Pharmacognostic and Phytochemical Investigation of *Juglans regia* Linn. bark

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**ABSTRACT**

*Juglans regia* Linn belongs to family *Juglandaceae*. It is commonly known as Walnut tree. *Juglans regia* bark has been claimed to possess anti-inflammatory, blood purifying, anticancer, depurative, diuretic and laxative activities. The bark is finely powdered and used to prevent bleeding gums and as a mouth rinse. The present investigation deals with microscopic evaluation of bark and establishment of its quality parameters, including physicochemical, phytochemical evaluation, HPTLC analysis & Microbial load. Chief microscopic characters include cork, phloem fibres with stone cells & calcium oxalate crystals. Phytochemical screening revealed presence of reducing sugars; alkaloids; tannins & phenols; steroids & saponins. The bark powder was found to be free from pathogenic organisms. The study will provide referential information for the correct identification of the crude drugs.

**Key words:** *Juglans regia* Linn, Pharmacognostic study, Phytochemical analysis, HPTLC analysis.

**INTRODUCTION**

*Juglans regia* Linn known as Akhort in India, a native of Eastern Europe to North Asia i.e. China, Iraq, Mexico, Spain, Turkey, Nepal, India (forests in Himalayas) is a member of *Juglandaceae* family. It is a woody, deciduous and frost-tender tree growing to 20m height. The wood is heavy, durable and polishes well. The bark is resinous and scented. This valuable tree has a long history of medicinal use to treat a wide range of health complaints. Almost all parts of the plant are medicinally important. The root and stem bark are anti-helmentic, astringent and detergent. The stem bark is dried and used as a tooth cleaner. The decoction of leaves and bark is used with alum for staining wool brown.[1]

Herbal medicine is a triumph of popular therapeutic diversity. Plants above all other agents have been used for medicine from time immemorial because they have fitted the immediate personal need are easily accessible and inexpensive.[2] Human population in countries around the world has been using plants from thousands of years for treating various ailments of humans & animals.[3]

Herbal medicines are promising choice over modern synthetic drugs. They show minimum/no side effects and are considered to be safe. Generally herbal formulations involve use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained.[4-8]

Though the traditional Indian system of medicine has a long history of use, they lack adequate scientific documentation, particularly in the light of modern scientific knowledge.[9] To ensure reproducible quality of herbal medicines, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication followed by creating numerical values of standards for comparison. Pharmacognostical parameters for easy identification like leaf constants, microscopy & physic chemical analyses are few of the basic protocol for standardization of herbs.[10-11]

The numbers of reports of patients experiencing negative health consequences caused by the use of herbal medicine has increased in recent year. Analysis & studies have revealed a variety of reasons for such problem. One of the major
cases of reported adverse events are directly linked to the poor quality of herbal drug and raw medicinal plant materials.[1][2]

This traditional knowledge about the plants can be transferred to several generations only by proper documentation of their botanical, physicochemical, phytochemical characters and along with their medicinal uses in the form of monographs. The monograph of these plants are prepared according to the WHO guidelines and presented as herbal pharmacopoeia. These guidelines enable to identify, authenticate, detect adulterants and standardize the plant material.[3]

This present work, thus aims to standardize *Juglans regia* Linn bark by pharmacognostic and preliminary phytochemical analysis.

**MATERIALS AND METHODS**

**Collection and authentication**

*Juglans regia* Linn dried bark was procured from the local market in Mumbai. It was identified & authenticated by Prof. Bindu of Botany Department of SVKM’S Mithibai College of Science & Commerce, Vile Parle (West), Mumbai. The dried bark was used for section cutting & the bark powder was used for phytochemical analysis.

The morphological studies such as colour, odour and taste of *Juglans regia* bark were studied.

Microscopic sections were cut by free hand sectioning method. The sections of bark were cleared with chloral hydrate solution & then stained with phloroglucinol & HCl & mounted in glycerine. Numerous mounts of the hydrate solution & then stained with phloroglucinol & method. The sections of bark were cleared with chloral. Microscopic sections were cut by free hand sectioning of bark were studied.

**Physicochemical analysis**

Physicochemical properties such as the percentage of total ash, Acid insoluble ash, Water soluble ash, Alcohol soluble extractive & water soluble extractive values were determined as per the standard procedure.[4] Percentage of ash value is indicative of the purity of the drug and extractive values represent the presence of polar and non polar compounds in the extract.

**Fluorescence Analysis**

Fluorescence study is an essential parameter for first line standardization of crude drug. The crude powders were subjected to these studies & their fluorescence patterns were noted. The powder material were treated separately with different reagents & exposed to visible, ultraviolet light to study their fluorescence behaviour.[5] The colors obtained by application of different reagents in different radiations were recorded.

