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"Wound healing potential of *Helicteres isora* Linn. fruits extract formulated into a topical gel"

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Abstract

The medicinal plants are widely used by the traditional medicinal practitioners for curing various diseases, but experimental demonstration of specific active compound is lacking. Recent research findings suggest that bioactive fractions derived from a reverberated medicinal plant, *Helicteres isora* Linn. possesses many therapeutic properties. Being a rich source of chemical constituents, different plant parts of this plant and their extracts are known to cure diarrhea, diabetes, snakebite, weakness and various skin ailments. Reports have shown that the extracts from bark, fruits and root possess antioxidant, anti-dysenteric, anti-diabetic and antimicrobial activities. The fruit extract of *Helicteres isora* L. have been reported to exhibit free radical scavenging activity, ability to induce toxicity to tumor cell and to protect normal cells. As the wound healing activity and free radical scavenging property go hand by hand holding each other, the present communication is to suggest to assessment the wound healing activity of fruit extract of *Helicteres isora* Linn. via *in-vivo* models.

Key Words: *Helicteres isora* Linn. gel, excision, incision and dead space wound healing model.

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1. Introduction

The medicinal plants having a great importance in human being show diverse pharmacological properties. Medicinal plants originated from India posses a plethora of therapeutic compounds useful for treating various diseases. Containing excellent composite of nutritive and medicinal properties many plants and herbs have been considered as a broad spectrum of their usage, the focus of research to find lead molecules full of high nutritious and rich source of antioxidants [1]. Extensive experimental studies and clinical researches have provided convincing evidences of association between bioactive compounds like reduction of cancer and other dangerous disorders.

Breaking in epithelial integrity of the skin altering the structure and functions of underlying normal tissue, caused by contusion, haematoma, lacerations or abrasions due to physical, chemical and microbial injury are commonly named as wound and its healing starts from the time of injury and continue for varying periods of time, depending on the degree of wounding [2] . Although wound healing is a normal biological process with numerous steps like coagulation, inflammation, granulation tissue formation, matrix formation, and connective tissue remodeling, collagenization and wound strength acquisition but according to severity treatment is recommended to increase the rate of healing and minimize the microbial growth around the wounded area. Researchers prove that due to the harmful effects reactive oxygen species (ROS) belong to deleterious for wound healing process of injured cells and tissues. It is also evident that the antioxidant supplementation helps in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases [3]. There are numbers of synthetic drugs and antibiotics available for this purpose but it create several unwanted effect. It is already reported that herbal medications are more effective, nontoxic in nature, nonresistant to microorganism, more available, affordable and cheap over the conventional medicine [4]. From this point of view there is a very much growing interest in research field to discover new potential herbal medicine for treatment of infectious diseases along with their diverse medicinal importance as it is safer in concern.

Among several indigenous medicinal plants, *Helicteres Isora* is a remarkable medicinal plant possessing a wide range of therapeutic activities. It is a tropical south-east Asian shrub cultivated

throughout India. Different parts of the plants are traditionally used in Indian System of Medicine (ISM) to cure various ailments [5]. All parts of Helicteres isora plant are used in several ailments including antimicrobial [6], antidiarhoeal [7, 8], anticancer [9], anti-diabetic [10, 11] and hepato-protective effects [12] with anti-oxidant property [9]. *Helicteres isora* has been employed in folk medicine for wound care traditionally. In Ayurvedic a paste prepared from the roots is used to cure wounds. Researchers also suggested that the roots extract of Helicteres isora is an essential remedy of wound [1]. They also evaluated that the specified chemical constituents of fruits are responsible for antioxidant activities [13]. So, the main attempt of this review is to identify existing research gaps with a view to prompt further research relevant to approach the study about the wound healing activity of fruit extract.

