



Design and evaluation of an herbal anti lice shampoo containing *Brassica juncea* (L.) Czern.

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Brassica juncea (L.) Czern. leaves have been ethnomedicinally used by the people of Assam for getting rid of *Pediculosis capitis* (lice infestations) since time immemorial. *Pediculosis capitis* is a widely occurring and highly transferable condition affecting millions worldwide. Both pharmacological and non-pharmacological approaches are utilized to get rid of this condition, but with the advent of lice becoming resistant to most chemical anti-lice agents, researchers are turning towards herbal alternatives. This current study is devoted to establish the potential present in *Brassica juncea* against this parasite and to use it when currently prevalent pediculocides fails to combat the parasite. In addition to this, a laboratory-based shampoo is formulated to provide an aesthetic, reliable, safe and patient friendly medium to deliver the agent. Lastly both the extract and the medicated shampoo are tested on lice following a definite protocol and the results are compared to that of a marketed formulation.

Keywords: Anti-lice, *Brassica juncea*, Ethnomedicinal, Pedulocidal, Shampoo

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Pediculus humanus capitis (or human head louse) is an ecto-parasite of mammals, with minute, dorsoventrally flat and wingless body, free horizontal head, claws for clinging to hair, and degenerated eyes¹. With piercing and sucking mouthparts, they feed on human blood and are found to transmit infections like typhus, trench and relapsing fevers. With a three stage life cycle, *Pediculus humanus capitis* can breed throughout the year in the favorable temperature of 32°C². Offspring emerge from eggs, known as nits, which are glued to hair by the ovipositor. Nymphs hatch out in 8 days and mature in about 11 days to completely resemble the adult^{2,3}. The most common treatment available for this lice infestation is benzyl alcohol, which works by stunning the respiratory spiracles of the louse, such that they remain opened; this led to blockage and subsequent asphyxiation. Other chemicals available include the likes of lindane, permethrin⁴. However, the efficacy of lindane has been reported to decrease by 200× and in another case permethrin by 20× within 3 years of introduction in Israel^{1,5}. This pharmacological resistance of lice has raised a serious

concern among healthcare personnel and researchers have turned to alternatives, especially herbal ones to tackle this ever-increasing problem.

Brassica juncea (L.) Czern. (commonly known as black mustard) has been used by the indigenous population of Northeastern part of India (especially Darrang District of the state of Assam) to treat lice infestations in females. *Brassica juncea* is an edible plant which is available throughout India, the seeds of which are used as condiment. The oil extracted from the plant is used as cooking oil in most parts of the country⁶. Scientifically the plant has been found to be useful in the management of many disorders like diabetes, cancer, worm infestations etc. It is also found to contain a very good antioxidant, antipruritic and wound healing properties⁷. The primary constituents of *Brassica juncea* include brassicasterol, sinapine, campesterol, vitamin a, sinigrin and sitosterol etc⁸.

The main objective of this study is a twofold one. Firstly, an attempt was made to establish the scientific potential of *Brassica juncea* to eradicate lice infestations Secondly, the extract of *Brassica juncea* was incorporated into a shampoo formulation

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followed by the evaluation of its stability and pedulocidal activity.

Materials and Methods

Plant material

The entire plant of *Brassica juncea* Lin. was collected from local vendors of a vegetable market located in Narengi, Guwahati, District Kamrup, Assam, India (N 26°10'52.32", E 91°49'56.26") during the month of March & April. During these months the vegetable is found in abundance in the local markets. The authentication of the plant specimen was carried out by the help of Botanical Survey of India (BSI), Eastern Regional Centre, Shillong, Meghalaya vide Accession No. BSI/ERC/Tech./2013/251.

The collected plant materials were thoroughly washed in running water, segregated from any unwanted material (grass, dirt, soil etc.) and the morphological characteristics of the plant (viz., height, flower colour etc.) were noted. Then leaves were separated from the rest of the plant and dried in shade for 30 days. The shade-dried leaves were made into coarse granules and were used for further investigation.

