Pharmacognostical and physicochemical standardization of ethnopharmacologically important seeds of *Lepidium sativum* Linn. and *Wrightia tinctoria* R. Br.

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Quality control standardizations of the various medicinal plants used in traditional medicine is becoming more important today in view of the commercialization of formulations based on these plants. *Lepidium sativum* Linn. seeds are used as tonic, carminative in chronic liver enlargement and spleen diseases. The bruised seeds mixed with lime juice are applied to relieve the local inflammation and rheumatic pain. *Wrightia tinctoria* R. Br. seeds are astringent, acrid, thermogenic, carminative, digestive, stomachic, antisynergic, constipating, depurative, anthelmintic, aphrodisiac, febrifuge and diuretic. WHO recommends various physicochemical and phytochemical evaluation parameters for quality control of medicinal plants. In view of their medicinal importance and taxonomic confusion, morphology and microscopy, physico-chemical parameters, fluorescence analysis, preliminary phytochemical screening and quantitative estimation were performed to establish the salient diagnostic characters. The morphological, microscopical and physicochemical standards developed in this study will provide referential information for identification of these crude drugs and standardization.

**Keywords:** *Lepidium sativum*, Garden cress, *Wrightia tinctoria*, Pharmacognosy, Physicochemical, Fluorescence, Phytochemical.

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**Introduction**

After decades of serious obsession with the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda, Siddha and Unani to treat the various types of ailments. This is because of the adverse effects associated with synthetic drugs. Herbal traditional medicines have gained considerable momentum worldwide during the past decade and play a paramount role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers all plant parts to be potential sources of medicinal substances. However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and non-compliance of GMP guidelines basically due to poor standardization status. It is very important that a system of standardization must be established for every plant medicine in the market because the scope for variation in different batches of medicine is enormous. Due to natural heterogeneity, plant material may vary in its phytochemical contents and therefore in its therapeutic effect according to different places of collection, with different times in a year for collection, with collection at the same time and places but in different years and with different environmental factors surrounding the cultivation of a particular medicinal plant. Adding to this variability it is the fact that in herbal medicine several plants may be used together in the same preparation. These factors substantiate basic need of standardized quality control tests for herbal preparations to ensure quality of the product. There is an internationally increasing demand for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material used as traditional medicine for proper marketing authorization and approval. Morphological authentication is not sufficient to ensure quantitative consistency of bioactive or marker compounds.
responsible for the therapeutic effects. Advances in chemical and instrumental techniques have made it easier to estimate phytochemical parameters of crude drugs. The process of standardization can be achieved by stepwise pharmacognostic studies⁵.

Keeping in view the above mentioned problems, an attempt has been made to standardize the ethnopharmacologically useful seeds of *Lepidium sativum* Linn. (Garden Cress) and *Wrightia tinctoria* R. Br., commonly available and widely used in central India, on the basis of pharmacognostical and other physio-chemical characteristics.

*L. sativum* Linn. (Family-Brassicaceae) is commonly known as *Chansur*. This is a small, herbaceous, glabrous annual, 15-45 cm high plant cultivated as salad supplement throughout India. The seeds are reddish in colour, oblong, somewhat angular and slightly curved on one side with rugous surface⁶. *Chansur* is traditionally reported to have aperient, alternative, tonic, aphrodisiac and carminative property. A cold infusion is used to relieve of hiccough⁵. The seeds are used in treatment of chronic liver enlargement and spleen diseases⁶. As a carminative, they are given as an adjunct to purgatives. The bruised seeds mixed with lime juice are applied to the relief the local inflammation and rheumatic pains⁵. The leaves are mildly stimulant, diuretic and serviceable in scorbutic diseases⁷.

*Wrightia tinctoria* R. Br. (Family-Apocynaceae), commonly known as *Indrajau* is a small deciduous tree distributed in Central India, Burma and Timor. This plant grows in abundance in dry, hilly and rocky areas of Tamil Nadu, Andhra Pradesh, Madhya Pradesh and Rajasthan. The seeds resemble the seeds of Jau (Barley), this is the reason it is known as *Indrajau*⁸. They are useful in vitiated conditions of pitta and kapha, dyspepsia, bilious affections, flatulence, colic stomach pain, diarrhoea, leprosy, psoriasis, haemorrhoids, helminthiasis, fever, burning sensation and dropsy⁹. The plant is very useful as stomachic, in the treatment of abdominal pain, skin diseases, as antidiarrhoeal and antihaeorrhagic⁹. Bark and seeds are astringent, acrid, thermogenic, carminative, digestive, stomachic, antiysenteric, constipating, deputative, anthelmintic, aphrodisiac, febrifuge and diuretic¹⁰.

Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality, which will provide safety and efficacy of herbal medicine. This study was undertaken to generate standardized data on various pharmacognostical, phyto and physico-chemical characteristics of the plant materials. The outcome of the present study will be helpful in identification, authentication and quality control of the plant materials.

**Materials and Methods**

**Plant material**

The studies were executed in the fresh specimens of seeds of *Chansur* and *Indrajau*, collected from their natural habitat, surrounding Bhopal, M. P., India during Oct-Nov of the year 2009-2010. The seed of *L. sativum* was authenticated by taxonomist Dr. H. B. Singh of Herbarium Department, NISCAIR, New Delhi (voucher specimen no. NISCAIR/RHMD/Consult/2009-10/1232/36) and seed of *W. tinctoria* was authenticated by Dr. T. Hussain of Herbarium and Angiosperm Taxonomy Department, NBRI, Lucknow and a specimen voucher no. 97314 was assigned. Voucher specimens of the seeds have been retained in the department for reference purpose. The collected seeds were washed, shade dried and pulverized with mechanical pulverizer for size reduction. The size reduced seed powder was then passed through mesh 40-60 and used for determination of physicochemical parameters and preparation of different solvent extracts. The fresh seed samples were used for macroscopic and microscopic studies.

**Macroscopic and microscopic analysis**

The external morphology of seed such as nature, color, odour and taste were noted and other structural peculiarities like size, shape and texture were observed by using simple microscope. In microscopy, the desired part of fresh seeds were cut into pieces of 2-5 mm without compression and immediately transferred into FAA solution (95% ethyl alcohol: glacial acetic acid: formalin: water in 50:5:10:35) for one day to kill and fix the tissues. The pieces were embedded with paraffin wax. The paraffin embedded specimens were sectioned with the help of rotary microtome having thickness of 10-12 μm. Dewaxing of the sections was performed by customary procedure¹². The sections were stained with hemalum and safranin. A drop of HCl and phloroglucinol were used to detect lignified cell in the cut sections. The microphotographs were captured using trinocular microscope (Jyoti Scientific, Gwalior) with digital Olympus camera.
Preparation of extracts

Coarse powders (25 g) of each seed were defatted individually with sufficient quantity of petroleum ether (40-60°C) with the aid of Soxhlet apparatus for 24 h. The defatted seed cakes (5 g each) were then extracted separately with 100 ml each of ethyl acetate, chloroform methanol, ethanol and water for 48 h by maceration and then filtered to obtain respective extracts. The petroleum ether fraction obtained after defatting was recovered as petroleum ether extract after filtration. The extracts in different solvent were collected separately and volume reduced under low pressure. 25 ml of the each extract was used to determine the percentage extractive values of seeds in different solvents. The remaining extract was stored in air tight glass container at 4-8°C for fluorescence analysis.

Physicochemical studies

The percentage of foreign matter, loss on drying, total ash and acid insoluble ash were determined according to the method described in Indian Pharmacopoeia and the WHO guidelines on quality control methods for medicinal plants materials. The dried seed powders were subjected to fluorescence analysis, as it is and also after treating separately with 1 N of HCl, HNO₃, H₂SO₄, NaOH, KOH, alcoholic NaOH, alcoholic KOH and ammonia against normal and ultra-violet light (254 nm). Colour reaction of petroleum ether, ethyl acetate, chloroform, methanol and water extract was also observed in normal light and UV light (254 nm).

Preliminary phytochemical screening

Preliminary phytochemical screening of the seed extracts in different solvents has been performed to detect the phytoconstituents like; alkaloid, amino acid, carbohydrate, glycoside, inulin, mucilage, tannin, starch, saponin, steroid, triterpenoid and flavonoid.

