Evaluation of *Illicium Verum* (Fruit) for Wound Healing Activity in Rats

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***Abstract*— The fruit of *Illicium verum* has long been used in traditional Chinese medicine and food industry with the actions of dispelling cold, relieving pain, antispasmodic, expectorants, aromatic, antiseptic, diureti, anti inflammatory stimulant, as well as diaphoretic properties. The present study was aimed for wound healing potential of ethanolic extract of *Illicium verum* (fruit part) by using various wound models, The results were comparable to standard in terms of wound contraction, tensile strength, and biochemical parameters such as hydroxyproline content, protein level, etc. significant (P < 0.05) increase in fibroblast cells, collagen fibres and blood vessels formation. All parameters were observed significant (P < 0.05) in comparison to control group.**

Aim of the review: This review summarizes the up-to-date and comprehensive information concerning the botany, traditional use, phytochemistry and pharmacology of *Illicium verum* together with the toxicology, and discusses the possible trend and scope for future research *of Illicium verum*.

***Index Terms: Illicium verum,* Wound healing activity, Phytoconstituent, Extraction yield, UV-Visible Spectrophotometer, Toxicity.**

INTRODUCTION

Herbal medicine is a part of human health care for thousands of years. Myriad of chemical constituents obtained from herbs are active against a number of diseased condition. As per the World Health Organization (WHO) reports more than 80% of the population in this world are dependent on herbal medicine Recently many plant extracts have been reported for wound healing activity and their cellular mechanism of wound healing has been studied extensively. It has been demonstrated that many plant extracts processed wound healing via angiogenesis activation of NF-κB, favoring pro-inflammatory cytokines up regulation of iNOS and alpha-1 type-1, fibroblast proliferation, and anti-oxidant activity.

Illicium verum belonging to the Magnoliaceae family, commonly known as Chinese star anise is one of the flavors used in china 5 spices, cultivated in mountainous region especially in Lanson province, Cochin, China (Southern china) and Vietnam. *The Illicium verum* fruits are capsule like aggregate with star shaped five to ten pointed boat shaped section about on eight averages. Each arm is a seed pod. The fruits s have tough skin and rust colored outer portion and seeds with high oil content. The oil is used in rheumatism.

Wounds are physical injuries that result in an opening or break of the skin that causes disturbance in the normal skin anatomy and function. They result in the loss of continuity of epithelium with or without loss of underlying connective tissue. Wound represents a significant burden on the patients and health care professional worldwide.

MATERIALS&METHOD

This chapter deals with the material and methods used for extraction, their preliminary chemical screening and in vivo wound-healing activity of *Illicium verum*.

Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C,55–65%). Rats received standard rodent chow and water ad libitum. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision

of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Toxicity Study

For the acute oral toxicity and LD50 determination the organization for economic co-operation and development (OECD) guideline 423 was followed. As per OECD guidelines a stepwise procedure with the use of 3 animals of a single sex per step was followed. Absence or presence of compound related mortality of the animal doses at one step will determine the next step i.e.

No further testing needed.

Dosing of three additional animals, with the same dose.

Dosing of three additional animals at the next higher or the next lower dose levels.

Experimental model

The rats were an anesthetized prior to creation of the wound, with 1 ml of intravenous ketamine hydrochloride (10 mg/kg body weight). The dorsal fur of the animal was shaved with an electric clipper and the area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of 2.5 cm in width (circular area = 4.90 cm2) and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors.

The entire wound left open. The animals were divided into three groups of 6 each. The group 1 animals were left untreated and considered as the control. Group 2 animals served as reference standard and treated with sulphathiazole ointment. Animals of groups 3 were treated with hydroalcoholic extract of *Illicium verum* respectively for 14 days.

Data Analysis

The data is expressed as mean ± Standard Deviation (SD). Results were analyzed using one-way ANOVA followed by Dunnet's test. Differences were considered as statistically significantat $P<0.05$, when compared with control.