Chemicals

All reagents and chemicals used to perform the analyses in this research work were analytical graded, procured from trusted manufacturers.

Preparation of extract

The shade-dried leaves of *Brassica juncea* (Lin.) Czern. were powdered to get a coarse granule in a mortar. About 500 g of dried powder was extracted with 400 mL of methanol: water mixture (4:1) at 55-60°C by continuous hot Soxhlet extraction procedure for 72 h. The methanolic extract was then filtered, concentrated by using rotary evaporator (Fig. 1a) and stored in a 4°C refrigerator after reconstitution with 3% dimethylsulphoxide (DMSO).

Organoleptic evaluation

The macroscopical characterization of *Brassica juncea* was done by analyzing the colour, odour, shape, taste and surface morphology. Structural features were determined using a simple microscope at 10× magnification.

Phytochemical screening

To qualitatively determine the presence of various phytoconstituents, preliminary tests were carried out as per established protocols⁹.

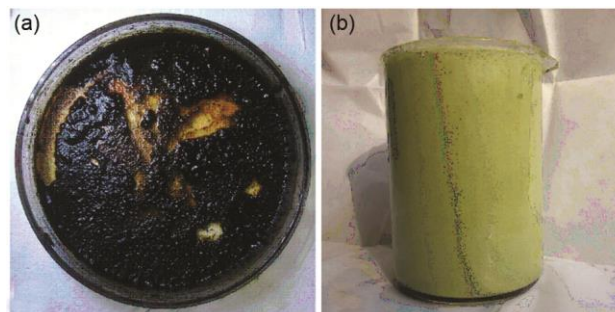


Fig.1 — (a) Dried methanolic extract of the leaves of *Brassica juncea*; (b) Shampoo formulation containing extract of *Brassica juncea* leaves.

Table 1 — List of detecting reagents used for specific detection of specific phytoconstituents

Sl. No.	Detecting Reagent	Compounds detectable
1.	Ferric chloride	Phenolic compounds, tannins and coumarins.
2.	Ninhydrin in acetone	Amino acids
3.	Dragendroff's reagent	Alkaloids
4.	3,5 dinitrobenzoic acid	Cardiac glycosides
5.	2,4 dinitro phenyl hydrazine	Aldehyde or ketones

Quantitative standards

The determination of the quantitative standards for the crude drug were examined as per the protocols mentioned in the Indian Pharmacopoeia^{10,11}.

TLC fingerprinting of *Brassica juncea* leaf extracts

The extracts were subjected to Thin Layer Chromatography using silica gel G as the stationary phase. Glass TLC plates of 15 cm × 20 cm dimensions were coated with a 0.25 mm thick layer of Silica Gel G (HiMedia Laboratories, Mumbai, India); air dried and heat activated at 110°C for 15 min. The TLC of the methanolic extract of *Brassica juncea* was carried out and the spots were visualized using specific reagents for specific phytoconstituents. The reagents and their corresponding phytoconstituents are depicted in Table 1.

Preparation of herbal shampoo

The herbal shampoo containing the extracts of *Brassica juncea* was prepared using the methods described by Mitthal and Saha¹² and Sharma¹³. A dispersion of extract in bentonite was prepared. Sodium lauryl sulphate (SLS) was heated with water; it was then stirred and heated to a temperature of 90°C. Half of this part was then added, with continuous stirring, to the bentonite dispersion to get a homogenous mixture. Then the rest of the SLS mixture was added to it. The mixture was cooled to 40°C and perfume and preservative was added (Fig. 1b).

Evaluation of herbal shampoo

Visual inspection

The prepared shampoo was evaluated for its visual appearance, clarity and foam producing capacity.

Foaming ability and foam stability

The foam volume produced by the formulated shampoo was performed by taking 50 mL of 1% aqueous solution of the shampoo in a stoppered 250 mL graduated cylinder and vigorously shaking it for 10 times. The data was recorded after 1 min of the last shaking process and continued for 4 min by taking data readings at every 1 min¹³.

pH

The pH of 10.0% shampoo solution in distilled water was determined at a temperature 25°C^{13,14}.