2. Plant profile of *Helicteres isora* Linn.

Helicteres isora Linn. (family-Sterculiaceae) also known as 'Indian Screw tree' is a large shrub or small tree containing hairy, ovate shaped leaves with serrate margins. Flowers are orange-red in color. The fruits are compound pod, twisted like screw with pointed end. The plant is found throughout India; from Punjab to Bengal, Jammu to South India. dry deciduous forests of central and western India up to 1500m found flora of central and Western India. It is also found in Mala Indonesia it is called "Buah Kayu Ules or Ulet-Ulet" on Java isla of the plant are used in herbal preparations like Gandharva Chhu due to presence of important antioxidants like polyphenols, tanni of nutrients.

Fig.1. *Helicteres isora* Linn.

2.1. Taxonomy hierarchy

Kingdom	Plantae
Class	Angiosperms
Subclass	Eudicots
Order	Malvales
Family	Sterculiaceae

Subfamily	Helicteroideae
Genus	*Helicteres*
Species	*H.isora*
Trade name	Avartani
Common names	English- East India screw tree, Indian screw tree Sanskrit- Avartaphala, Murva, Avartani Hindi- Marodphali, Marophali, Enthani, Gomathi Marathi- Kewad, Muradsheng Bengali- Antmora

3. Different parts of *Helicteres isora* Linn. plant and their pharmacological activities

Relative to random approach, prior information on the folk medicinal use of the plant substantially increases possibilities of finding a herb/drug of marked therapeutic value. Therefore here we attempted to find and gather through tabular form of various parts of the plant by talking to native people. Most of the probable mode of actions of various bioactive fractions derived from this plant is suggestive only; in depth, comprehensive laboratory studies are warranted to confirm the indigenous medicinal claims.

Table1: Brief explanation of ethno- medicinal uses and possible Pharmacological activities

Plant Parts	Ethno- medicinal uses	Possible Pharmacological activities
BARK	Bark boiled with water taken orally thrice per day	**Anti diarrheal/** antimicrobial/antispasmodic[1]
FRUITS	1 fresh fruit each taken orally	Antioxidant activity/**anti-hyperglycemic and hypolipidemic effects** Decreased level of glucose, glycosylated hemoglobin and plasma insulin, hemoglobin[1]
	Approx. 5g fruit powder with salt is to be taken thrice daily with water	**Gastrointestinal** antimicrobial/ antioxidants effects[1]
	1. Fruit paste mixed with mustered oil and turmeric paste is used for massaging in	Antioxidants activity/antispasmodic action[1]

	new born baby to cure profound weakness. 2. Fruits are dried in mustered oil, used on new born to remove body pain.	
	Fruit powder along with other herbs and spice mixed sweet dish is given to women after child birth. It may be given to them during pregnancy.	Reduction of **post delivery weakness** by antioxidant activity/antispasmodic action[1]
	Fruits are made into liniment for sores of ear	Antioxidant activity/antimicrobial activity[1]
SEEDS	5g seed powder boil with water, take twice a day	Antimicrobial activity for diarrhea and dysentery due to **amoebiasis[1]**
ROOTS	Fresh root juice taken twice a day	Anti-hyperglycemic activity[1]
	Fresh root paste with turmeric paste is applied externally on cut and wounds	Antioxidant/antimicrobial activity in recovery of **cut and wounds[1]**
	Root decoction	Antioxidant/antimicrobial/**anti diarrheal** activity[1]
	Root paste is applied externally twice per day till cure on infection area of scabies	Antimicrobial effects against **Scabies[1]**
LEAVES	Fresh leaves paste applied thrice a day	Antioxidant/antimicrobial properties in the remedy of **Skin infection[1]**
	Fresh leaves paste applied on affected area	Free- radical scavenging activity might be playing an important role in inflammation in response to **Snakebite[1]**

