoxia* seed oil revealed the presence of 10 long chain fatty acids which is displayed in table 9 and figure 13.

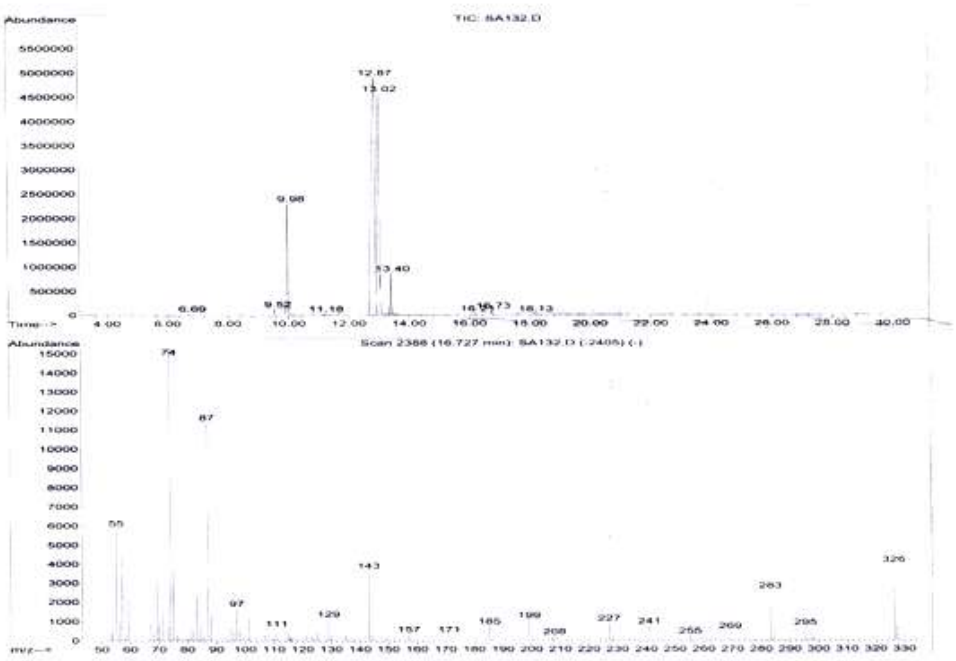

Figure 13: GC-MS report of D.innoxia seed oil

Table 9: GC-MS report of the identified fatty acids

S.No	Chemical constituents	RI	% Peak area
01	Methyl tetra decmoate	06.69	00.10
02	9-Hexa decmoic acid	09.53	00.42
03	Hexa decanoic acid	09.98	10.10
04	7-Hexa decmoic acid	11.18	00.11
05	8,11-Octa deca diennic acid	12.86	54.68
06	11-Octa decmoic acid	13.02	31.07
07	Octa decanoic acid	13.40	02.88
08	Cyclo propaneoctanoic acid	16.21	00.13
09	Ecosanoic acid	16.73	00.34
10	Dodecanoic acid	18.13	00.17

Screening of Antilice activity

The *invitro* pediculocidal activity by filter paper diffusion study showed both plants as potent anti lice agents. It was observed that *D.innoxia* seed oil at a concentration of 3% showed a potent activity equal to that of standard 1% permethrin. *C.peltata* leaf extract also showed significant activity but it was observed to be less when compared to *D.innoxia* seed oil and standard permethrin(Table 10). The petri dish with adult lice at 300mg/2ml died within 10 minutes of commencement of the activity, whereas the adult lice in the petridishes with 3% *D.innoxia* seed oil and 1% permethrin died by 5 minutes and 3 minutes respectively. The nymphs took less time to die when compared to the adults. It took 7 minutes for *C.peltata* at 300mg/2ml, 3minutes for *D.innoxia* seed oil and 2 minutes for 1% permethrin.In the control treated group all the insects were found to be alive and active. It was observed that all the nits were hatched in the group treated with distilled water by 14 days of incubation where as 3%*D.innoxia* seed oil and 1% permethrin has shown 100% hatching inhibition. The *C.peltata* leaf extract has shown 98% hatching inhibition at 300mg/2ml on the 14th day of incubation. The lower concentrations of both the plant extracts delayed the emergence of the lymphs. The potent pediculocidal activity of both plants can be attributed to the high alkaloid fraction present in the extracts. The nit with nymph was observed in the microscope and the microscopic picture was taken (figure 14). The hatching of a nit in the control treated group was observed under the microscope and the photograph is displayed in figure 15. The legs and eyes of the insect can be clearly observed in the figure 15. The empty shell without the larva is also displayed in figure 16.

Table: 10 Screening of Antilice activity

Test sample	Concentration	Died (Mean±SEM)		%inhibition of egg hatch	
		Adults	Nymphs	Nits	Nits
Cyclea peltata	100(mg/2ml)	04.75±0.25*	3.75±0.25*	7.6±0.3*	38
extract	150(mg/2ml)	09.75±0.25*	11.75±0.25*	12.3±1.45*	61
	250(mg/2ml)	15.75±0.25*	14.75±0.25*	15.33±0.66*	76
	300(mg/2ml)	18.25±0.25**	19±0.25**	19.45±0.57**	98
D.innoxia nil	1%	08.33±0.33*	11.66±0.3*	13.25±0.25*	65
	2%	16.67±0.33**	18.66±0.3**	18.60±0.6**	78
	3%	20±0	20±0	20±0	100
Standard	1%	20±0	20±0	20±0	100
Distilled water (0.5 ml)	0.5ml	00	00	00	00

The results represent mean ± SEM, where n=3.One way ANOVA followed by Dunnett Multiple comparison, Test Vs control was perfomed.**P<0.01and *P<0.1 were considered statistically significant.

Figure 14: A typical nit

Figure 15: A nit in the hatching stage

Figure 16: An empty nit shell

CONCLUSION

The pharmacognostical standardization was performed for the seed and leaf of *Datura innoxia* and *Cyclea peltata* which has traditional claim of anti lice activity. Hence these parameters will help in proper identification and authentication of the plant parts, moreover it will also aid in the development of plant profile. Both the plants have proven to be a potent anti lice agents.These plants can be very well substituted with the synthetic anti lice agents which are

reported with several side effects.

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