Determination of percent of solids contents

The total weight of the solids left after evaporating 4 g of the shampoo on a pre-weighed evaporating dish over a hot plate was determined to estimate the solids present in the formulated shampoo¹⁴.

Dirt dispersion

1 drop of India ink stain along with 10 mL distilled water and 2 drops of the formulated shampoo was taken in a stoppered test tube and shaken vigorously for ten times. The presence of the black ink colour in the foam was estimated as None, Light, Moderate, or Heavy^{13,14}.

Cleaning action

The cleaning power of the shampoo was estimated by shaking 5 g of greased wool yarn in a flask containing 200 mL of water and 1 g of shampoo at 35°C with vigorous shaking for 4 min at the rate of 50 times per min. A control test was performed in the same manner excluding the shampoo in the water. Both the wool yarn samples were removed, dried and weighed to estimate the amount of grease removed by using the following equation¹⁴.

$$DP = 100 - \left(1 - \frac{T}{C}\right)$$

Where, "DP" is the percentage of detergency power, "C" is the weight of sebum in the control sample and "T" is the weight of sebum in the test sample.

Surface tension measurement

The surface tension was measured using Stalagmometer on a 10% shampoo solution prepared in distilled water, at 20°C. The number of solution drops between two points previously set was counted

(3 times) and then the surface tension was calculated using the formula¹⁵:

$$\sigma = \frac{V}{2} \times \pi \times r \times n \times \rho \times g$$

Where, "σ" is surface tension, "ρ" is the density of the measured liquid, "g" is acceleration due to gravity, "V" volume of the liquid, "r" radius of the tube, and "n" number of drops counted.

Viscosity measurement

The viscosity of the shampoo was determined using Brookfield viscometer (Brookfield Engineering Laboratories Inc., MA). The samples volume was fixed at 3 mL and measurements were made at 50 rpm¹⁶.

Pediculocidal activity of shampoo

The extract and the formulated shampoo were tested separately for head-lice activity according to the methods described previously^{17,18}. The extract and benzyl alcohol were separately dissolved in distilled water to prepare concentrations of 0.1%, 0.5% and 1.0% v/v. From these solutions, 0.5 mL was put in different Petri discs containing filter paper placed inside it and spread in a thin layer over a 2 cm² area. It was then covered to saturate the environment inside. Seven equal sized head lice were then placed in the Petri discs and covered. The mortality of lice on filter paper was detected under dissecting microscope and the non-moving head-lice were declared as dead. The counting was performed after every 5 min until all the lice were dead. Virtually complete lice killing was noted for the experiment to 5 h post dose¹⁹. Benzyl alcohol was the positive control and distilled water was used as a negative control.

For determining the efficacy of shampoo, the test was performed using 0.1% & 1.0% of the shampoo solution in distilled water, using the same method. A commercial anti-lice shampoo of the same concentrations in distilled water was used as a positive control and laboratory scale shampoo without extract as a negative control. In the entire experiment the temperature & relative humidity (RH) were maintained at 33°C (temperature of the human head) & 60-80% RH.

Results and Discussion

Organoleptic evaluation of the collected plant material

The collected leaves of *Brassica juncea* were evaluated for their organoleptic characteristics. The leaves were found to be approximately 30.50 - 48.30 cm long and 21.40 - 29.73 cm in wide.

Leaves were ovate in shape, with toothed lobes and frilled edges, constricted at various intervals along the sides with a tapering apex. The leaves were procured from the local markets and hence presence of some soil was observed on the leaf surfaces which were cleaned, shade dried and stored for further uses. After extraction the yield was found to be 22.42%.

Phytochemical screening

The methanolic extract of *Brassica juncea* obtained exhibited a greenish brown colour and characteristic odor. The extract was analyzed for their different phytochemicals and it was observed that the extract contained alkaloids, amino acids, carbohydrates, flavonoids, proteins, triterpenoid, tannins and phenolic compounds. The results are tabulated in Table 2.