4. Phytocontituents of Helicteres isora Linn. fruits

The essential chemical constituents of *Helicteres isora* Linn. are Isoscutellarein and their derivatives; Helisterculins A and B, Helisorin Gallic acid, Caffeic acid, vanillin, p-Coumaric acid [1]. Some researchers isolated three new compounds which are 49-O-b-D-glucopyranosyl rosmarinic acid (2), 4,49-O-di-b-D- glucopyranosyl rosmarinic acid (3) and 2R-O-(49-O-b-D-glucopyranosyl caffeoyl)-3-(4-hydroxyphenyl), lactic acid named as 49-O-b-Dglucopyranosyl isorinic acid (4) were isolated together with rosmarinic acid (1) from the fruit of *H.isora* (Sterculiaceae), an Indonesian medicinal plant. The structures of these compounds, including the absolute stereochemistry of (4), were elucidated by spectroscopic analysis and chemical means

[14]. Compoud (3) had greater scavenging activity against superoxide anion produced with xanthine and xanthine oxidase than rosmarinic acid (1).

5. Estimation of total flavonoid and total phenolic content of Helicteres isora Linn. fruits

Estimation of total flavonoid content [13] of fresh and dry fruit extract soaked in specific solvent system is done with the help of aluminium chloride colorimetric method with little modification, where Quercetin is used to make the calibration curve. Result was expressed as milligrams of Quercetin equivalents (QE) per gm of sample (mg QE/g).

Estimation of total phenolic content [13] of fresh and dry fruit extract soaked in specific solvent system is done using Folin-Ciocalteu reagent based on procedure with some modifications. Gallic acid (GA) was used for constructing the standard curve using the same process mentioned above and the total phenolic contents in the fraction was expressed as milligrams of Gallic acid equivalent (GAE) per gm of sample (mg GA/g).

6. Experimental evidences for biological activities of fruits

Therapeutic importance of the different parts of whole plant of *Helicteres isora* have presented previously by tabular form. But here different solvent extracts of fruits are reported to display pharmacological properties such as antimicrobial, antioxidant and anticancer activities elaborately which are related to wound healing as supportive study.

6.1. Antimicrobial activity

Some researchers have demonstrated antimicrobial activity from aqueous and alcoholic of fruits of *Helicteres isora* against a number of bacterial strains [7]. Another group of researchers reported that the acetone extract of the fruit is capable of removing antibiotic resistant R-plasmid of many strains of bacteria thus making them more sensitive towards low antibiotic doses. Such plasmid loss reversed the multiple antibiotic resistances in cured derivatives making them sensitive to low concentrations of antibiotics. Hence after, it was suggested that acetone extracts of *Helicteres isora* may be a source to develop antiplasmid agents of natural origin; and a sensitizer of multidrug resistant genes of pathogenic bacteria [8].

6.2. Antioxidant activity

Aqueous and alcoholic fruits extracts of *Helicteres isora* showed the antioxidant properties such as free radical scavenging, toxicity to tumor cells and protection to normal cells. However, most of them are limited to initial analysis in cell free systems. Another group of researchers suggested differential cellular response of methanolic fruits extract (50%) of *Helicteres Isora.* They showed that the extract displayed significant antitumor activity in melanoma cells, but in contrast protected normal human blood lymphocytes [1, 9].

6.3. Anticancer activity

Cumulative research findings on fruit extract prepared from acetone soaking showed presence of antioxidants by DPPH free radical scavenging assay and strong anticancer/cytotoxicity activity by several steps process including cell viability test, measurement of intracellular ROS by fluorescence microscopy, assessment of mitochondrial membrane potentiality [1, 9].

7. Evaluation of wound healing properties of *Helicteres isora* Linn. formulated into a topical gel

It is already mentioned the wound healing activity of fresh root paste with turmeric paste applied externally on cut and wounds. In Ayurvedic a paste prepared from the fruits is used to cure wounds. A herbal gel formulation generally give smooth feel on application with less variation, easy to spread, shows the highest percentage of rapid effectiveness with no signs of skin irritations during and after the treatment [15, 16]. So after formulating into topical gel assessment of wound healing activity may be established using the following methods treated with mice,

7.1. *In-vivo* Wound Healing Studies

Animals

Swiss albino mice (23-26gm) housed under condition of 22± 1ºC, 50± 10% humidity and 12 hrs light and 12hrs dark cycle. During maintenance the animals were received food and water as required [17].

