FORMULATION AND EVALUATION OF WOUND HEALING PROPERTIES OF TERMINALIA ARJUNA OINTMENT

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Abstract
A standardized topical formulation containing bark extract of Terminalia arjuna was developed and evaluated. Physicochemical standardization, extractive profiling, phytochemical screening, formulation development, and pharmaceutical evaluation were performed. Results demonstrated acceptable physicochemical quality, favorable spreadability, skin-compatible pH, and non-irritant behavior, supporting the suitability of the formulation for wound healing applications.

Keywords: Terminalia arjuna, wound healing, herbal ointment, phytochemistry, topical formulation.

1. Introduction
Ayurveda is the oldest surviving complete medical system in the world. Derived from its ancient Sanskrit roots ayus' (life) and 'veda" (knowledge) and offering a rich, comprehensive outlook to a healthy life, its origins go back to nearly 5000 years. Terminalia arjuna is a large, evergreen tree, with a spreading crown and dropping branches. It has been grown in most parts of India and used in Ayurvedic formulations since ancient times.

Besides its wide range of medicinal uses, T. arjuna is planted for shade and ornamental purposes. Terminalia's active constituents include tannins, cardenolide, triterpenoid saponins (arjunic acid, arjunolic acid, arjungenin, arjun glycosides), flavonoids (arjunone, arjunolone, luteolin), gallic acid, ellagic acid, oligomericproanthocyanidins (OPCs), phytosterols, calcium, magnesium, zinc, and copper. Improvement of cardiac muscle function and subsequent improvement in the pumping activity of the heart seems to be the primary benefit of Terminalia.

It is thought that the saponin glycosides might be responsible for the inotropic effect of Terminalia, while the flavonoids and OPCs provide free radical antioxidant activity and vascular strengthening.3 A dose-dependent decrease in heart rate and blood pressure was noted in dogs given Terminalia intravenously.4 Recently, two new cardenolide cardiac glycosides were isolated from the roots and seeds of Terminalia.

Various parts of plant have been investigated for the presence of phytoconstituents and pharmacological activities. Many useful phytoconstituents have been isolated from T. arjuna. Triterpenoids are mainly

responsible for cardiovascular properties. Tannins and flavonoids are responsible for its anticancer properties. The present review summarizes the ethnic use, pharmacological activities of the extracts and phytoconstituents of T. arjuna for last 90 years. To cure human diseases, medicinal plants have been a major source of therapeutic agents since ancient time."

2. Introduction

plant of Arjuna botanically named as Terminalia arjuna linn.; family Combretaceae, has traditionally been used to treat many diseases especially heart disease for centuries, that's why it is called as "Guardian of the heart".

kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Oder	Myrtales
Family	Combretaceae
Genus	Terminalia
Species	Terminalia Arjuna
Common Name	Arjuna

Table No. 1 Plant Profile of Terminalia Arjuna
Image No.1 Terminalia Arjuna

- **Synonyms:** Arjun bark, arjun.
- **Biological name:** Terminalia arjuna
- **Biological Source:** Arjuna consists of dried stem bark of the plant known as Terminalia arjuna belonging to family Combretaceae.

- **Geographical Source:** The tree is common in Indian peninsula. It is grown by the side of streams anc common in Chotta Nagpur region.
- **Uses:** Arjuna bark is used as a diuretic and astringent. The diuretic properties can be attributed to the triterpenoids present in fruits. It causes decrease in blood pressure and heart rate. It is used in the treatment of various heart diseases in indigenous systems of medicines. The bark was extensively used in the past by the local tanneries for tanning animal hides. It yields a very firm leather of a colour which is similar babool tanned leather.