Quantitative estimation of phytoconstituents

Alkaloid estimation

Seed powder was weighed (5 g) into a 250 ml flask; fitted with stopper and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 h. This was filtered and concentrated on a water bath to one-quarter of its original volume. Concentrated ammonium hydroxide solution was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle down to collect the precipitate, which was then washed with dilute ammonium hydroxide and filtered. The total alkaloid residue was dried and weighed.

Flavonoid estimation

Aluminium chloride colorimetric technique was used for flavonoids estimation. Each extract (0.5 ml of 1:10 g/ml) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm with a double beam UV/Visible spectrophotometer, (SHIMADZU, model no. 1700 series, Japan). The calibration curve was plotted by preparing the quercetin solutions at concentrations 12.5 to 100 g/ ml in methanol.

Saponin estimation

Crude sample (20 g) was put into a conical flask and 100 ml of 20% aqueous ethanol added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% aqueous ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated three times, and then 60 ml of n-butanol was added. The combined n-butanol extracts was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage.

Estimation of total phenols

The total phenols of both the extracts were measured at 765 nm by Folin Ciocalteu reagent. The dilute methanolic extract (0.5 ml of 1:10 g/ml) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous sodium carbonate (4 ml, 1 M). The mixture was allowed to stand for 15 min and the total phenols were determined by spectrophotometer at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/ ml solutions of gallic acid in methanol: water (50:50). Total phenol values were expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound.
Determination of swelling index

A fine seed material (1 g) was placed into a 25 ml glass-stoppard measuring cylinder. The internal diameter of the cylinder was 16 mm, the length of the graduated portion was approximately 125 mm, marked in 0.2 ml divisions from 0 to 25 ml in an upwards direction. 25 ml of water was added into the cylinder containing material and mixture was shaken thoroughly at interval of every 10 min for 1 h. Sample was allowed to stand for 3 h at room temperature. Volume was measured in ml occupied by the plant material, including any sticky mucilage. The mean value was calculated, related to 1 g of plant material.

Results and Discussion

Morphological evaluation of seeds

The organoleptic studies indicated the importance of characteristics such as typical tongue sensitizing aromatic taste, aromatic odour, etc. which are useful diagnostic characters. Morphological characters of both the species are given:

*L. sativum* (Chansur)

Seeds are small, oval-shaped, pointed and triangular at one end, smooth, about 3-4 mm long, 1-2 mm wide, reddish brown in colour. A furrow present on both surfaces extending up to two thirds downward, a slight wing like extension present on both the edges of seed. Soaked in water seed coat swells and gets covered with transparent, colorless, mucilage with mucilaginous taste (Plate 1).

*W. tinctoria* (Indrajau)

Seeds are 1.3-2 cm long, 1-2 mm wide, pointed at apex, linear, glabrous, light yellowish-grey in colour. Seeds are crowned with a deciduous coma or a tuft of white silky hairs of more than 3.8 cm long at the base by which they can disseminate in air. The seed resemble the seed of *jau*, thus it is known as *Indrajau* (Plate 2).

Microscopic characters of seeds

Detailed microscopic examination of a drug helps to identify the organized cellular structure of drugs material by their known histological characters. Use of various reagents or stains help to distinguish cellular structures depending on their chemical nature.

Histological studies were made from microtome sections of fresh crude drugs to characterize testa, endosperm, embryo, vascular bundles, trichomes, seed oil, pericarp, sclerenchyma, epidermis, cotyledons, aleurone grains and lipid globules. The transverse sections of seed showed the following characteristics:

Chansur

The transverse section of seed showed presence of testa, tegmen, aleurone layer, endosperm and embryo (Plates 3.1 & 3.2). Testa was thick, 1-2 layered and appeared yellowish brown whereas; tegmen layer was attached to inner side of testa layer and appeared as single layer. Endosperm was composed of thick walled polygonal cells. Embryo appeared as innermost structure surrounded by endosperm cells. The cells of embryo were small in size and polygonal in shape.
In normal seed, the outer cover testa was present and the inner layer tegmen is attached to inner side of testa layer. Transverse section showed distinct endosperm layer from embryo provided with aleurone layer. Small embryo occurs in groves, consisting shield shaped cotyledon known as scutellum (Plates 4.1 & 4.2).