7.1.1. Excision wound model

Group the animals as untreated (negative control) mice, wound treated topically with specified dose of extract (test) gel maintaining the LD_{50} dose, wound treated topically with Mega gel (positive control). After anaesthetization by open mask method using anesthetic ether, mice have to be depilated on the dorsal back, Ethanol (70%) is used as antiseptic for the shaved region before making the wound, and an excision wound is made by removing a 7mm × 7mm full thickness piece of the skin from a predetermined shaved area on the back of each animal. The wound is to leave undressed to the open environment and no local or systemic anti-microbial agents are used. The mice are distributed in groups and each mouse is placed in separate cage. Throughout the study period, the excision wounds have to clean with normal saline every morning prior to medication. In each animal group the wound inflected to animals treated with Mega gel (positive control), test gel, normal saline (negative control), respectively. The size of wounds is to be maintained to trace at 0^{th}, 4^{th}, 8^{th}, 12^{th}, 16^{th}, 20^{th}, 24^{th}, 28^{th} and 32^{nd} days and the evaluated surface area then employed to calculate the percentage of wound contraction. This model is used *to monitor rate of wound contraction* [17, 18].

Determination of wound contraction is done by using formula,

Percentage of wound contraction= (wound area on 0^{th} day- wound area on n^{th} day) / wound area on 0^{th} day × 100

7.1.2 Incision wound model

Animals in each group are anaesthetized and one 'Para vertebral long incision' is made through the skin and cutaneous at a distance of 1cm on each mouse with depilated back. Full aseptic measures are not taken and no local or systemic antimicrobial agents are used. After the incision is made, the parted skin kept together and stitched with surgical thread and curved needle. The wound is left undressed. Test extract gel, standard and control drug are topically applied once daily for 11^{th} day; when wounds are cured thoroughly the sutures are removed on the 11^{th} day and tensile strength is measured. This model is used *to measure the tensile strength, resisting the breaking under tension* [17, 18].

Measurement of tensile strength is done by using formula,

Percentage Tensile Strength standard gel = (Standard gel TS- Control TS) / control TS × 100.

7.1.3. Dead space model

The model is used for the *study of granuloma tissue.* Animals of each group are anaesthetized by light ether and wound is made by implantation of two polypropylene tubes (2.0×0.5 cm) one on either side, in the lumber region on the dorsal surface in each animal on the ninth post wounding day, granuloma tissue formed on an implanted tube is dissected out carefully. Granuloma tissue from one tube is dried (60°C) and stored in 10% formalin for the biochemical parameters and histopathological study. While the other part of granuloma tissue is used for determination of tensile strength [19].

Measurement of tensile strength is done by using formula,

Percentage Tensile Strength standard gel = (Standard gel TS- Control TS) / control TS × 100.

7.2. Histopathological Examination

After deep ether anesthesia, the cross-sectional full thickness skin specimens from each group are collected at the 10^{th} day of the experiment to evaluate for the histopathological examinations. Tissues are fixed in neutral buffered formation (10% formaldehyde in Phosphate buffered saline) overnight. After fixation, the tissues are placed in 70% isopropyl alcohol for 2 hr and then in each ascending strength (80%, 90%, 100% isopropyl alcohol) for 1 hr each. The amount of alcohol used should be 15 times of the size of the tissue. After that, xylene is to added to check for the appearance of milkyness. If milkyness appeared then repeat the dehydration procedure. The dehydrated tissue has to impregnate in paraffin wax (m.p.56°C) for a period of 1 hr at 58-60°C. Molten parafin poured into L-block along with the tissues and allowed it to become hard.The tissue have to sectioned into very thin (2-8 or 5-10 micrometer) sections using a microtome. The tissue Mounted on the slides with Mayer's albumin solution (a mixture of equal parts of egg white