2.1 Chemical constituents: The dry bark from the stem contains about 20 to 24% of tannin, whereas that of the bark obtained from the lower branches is up to 15 to 18%. The tannins present in arjuna bark are of mixed type consisting of both hydrolysable and condensed tannins. The tannins are reported to be present are (+) catechol, (+) gallocatechol, epicatechol, epigallocatechol, and ellgic acid. The flavonoids such as arjunolone, arjunone, and baicalein have been reported from the stem hark. The triterpenoid compounds arjune-tin, arjungenın, arjunglucoside I and II, and terminoic acid have also been reported from the bark. The root contains number of triterpenoids such as arjunoside I and II. Terminic acid, oleanolic acid, arjunic acid, arjunolic acid, etc. The fruits also contain 7 to 20% of tannins. A pentacyclic triterpene glycoside arjunoglucoside III has been reported from the fruits along with hentriacontane, mynistyloleate and arachidic stearate.

2.2 Phytochemistry: It was initially reported that the bark had 34% ash content consisting entirely of pure calcium carbonate. The water extract existence 23% calcium salts and 16% tannins, whereas the alcoholic extract contained very little coloring matter and tannins (Dymock et al., 1891). The chemical analysis of the bark showed confirmation of sugar, tannins (12%), coloring matter, glycoside, and carbonates of calcium, sodium and traces of chloride of alkali metals (Ghoshal, 1909). The chemical constituents of T. arjuna are shown in Table No. 2

Compounds	**Stem/bark**	**Root**	**Activity of compounds**	**References**
Triterpenoids	Arjunin, arjunic acid, arjunolic acid, arjungenin, terminic acid	Arjunic acid, Arjunolic acid, Oleanolic acid, terminic acid	Antifungal,Cardioprotective	Zhouet al.,2011a; Dwivedi, acid 2007
Gycosides	Arjunetin, Arjunaphthanoloside,Arjunoside I, II andTerminoside-A	Arjunoside,IVGlucopyranoside	ICardioprotective	Dwivedi, 2007

Sitsterol	Sitosterol	Sitosterol	Antimutagenic, Antiinflammato-ry,antitussive.	Zhou et al.,
Flavo-noids	Arjuno-lone,arjunone,bicalein,luteolin,gallic acid,ethyl gallate kempfero,proanthocyaninids,quercetin,pelorgonidin,		Antialler-gic,antibacterial, cytotox-ic,antiasthmatic,antifungal,antioxidant,	Zhou et al., 2011b,c,d anddwivedi 2007
Tannis	Pyrocate-chols,casuarinin,casurin,punicallin,	Puni-calagin,castalagin,Terchebulin,Terflavin C,	Woundhealing and antimicrobial	Dwivedi 2007
Trance	Calci-um,Aluminium,Magnesium,Silica,Zinc,Copper		To fill up ion re-quirement	Dwevedi,2007

Table No. 2

2.3 Bioactive Compounds: T. arjuna has medicinal and economic value due to the presence of different bioactive compouShowing biological activities in human and animal body (Zaidi, 1998). Some bioactive compound Showing biological activities reported so far are summarized in Table No 3.

Compounds	Biological Activity	References
Arjunolicacid (C30H4805MW: 488.71)	Antifungal, cardioprotective	Zhou et al., 201 la
Castalagin MW: 934.65) (C41H26026	Antihypertensive, cytotoxic	Zhou et al., 2011a

Ethylgallate [C9H1o05,MW:198.1	Antibacterial[Bacillus dysenteriae],antifibrotic,platelet aggregation inhibitor, collegenase inhibitor,analgesic	Zhou et al.,2011b
Gallic aci [C7H05,MW:170.12]	Antiallergic, antibacterial,antineoplastic, cytotoxic,antifungal,antiinflammatory,	Zhou et al.,201 1b
	Antimultagenic, antiviral,astringent,antiasthmatic,choleretic, antioxidant cell. Growth inhibitor,controlphosphoramidon	
	antimutagenic, , antiviral astringent, antiasthmatic; choleretic, antioxidant cell growth inhibitor, control phosphoramidon	
Luteolin (C15H1006, MW: 286.24	Antiallergic, antibacterial, antineoplastic, antifungal, inflammatory,antispasmodic, antitussive, immunoenhancer, increases coronary flow, protein kinase C inhibitor, succinic oxidase inhibitor, antihypercholesterolemic	Zhou et al., 2011c
Kaempferol(C15H10O6MW: 286.24)	Anti-HIV-1, antibacterial,antitussive to cure trachitis, antioxidant, thyronine iodinate deiodinase inhibitor, aldose reductase inhibitor, anti-inflammatory	Zhou et al., 2011c
Proanthocyanidin(C30H26012,MW: 578.53)	Anticomplement activity, antihypertensive, protein kinase C inhibitor, reverse transcriptase antioxidant inhibitor.	Zhou et al., 2011d