**HPTLC analysis**

Chromatographic finger-printing of phytoconstituents can be used for the assessment of quality consistency and stability of herbal extracts or products by visible observation and comparison of the standardized fingerprint pattern. The fingerprint has potential to determine authenticity and reliability of chemical constituents of herbal drug and formulations.

Chromatographic separation of hot & cold methanolic extracts of *Juglans regia* bark were performed on 10 cm × 10 cm aluminum-backed HPTLC plates coated with 200 μm layers of silica gel 60GF254 (Merck, Darmstadt, Germany). Standard solution of Gallic acid & Methanolic extracts (10 μL each) were applied on to HPTLC plate as 8 mm wide bands and 12 mm apart from middle of bands by spray-on technique along with nitrogen gas supply for simultaneous drying of bands, by means of a CAMAG Automatic TLC Sampler 4 (ATS4). A constant spot application rate of 10 μL/sec was used. Plates were developed to a distance of 80 mm at room temperature (28 ± 2 °C) with CHCl₃: Ethylacetate: Formic acid (7.5:6:0.5) (v/v) as mobile phase in a CAMAG glass twin-trough chamber previously saturated with mobile phase vapour for 20 min. Chromatography was performed in CAMAG’s twin-trough chamber. After development, the plates were dried in air & then scanned at 340nm with CAMAG TLC scanner with CAMAG winCATS planar chromatography manager software (version 1.4.2). The plate was later on derivatized with anisaldehyde sulphuric acid & heated at 105 °C till bands develop.

**Determination of Microorganisms**

Medicinal plant materials normally carry a great number of bacteria & moulds, often of soil origin. While a large
range of bacteria & fungi form the naturally occurring microflora of herbs, aerobic spore forming bacteria frequently predominate. Current practices of harvesting, handling & production often cause additional contamination & microbial growth. Determination of Total Viable count & detection of pathogens was performed as per the method in WHO guideline on “Quality Control methods for medicinal plant materials”.[19]

RESULTS AND DISCUSSION

Morphology
Bark of *Juglans regia* was dull blackish brown in colour. It was Thin with whitish epidermal layer tough and fibrous and somewhat mealy. Inner fibers were tough and flattened; the outer ones were white and silky. The taste of bark slightly Bitter and astringent

Microscopy
The transverse section of *Juglans regia* showed one cell layer thick cork on the outermost side of the bark. It also showed presence of phloem fibres with stone cells present in them. Crystals of calcium oxalate were found to be scattered amongst the stone cells (Figure 1-3).

Powder Microscopy
*Juglans regia* powder was brown in colour & showed presence of stone cells, fibers & calcium oxalate crystals (Figure 4).

Preliminary phytochemical test
Preliminary phytochemical test for hot & cold methanolic extract of the drug was carried out. Both the extracts showed the presence of reducing sugars; alkaloids; tannins & phenols; steroids & saponins (Table 1).

Physico-chemical constants
The powdered bark of *Juglans regia* was studied for their physico-chemical constant which included percentage of
total ash, acid-insoluble ash, water-soluble ash, alcohol soluble extractives (Table 2).

**Fluorescence analysis of extract and drug powder**

The fluorescence analysis of the powdered drug of *Juglans regia* in various solvents and chemical reagents were performed under normal and UV light. There was no fluorescence observed under UV long (365nm) with any of the chemicals (Table 3).

**HPTLC analysis**

HPTLC analysis of methanolic extracts was carried out using CHCl₃: Ethylacetate: Formic acid (7.5:6:0.5) (v/v) as a mobile phase. HPTLC screening of the extracts was established to substantiate the standardization data on *Juglans regia* Linn (Figure 5). As Gallic acid was used as standard, quantification of gallic acid in the extract was carried out. Hot & cold methanolic extract of *Juglans regia* showed 1.4% & 1.08% of gallic acid respectively.

**Determination of Microorganisms**

Total aerobic plate count of *Juglans regia* bark powder was found to be $2.41 \times 10^5$ cfu/ml & no fungal propagules were observed in total fungal count (Table 4). The bark was also found to be free from objectnable pathogens.

**DISCUSSION**

The information obtained from preliminary phyto-chemical screening will be useful in finding out the genuity of the drug. Ash values; extractive values & fluorescence analysis are few parameters, which normally are adopted to get the qualitative information about the purity & standard of the crude drug. The percent extractives indicate the quantity and nature of constituents in the extracts. Morphological and anatomical studies discussed can be considered as a distinguishing parameter to identify & decide the authenticity of this drug. These simple but reliable standards will be useful to a lay person in using the drug as a home remedy.

**CONCLUSION**

The data produced in the present investigation is also helpful in the preparation of the crude drug’s monograph and inclusion in various pharmacopoeias. Also the manufacturers
can utilize them for identification and selection of the raw material for drug production.

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