**Physicochemical evaluation**

The physico-chemical parameters help in judging the purity and quality of the drug. The powder drugs were evaluated for its physico-chemical parameters like foreign matter, loss on drying, total ash, acid insoluble ash and different extractive values. Foreign matters were found to be 1.25% in Chansur and 1.36% in Indrajau. This may be due to first hand collection of plant material from non polluted area. Loss on drying turned out to be 6.44% in chansur and 3.42% in indrajau which is not too high, hence could discourage bacterial, fungal or yeast growth.

As ash value is useful in determining authenticity and purity of drugs and also these values are important quantitative standards. Content of total ashes in all powder were found to be relatively lower i.e. 5.40% in Chansur and 5.02% in Indrajau which may be due to low content of carbonates, phosphates, silicates and silica. This is also in accordance with low content of acid insoluble ash in Chansur (0.34%) and Indrajau (1.06%). The total ash is particularly important in the evaluation of purity of drugs, the presence of or absence of foreign inorganic matter such as metallic salts or silica. The results suggest that
Chansur and Indrajau seeds have high water soluble extractive value (mucilaginous and 39.84%) in comparison to the alcohol (15.36 and 29.92%), petroleum ether (1.288 and 1.820%), chloroform (1.656 and 1.956%), ethyl acetate (1.288 and 1.940%) and methanol soluble (6.540 and 5.436%) extractive values, respectively. It indicates the possibility of considerable amount of polar compounds and presence of large quantity of water soluble constituents such as sugar, glycosides, phenolics and tannins in the seeds.

Fluorescence analysis of drug powder and extracts

The result of fluorescence studies of seed powder using different reagents are given in Table 1 and that of the extracts is compiled in Table 2. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Many phytochemical fluorescence are seen when suitably illuminated. The fluorescence colour is specific for each compound. A nonfluorescent compound may fluorescent if mixed with impurities that are fluorescent. Some constituents show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which is not visible in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives after reacting with different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation.
results of fluorescence analysis of seed powders showed their characteristic fluorescent colour.

**Preliminary phytochemical test for extracts**

Preliminary phytochemical investigation was undertaken for the identification of different type of chemical constituents present in the seeds. Results of preliminary phytochemical screening are compiled in Table 3. Screening of chloroform extract indicated the presence of carbohydrate, glycoside, mucilage, starch and steroid in both the seed extracts. Methanolic extract of seeds showed the presence of alkaloid, inulin, flavonoid and amino acid. Tannin was found only in methanolic extract of Chansur. Whereas, aqueous extracts of both the seeds showed the presence of carbohydrate, inulin and mucilage. Triterpenoid and flavonoid was present in aqueous extract of both the seeds. Amino acid, mucilage and triterpene were present in ethyl acetate extract of both the seed. Petroleum ether extract of both the seeds showed presence of amino acid, inulin, mucilage and triterpene, whereas Indrajau also contains tannin and saponin.

**Quantitative estimation of phytoconstituents**

Quantitative estimation indicate that Chansur seed have higher percentage yield of flavonoid (2.50%) and saponin (2.96%), whereas the percentage yields of alkaloid and total phenol recorded were minimal (0.29 and 0.94, respectively). The percentage yield of flavonoid and saponin in indrajau was found to be 4.4 and 1.96% whereas yield of alkaloid and total phenol were 0.17 and 0.78%, respectively.

**Swelling index**

Swelling index of Chansur was observed 15 ml possibly due to presence of mucilage in seed.

*W. tinctoria* is commonly used as adulterant of an important antidysentric drug *Holarrhena antidysenterica* (Linn.) Wall., another Apocynaceae plant. Therapeutic properties of both the plants are quite similar. The differentiation of species are mostly done by morphological basis like size of leaf, colour of flower and root, taste of root and bark etc. Seeds of *H. antidysenterica* have hair at apex whereas *W. tinctoria* has tuft of hair at the base of seed.

**Conclusion**

This study establishes not only pharmacognostic and phytochemical characterizations of seed but also microscopic and fluorescence characters of both the seeds. These characteristics can be used further as identification and authentication parameters of the seeds. Both the seed are found to be rich in flavonoid and saponins having wide spectrum of bioactivity. The seed studied here can be seen as a potential source of useful therapeutics. Further studies are going on these seeds in order to isolate, identify, characterize and elucidate the structure of bioactive compounds along with their pharmacological activity.

**References**