Table No. 3

3 Pharmacological Action:

A number of previous studies reported a wide number of pharmacological activities of T. aquna can be used to treat diabetics, beart diseases as well as for the treatment of wound. It has antiviral. Antibacterial, anticancer and other potential anti-alment properties

3.1 Antimicrobial activity:

Perumalsamy et al (1998) reported that the aqueous extracts of T. arjuna bark holds major antimicrobial activity against Proteus vulgaris, Klebsiellaarrogenes, Escherichia coli and Pseudomonas aerogenes. The presence of antibacterial activity in the bark of T. arjuna exhibiting selectively maximum activity against S. epidermidis (Singh et al, 2008). Antimicrobial activity of different solvent extracts from T. arjuna reported previously are summarized.

3.2 Anticancer activity:

Different types of cancer reported to treat by T. arjuna extracts are compiled in Table 4. Herbal extracts of T. arjuna reported to enhance increased percentage of life span of experimental animals induced with DIA (Dalton's Lymphoma Ascites) tumour cells and in some cases induced with carcinogens (Muthuchelian et al., 2010). Arjuna extract inducing DNA damage in HepG2 cells indicated that T. arjuna extract induces ROS production in HepG2 cells and consequently causes apoptotis (Sarveswaran et al., 2006)

3.3 Against car infection:

T. arjuna plants extracts having a great potential to be developed as herbal ear drop to control the bacterial car infections. Ancja et al (2012) reported the leaves and bark extracts as potent and effective medicine against tested bacteria responsible for ear infections than that of standard ear drop

3.4 Antifungal activity: The organic extracts of five Terminalia species (T. arjuna, T. chebula, T. bellerica, T. catappa and T. alata) were tested with plant pathogenic fungi ie. A flavus, A. alternata, A. niger, A. brassicicola, and H. tetramera. The leaves extracts of all five plants found to inhibit these plant pathogens (Shinde et al., 2011). The bark extracts were more effective than fungicide (control) used in this antifungal test. Moderate antifungal activity against C. albicans, C krusei and C. parapsilosis was exhibited by a mixture of arjunolic acid with minimum inhibitory concentration (MIC) values in the range of 50-200 µg/ml (Puvanakrishnan et al., 2010).

3.5 Antidiabetic activity:

The T. arjuna extracts have potential effects on diabetic. In the experimental diabetic rats model treated with T. arjuna extracts showed two enzymes (glucose-6- phosphatase, fructose-1, 6- diphosphatase) significantly reduced in liver and kidney. This has effects on increasing insulin secretion which can effects on repression of the gluconeogenic key enzymes (glucokinase and phosphofructokinase) (Ragavan et al., 2006). Terminalia arjuna bark extract exhibited antidiabetic activity by enhancing the peripheral utilization of glucose which have the ability to kidney glycolysis and correcting the impaired liver and by decreasing its gluconeogenic formation as like as insulin. This effect may be due to the presence of tannin, saponin, flavonoids and other constituent's presence in the bark, which could act synergistically or independently in enhancing the activity of glycolytic and gluconeogenic enzymes (Ragavan et al., 2006). Manna et ol (2009a; 2009b) have investigated the prophylactic role of arjunolic

acid against streptozotocin (STZ) induced diabetes in the pancreatic tissue of Swiss albino rats. STZ administration (at a dose of 65mg/kg body wt, injected into the tail vein) causes an increase in the production of both ROS and reactive nitrogen species (RNS) in the pancreas of experimental animals. Formation of these reactive intermediates decreases the intracellular antioxidant defense, increases the levels of lipid peroxidation, protein carbonylation, serum glucose and TNF-a (Puvanakrishnan et al., 2010)