RESULT AND DISCUSSION

Determination of Percentage Yield

To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extract obtained from samples using hydroalcoholic solvent is depicted in the table 1.

Table 1: % Yield of *Illicium verum* extract.

S. No.	Extracts	% Yield (w/w)
1.	Pet. Ether	2.63%
2.	Hydroalcoholic	11.34%

Percentage yield of pet. ether and hydroalcoholic extract of *Illicium verum* exhibited in 2.63% and 11.34% respectively.

Phytochemical screening of extract

Small portion of the dried extract was subjected to the phytochemical tests using standard methods to test for alkaloids, glycosides, saponins, flavonoids and phenol separately for extracts of all samples. Small amount of each extract was suitably resuspended into the distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed in the table 2

Table 2: Phytochemical screening of extracts of *Illicium verum.*

S.R No.	Constituents	Hydro alcoholicextract	Observation
1	AlkaloidsDragendroff's testHager'stest	-ve -ve	Green colouredNot Yellow coloured
2	Glycosides Legal'stest	-ve	Greencoloured
3	Flavonoids Lead acetateAlkalinetest	-ve +ve	Yellowcolouredppt Yellow colour
4	Phenol Ferricchloridetest	+ve	Blackcoloured
5	Proteins Xanthoproteictest	+ve	Yellowcoloured
6	Carbohydrates Fehling'stest	+ve	Redcolouredppt
7	Saponins Foamtest	+ve	Layeroffoam
8	Diterpenes Copperacetatetest	+ve	Greencoloured
9	Tannins GelatinTest	-ve	Browncoloured

Results of phytochemical screening were found to be flavonoids, phenol, proteinsditerpenes, carbohydrates

and saponins were detected in hydroalcoholic extract of *Illicium verum.*

Estimation of total phenol content (TPC)

Total phenol content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: y = 0.021x + 0.002, R^2= 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Calibration Curve of Gallic acid

S. No.	Concentration (µg/ml)	Mean Absorbance
1	10	0.227
2	20	0.434
3	30	0.649
4	40	0.855
5	50	1.097

Table 3: Preparation of calibration curve of Gallic acid

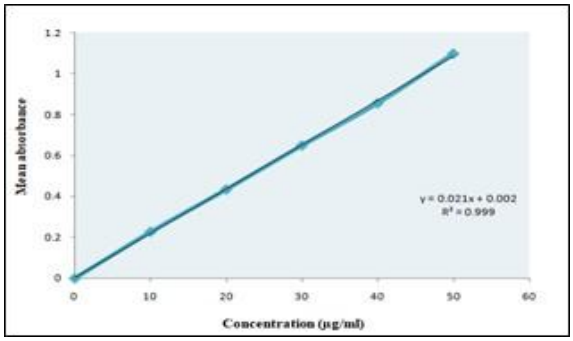


Figure1: Graph of calibration curve of Gallic acid.

Estimation of total flavonoids content (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: y = 0.036x + 0.002, R2=0.999, where X is the quercetin equivalent (QE) and Y is the absorbance.

S. No.	Concentration (µg/ml)	Absorbance
1	5	0.185
2	10	0.362
3	15	0.543
4	20	0.732
5	25	0.896

Table 4: Preparation of calibration curve of Quercetin

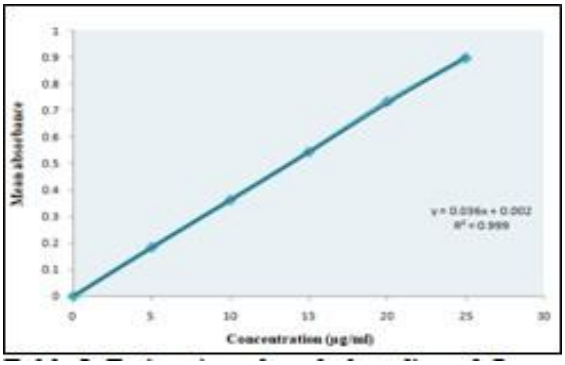


Figure 2:Graphofcalibration curveofQuercetin.