Quantitative standards

Quantitative standards are numerical values which measures the quality and efficiency of the crude drugs. These values are tabulated in Table 3.

TLC fingerprinting of *Brassica juncea* extracts

The methanolic extract of the leaves of *Brassica juncea* were subjected to TLC using various mobile phase systems and detected using various reagents (Table 1). The results of the TLC fingerprinting along with the phytoconstituents detected are tabulated in Table 4.

Preparation and evaluation of shampoo

The laboratory scale shampoo that was formulated using the various ingredients as mentioned in Table 5. The methanolic extract of the leaves of *Brassica juncea* was incorporated into the shampoo as the active ingredient. The shampoo was found to be aesthetic and appealing to the eyes with a pale green color. The shampoo was evaluated for its visual aspects, foaming ability and foam stability, pH, determination of percent of solids contents, dirt dispersion, cleaning action, surface tension measurement, viscosity measurement and pediculocidal activity. The results are tabulated in Table 6.

Pediculocidal activity

The collected human head lice were treated with *Brassica juncea* leaf extract and anti-lice shampoo containing the methanolic extract of *Brassica juncea* leaves separately. Two different positive controls were maintained in the experiment. One; benzyl alcohol treated group which served as a positive control for *Brassica juncea* leaf extract; and the other

Table 2 — Phytochemical screening of methanolic extract of *Brassica juncea* leaves

Plant Constituents	Test	Methanolic extract of <i>Brassica juncea</i> .
Alkaloids	Dragendroff's test	Present
	Mayer's Test	Present
Amino acids	Ninhydrin Test	Present
Carbohydrates	Molisch Test	Present
	Fehling's Test (for reducing sugars)	Present
	Benedict's Test (for non-reducing sugars)	Absent
Fats and Oils	Saponification Test	Absent
Flavonoids	Shinoda Test	Present
	Alkaline reagent test	Present
Anthraquinone glycosides	Borntrager's test	Absent
	Modified Borntrager's test	Absent
Cardiac glycosides	Legal's test	Absent
	Liebermann's test	Absent
	Keller–Killiani Test	Absent
Saponin glycosides	Foam test	Present
Coumarin glycosides	Alkaline treatment of alcoholic extract	Absent
Gums	Sample hydrolysis followed by Benedict's test	Absent
Lignins	Sample+alcoholic phluroglucinol+HCl	Absent
Proteins	Biuret test	Present
	Million's test	Present
Steroids	Liebermann–Burchard's Test	Present
	Salkowski Test	Present
Triterpenoids	Noller's test	Present
Saponins	Foam test	Absent
Tannins and Phenolic Compounds	Sample+5 % FeCl ₃ Solution	Present
	Sample+Dilute HNO ₃	Present

The results depicted herewith are obtained from three replicates of the same test.

Table 3 — Ash values, extractive values and loss on drying of dried leaves of *Brassica juncea*.

Sl. No.	Parameters	<i>Brassica juncea</i> leaves
1	Total ash	0.67 ± 0.19
2	Acid insoluble ash	0.50 ± 0.18
3	Water soluble ash	0.13 ± 0.05
4	Water soluble extractive	10.26 ± 0.78
5	Alcohol soluble extractive	12.26 ± 1.67
6	Loss on drying	22.89 ± 2.36

Values are mean % ± S.D. The values were from three independent replicates

a commercial anti-lice shampoo served as a positive control for the fabricated shampoo containing the methanolic extract of *Brassica juncea* leaves. A distilled water treated group (negative control) was

Table 4 — Thin layer chromatography (TLC) of methanolic extract of *Brassica juncea* leaves.

Sl. No.	Phytoconstituents detected	R _f value
1.	Carbohydrates	0.75±0.09
2.	Phenolic compounds	0.59±0.05
3.	Flavonoids	0.73±0.06

Table 5 — Formula for anti-lice shampoo containing *Brassica juncea* leaf extracts.