3.6 Antiacne activity:

Topical formulations (cream) of T. arjuna extract containing flavonoid (FF-1 to III) and tannin fraction (TF-1 to III) have been developed, which were examined for antimicrobial activity against Propionibacterium acnes and Staphylococcus epidermidis. The famulation of FF-III (cream containing 2% flavonoid fraction) has showed higher antihacierial activity against P. acnes (zones of inhibition 17 mm) and S. epidermidis (zones of inhibition 20 mm) than other formulations and which is comparable to that of standard marketed topical herbal perparation (Vijayalakshmi et al. 2011) Herbal anti-acne cream is non-toxic, safe, effective and improves patient compliance by the utilization of herbal extracts from T. arjuna would be highly acceptable (Vijayalakshmier al, 2011).

3.7 Anthelmintic activity:

Crude methanolic extracts of T. arjuna bark exhibited anthelmintic activity both in vitro (eggs, larvae and adult of Haemonchuscontortus and in vivo studies against mixed gastrointestinal vichostrongylidar nematodes of sheep (Bachaya et al., 2009). Anthelmintic activity of T. arjuna bark may be mainly attributed to its tannin content that binds with a free protein existing in the tubes for larval nutrition and reduced nutrient availability resulting in larval starvation decreased gastrointestinal metabolism by directly inhibiting the oxidative phosphorylation thereby causing larval death (Bachaya et al, 2009).

3.8 Wound healing activity:

The hydroalcoholic extract of T. arjuna bark phytoconstituents was reported to be used in topical application on healing rat dermal wounds. Wounds created on the back of rats under anesthesia have been treated with various fractions applied topically as simple cintment. Results prove that fraction III prepared as 1% simple ointment shows complete epithelialization on day 20, whereas fraction I show complete epithelialization on day 9, which essentially consists of tannins (Puvanakrishnan et al., 2010) Mengi et al (2003) reported the capability of T. arjuna to complete epithelisation of excision wounds and increased tensile strength of incision wounds.

3.9 Cardioprotective activity:

There are different types of therapeutic use of T. arjuna for cardiac disease that based on empirical explanation recorded in various treatment of ancient medicine.

3.10Candiotonic activities:

Arjunolic acid is used as a cardiac tonic in ayurvedic medicinefor Centuries and it has been first isolated from T. arjuna. The bark extracts have major component triterpenoidsaponin is an arjunolic acid

(Puvanakrishnan et al., 2010). Physiological studies carried on the isolated rabbit and frog heart exposed that T. arjuna bark had cardiotonic and stimulatory eflect (Ghoshal, 1909). It was consequently found that intravenous administration of the glycoside, obtained from the bark of T. arjuna, resulted in rise in blood pressure (Ghosh, 1926). It was indicated that the bark powder has a cardiotonic property, also possessed diuretic properties.

3.11Coronary flow:

Bhatia et al. (1998) reported to inject aqueous extract of the bark injection into neland rabbit heart (Langendorff' si to increase in coronary Bow. The dose was 1024 µg/ml thar causes highest increase in coronary flow

3.12 Hypotensive effects:

Singh et af (1982) reported intraverschral and intracerebro-ventricular injection of alcoholic and aqueous extract of bark that was dose-dependent persistent beadycardia and hypotension. Further the alcoholic extract causes the hypotensive effect in dogs was abolished try pre-innatnsent with atropine. In another study the observation in dogs where intravenous administration of aqueous extract of T arjuna resulted in dose-dependent fall in blood pressure (Srivastava vt al., 1992)

3.13 Di Effect on aortic prostaglandins:

Aortic prostaglandin E2 like activity was enhanced in those rabbits that were administered T. arjuna compared to those who were on placeho. The finding of miint PGE2 like activity was significant because PGE2 is known to produce coronary vasodilation. This may possibly explain the pharmacological basis of the increased coronary flow following T arjuna infasion (Bhatia et al., 1998). This may also be contributing to the beneficial effect of T arjuna in coronary artery disease (CAD) patients.