and glycerin, beaten and filtered with the addition of 1% sodium salicylate) and incubated in warm oven for 2hr at 60°C. Slides containing paraffin sections are placed on a slide holder. Slides are deparaffinized with Xylene for 30 min and the excess xylene blotted. The tissue has to rehydrate sucessively with 100% ,90%, 80% isopropyl alcohol for 2-3 min.each and put into water for 1-2 min.and then kept in tap water for 1-2 min. The slides containing tissue sections is dipped into 1N HCL followed by Scott's water (Sodium Bicarbonate 3.5g, Magnesium sulphate 20gm, distilled water1L) for 1min each. The tissue has to immerse in Eosin stain for 30 sec.The tissue is dipped into 70% alcohol and then into 90% alcohol and pure alcohol subsequently and left for 2mins each.Finally one drop of gum (DPX) is poured onto the slide and section has to cover with cover slip [19, 20].

8. Discussion

Being a complicated biological process in cellular and molecular level for regeneration of the damaged tissue [2], wound healing involve four phases, coagulation which prevents blood loss; inflammation and debridement of wound; epithelial repair including proliferation, mobilization, migration and differtiation; tissue remodeling and collagen deposition. It is condensed that reactive oxygen species (ROS) are deleterious to wound healing process due to the harmful effects on cells and tissues. It is also evident that the antioxidant supplementation helps in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases [3]. Table1 epitomizes the possible modes of various bioactive functions of different plant parts of *Helicteres isora* Linn [1]. However, most of the probable modes of action of various bioactive fractions derived from *Helicteres isora* are suggestive since they lack systematic research based validation. Therefore, in depth, comprehensive laboratory studies are warranted to confirm the indigenous medicinal claims and for establishment of *Helicteres isora* based effective therapeutic modalities. Fruit extract of Indian screw tree is reported to induce toxicity to tumor and protection to normal cells. The major therapeutic effect of extract is attributed to free radical scavenging mechanism. Studies have suggested that alcoholic and acetone extracts of fruits showed strong antioxidant and free radical scavenging activities by bioactive compounds such as rosmarinic acid, gallic acid. Reports also

point to promising antitumor activity of fruits which may prove useful in chemoprevention as well as chemotherapy. Reports have appeared showing that compounds like cucurbitacin B, kaempherol induce apoptosis by inhibition of JAK/STAT, MAPK pathways, potentiate anti-proliferative effects of gemcitabine and activation p53 in tumor cells [1].

In general, it may be suggested that due to the different experimental approaches and models used, investigations have sometimes yielded different results. Hence, the use of a standardized and reproducible model is inevitable to obtain objective information of the wound healing process as well as to better understand the pathological process and to improve medical technologies. Hence two different *in vivo* models (Excision wound model, Incision wound model and Dead space model) has been chosen in our study to assess the effect of herbal gel of most active fraction on wound healing[17-19] .

It however, needs to be noted that most of these studies are lacking for evaluation of anti-inflammatory and analgesic activity. We know when injury occurs and inflammation create then inflammation markers histamine release at 1^{st} hour, serotonin at 2^{nd} hour, bradykinin at 3^{rd} hour and prostaglandin release at 4^{th} hour, which are useful for body defense mechanism. Practical experience has shown that once a plant extract is found to possess anti-inflammatory activity, it is better to test whether it also possesses wound healing activity because it is now pretty well established that inflammation and wound healing go hand in hand. So, the anti inflammatory activity is useful for wound healing activity. When wound occurs, it is accompanied by pain, so the analgesic activity is supportive to wound healing activity [21]. We strongly advocate that further detailed cohort studies are warranted both at laboratory and clinical level for the development of herbal formulations containing *Helicteres isora* Linn. alone or in combination with other herbals to fight against several diseases.

9. Conclusion

Present paper provides a summary of diverse medicinal uses of *Helicteres isora* Linn. Both laboratory and epidemiological studies have provided considerable evidence that both fresh and

dry fruit of this plant possesses medicinal properties. So, the current study has suggested fulfilling the assessment of wound healing activity in ambient atmosphere *in-vivo*.

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