Sl. No.	Ingredient	Utility	Composition (g/100 g)
1.	Bentonite	Absorbent, antimicrobial	5.0
2.	Hydroxy propyl methyl cellulose	Thickener	10.0
3.	Methanolic extract	Active pediculocidal agent	1.0
4.	Sodium lauryl sulphate	Surfactant	40.0
5.	Methyl paraben	Preservative	1.5
6.	Perfume	-	0.5
7.	Water	Vehicle	quantity sufficient

Table 6 — Evaluation of shampoo containing *Brassica juncea* leaf extracts.

Parameter	Result
Physical appearance	Pale green
Foam ability	Good
	0 min 15.0 mL
Foam stability	3 min 7.0 mL
	6 min 0.9 mL
	10 min 0.9 mL
pH	7.70±0.26
% of solid contents (% w/w)	2.02±0.17
Dirt dispersion (% detergency power)	Moderate
Cleaning action	25.21±2.91
Clarity	Moderate
Viscosity (cPs)	45.00±2.91
Surface tension (N/m) × 10 ³	45.96±1.77

also maintained. A placebo shampoo group was maintained to observe any effects of pediculocidal activity (if present) of the other ingredients present in the shampoo. The placebo shampoo formulation contained all the ingredients except the methanolic extract of *Brassica juncea* leaves.

It was observed that at both 0.1%, 0.5% and 1.0% concentrations, the extract required lesser time to paralyze or completely kill the head lice. The same was also found to be true for the laboratory fabricated shampoo containing methanolic extract of *Brassica juncea* leaves. The results are displayed in Fig. 2. The lice treated with distilled water and placebo shampoo formulation survived the treatment even after 5 h of treatment.

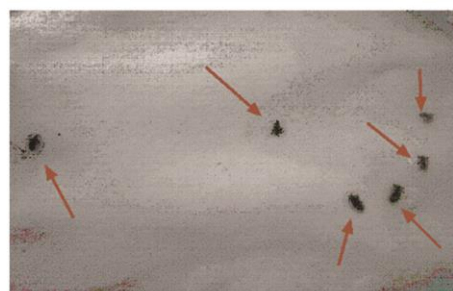
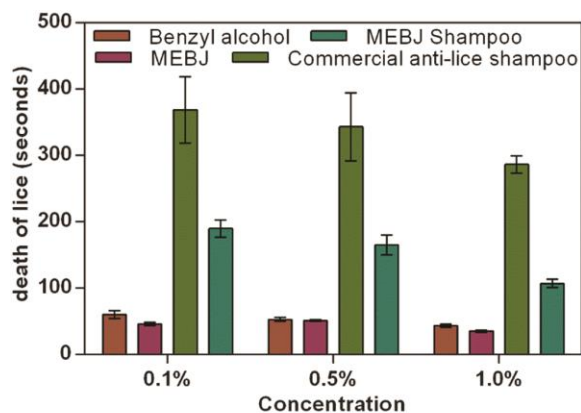


Fig. 2 — Pediculocidal activity of methanolic extract of *Brassica juncea* leaves and shampoo containing *Brassica juncea* leaf extract

Conclusion

A vast array of plant products have surfaced in the recent years to offer new avenues for the treatment of head lice. However the commercially available products for head lice consist of defined chemical insecticides like pyrethrin, permethrin and malathion. Since the prevalence of resistance of head lice to these chemical entities has increased, alternative topical therapies for pediculus have become the need of the hour. The results of the current study envisage that the extracts of the leaves of *Brassica juncea* (Lin.) Czern. and its shampoo has good pediculocidal activity. Thus, the topical application of the plant and its combination can be a good alternative therapy for management of head lice infestation. The formulated shampoo was also found to have good dirt dispersion, cleaning ability and anti-pediculocidal activity,

making it an ideal candidate for an OTC medicine for head lice infestations.

Conflict of Interest

The authors declare that there is no conflict of interest.

Author's Contributions

AS performed the experiments and acquired the data, BJS conceptualized the research and gave final approval for publication, NSB analysed the acquired data statistically, prepared the manuscript and handled all revisions of the manuscript as per the journal policies.

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