3.14 Insecticidal property: Arjunolic acid isolated from the stem of T. arjuna exhibits significam inhibitory activity towards fourth instar larvae of Spilarctiaobliqua. Effective concentration to reduce feeding and growth of the larvae has been found to be 617.8 and 666.9 ppm, respectively (Pavanakrishnan et al., 2010) (xii) Antioxidant activity: In antioxidant activity test, the methanol extract of T. arjuna bork exhibited significant antioxidant activities with the IC50 value of 7.05apind Methanol extract of arjuna base antioxidant activity and may have petentialwri medicine (Rahman et al, 2011)

3.15 Astiasthmatic activity:

Arjunolic acid and alcohol extract of Taryuns have vignificant mast cell stabilization activity and specifically, arjunolic acid exhibits comparatively better uabilization activity than alcoholic extract of TA (Proud er al., 2004) The antisthumatic and antianaphylactic activity may be due to the mast cell stabilizing pounsial and inhibition of antigesinhand histamine and acetylcholine release (Prasad et al, 2004, Puvanakrishnaneraf, 2010)

3.16 Gamepective effect: T. arjuna scs as an gastroprotective agent probably due to its free sdical scavenging activity and cytoprotective nature (Devi eraf, 2007)

4 Material and Method:

4.1 Preparation of Extract: The fresh Bark of the plant is taken powder and get for further extraction.

Extraction of the Arjua Bark:

Image No.3 Arjuna Extract

Image NO.2 Soxhlet Apparatus

4.2 Soxhlet extractor:

Soxhlet extraction or hot continuous extraction. In this method, finely ground sample was placed in a porous bag or "thimble" made from a strong filter paper or cellulose Extraction solvent Le. Ethanol was heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. When the liquid content reaches the siphon arm, the liquid contents emptied into the bottom flask agam and the process was continued. The final Ethanolic extract is collected. Then it was allowed to settle down in open beaker for 24 hours at room temperature.

5 Formulation Ointment bases

Bases can be conveniently categorized into the following types.

5.1 Hydrocarbon bases: Soft paraffin; hard paraffin; liquid paraffin

5.2 Absorption bases: Wool fat (anhydrous lanolin): hydrous wool fat; wood alcohol; beeswax

5.3 Neutral oil bases: Almond oil; coconut oil; olive oil; vitamin E, wheat germ

6. METHOD OF PREPARATION OINTMENT BASES

1. Melt petroleum jelly (500 gm.) or wax in a glass bowl set in a pan of boiling water.

2. Add the finely cut herb (60 gm. Dried or 150 gm. Flesh herb) and simmer for fifteen minutes with continuous stirring.
3. Pour the mixture (herb mixture) into a jelly bag secured to the rim of a jug with a string and allow the liquid to filter through.
4. Squeeze as much as of the hot herb mixture (by wearing rubber gloves) as possible through the bag into the jug.
5. Quickly pour the molten ointment into jars before it sets in the jug. Place the lid on jar without securing it firmly, when cool, tighten the lids and label.

7. Evaluation Parameter for Ointment:

7.1 Colour and Odour:

Physical parameters like colour and odour were examined by visual examination

7.2 Consistency:

Smooth and no greediness is observed.

7.3 PH:

PH of prepared herbal ointment was measured by using digital PH meter. The solution of ointment. Was prepared by using 100ml of distilled water and set aside for 2hrs. PH was determined inTriplicate for the solution and average value was calculated.

7.4 Spreadability:

One of the criteria for a cream, ointment or gel is that it should possess good spreadability. SpreadabilityIs a term expressed to denote the extent of area to which the formulation readily spreads on applicationTo skin or affected part. The therapeutic efficacy of a formulation also depends on its spreading value.