S. No.	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	1.025	0.577

Table 5: Estimation of total phenolic and flavonoids content of *Illicium verum*
hydroalcoholic extract.

The presence of phytochemicals (Phenols, Flavonoids) was quantitatively screened. The extract quantitative analysis revealed total phenolic content (equivalent to gallic acid) of 1.025mg/100 mg. The total content of flavonoid (equivalent to quercetin) was found 0.577mg/100 mg in *Illicium verum extract.*

Results of wound-healing activity

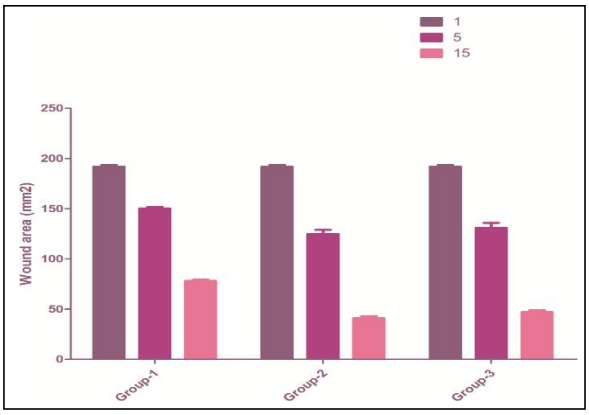
In this study, significantly improved wound-healing activity has been observed with the hydroalcoholic extract of *Illicium verum*, compared to that of the reference standard and control group of animals

Grp	Treatment	Wound area (mm^2) ± SD		
		1	5	15
1	Control	192.0 ± 1.80	150.3± 1.50	78.2 ± 1.24
2	Standard	192.0 ± 1.80	125.0 ± 4.00	41.0± 2.00
3	Hydroalcoholic extract fruit of *Illicium Verum*	192.0 ± 1.80	131.0± 5.00	47.0± 2.00

Table6: Evaluation of wound healing activity of hydroalcoholic extract of *Illicium verum*onrats

All values are expressed as mean ± SD

Figure 3: Evaluation of wound healing activity of hydroalcoholic extract of *Illicium verum* on rats.



SUMMARYANDCONCLUSION

The aim of wound healing is first to improve the rate of healing process, causing minimal pain and reducing the probable complications. Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. It mainly depends on the repairing ability of the tissue, type and extent of damage and general state of the health of the tissue. The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema, and small new blood vessels.

The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin strands. The collagen composed of amino acid (hydroxyproline) is the major component of extra cellular tissue, which gives strength and support. Breakdown of collagen liberates free hydroxyproline and its peptides. Measurement of the hydroxyproline could be used as an index for collagen turnover. The data depicted in table 6 showed that hydroalcoholic extract of Fruit of *Illicium verum* was significantly increased when compared to the control. The preliminary phytochemical analysis of wound healing activity of hydroalcoholic extract of Fruit of *Illicium verum* on ratsrevealed the presence of active constituents. Flavonoids,are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation.

Thus, wound-healing property of wound healing activity of hydroalcoholic extract of Fruit of *Illicium verum* on rats may be attributed to the phytoconstituents presentinit. However, further phytochemical studies are needed to isolate the active compound (s) responsible for these pharmacological activities.

The wound healing activity of hydroalcoholic extract of Fruit of *Illicium verum* on rats promote wound healing activity. The wound healing activity of hydroalcoholic extract of Fruit of *Illicium verum* on rats showed remarkable wound healing activity and it may be suggested for treating various types' wounds in human beings. Further studies with purified constituents are needed to understand the complete mechanism of wound healing activity of hydroalcoholic extract of Fruit of *Illicium verum* on rats.

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