The time taken for the upper slide to travel the distance of 6.0 cm and separated away from the lower slide under the influence of the weight was Noted.8 The experiment was repeated by three times and the mean time was taken for calculation. Spreadability was calculated by using the following formula:

Formula:- m x I / t (spread ability)

Where,

S – Spreadability

m-Weight tied to the upper slide

l – Length of the glass

t – Time taken in seconds

7.5 Extrudability

The formulation was filled in collapsible tube container. The extrudability was determined in terms of Weight of ointment required to extrude 0.5cm of ribbon of ointment in 10 seconds.

7.6 Diffusion study

The diffusion study was carried out by preparing agar nutrient medium. A hole board at the center ofMedium and ointment was by placed in it. The time taken by ointment to get diffused through was noted (after 60 minutes]

7.7 Solubility

Soluble in boiling water, miscible with alcohol, ether, chloroform

7.8 Washability

Formulation was applied on the skin and then ease extend of washing with wate

7.9 Stability study

Stability study was performed as per ICH guideline.13 The purpose of stability testing is to provide Evidence on how the quality of a drug substance or drug product varies with time under the influence of a Variety of environmental factors such as temperature, humidity and light. Therefore, stability studies Provide data to justify the storage condition and shelf-life of the drug product. For drug substance, such Studies establish the retest date in addition to the storage condition of raw material. Stability studies Were performed for selected formulation with 25 ± 2° C and 60 ± 5% RH and 40 ± 2° C and 75 ± 5% RH Conditions for 6 months. The samples were analyzed at 0, 3 and 6 months interval for colour, physical Appearance and pH.

7.10 Drug Content

1 g of prepared formulations were weighed and dissolved in 100 ml methanol. They were filtered andNecessary dilutions were made and the drug content was then determined spectrophotometrically.11

8. Result and Discussion

Test	Poor	Good	Very Good	Excellent
Irritancy				✓
Washability			✓	
PH		✓		
Viscosity		✓		
Phase separation				
Spreadability				✓

In this modern era, the knowledge and experience of usage of herbs are being blend with advanced cosmetic technology to develop a safe and effective product.

From the present study it can be concluded that it is possible to develop creams containing berbal extracts and can be used as a barrier to protect skin.

9. Conclusion:

On the basis of the result the Arjuna bark powder have all the parameter within the limit and satisfactory select some Physicochemical, Phyto-Chemical, Macro & Microscopic and TLC examinations, which are found to be very useful tools for the identification and characterization of T. arjuna hark. A siniple, accurate and precise analytical method is used for the analysis of T. arjuna bark, which could be uneful in future Forensic identification of unknown plant material. It is found that the Physicochemical, PhytoChemical, Macro Microscopic, and TLC examinations are very useful tools for the identification of T. arjuna hark Phyto chemical studies were carried out for the identification of Arjuna bark with standard plant hack. Thun layer chromatographic studies showed the presence of active principles of T. arjuna, this is further suggesard that the proposed methods are simple, sensitive and reproducihin. The suggested protocol can also be used for the qualitative evaluation of Arjuna bark in laboratory with very less equipments and expenses. These can be employed successfully for routine forensic analysis of T. arjana. As the evaluation expenses are lesa as compared to other instrumental methods, this could be a method of choice for official monographs in Forensic Toxicology. The wound healing activity of two herbal formulations (Mimaxcintment and lotion) containing Indradaru extract, Le. Arjuna bark (Terminalia arjuna, Family-Combretaceae), extract was evaluated for its wound healing potential in two types of wound models in racs (1) excision wound model and (ii) incision wound model. Both the formulations responded significantly in both the wound models tested The results were also comparable to that of the standard drug nitrofurazone used as a standard drug for comparison in this present investigation. The results were also comparable in terms of wound contracting ability, epithelization period, tensile strength and regeneration of tissues at the wound aren. Thus, this investigation confirms the use of the Himax ointment and lotion containing Terminalia arjuns extract as a wound-healing agent.

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