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Review Article

Stem Bark of Albizia Lebbeck (L.) Benth.: A Comprehensive Review of Its Phytochemistry, Wound Healing Potential and Pharmacological Properties

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ABSTRACT

Albizia lebbeck (L.) Benth. (Fabaceae) commonly known as siris or lebbeck tree, is a deciduous tree that has been traditionally used in Ayurvedic, Unani and folklore medicine. Stem bark is traditionally used in the treatment of inflammatory disorders, dermatological disorders, pulmonary diseases, periodontal and dental problems. However, there is no available compilation of information related to the bark. This review provides an overview of the available evidence regarding the phytochemical composition, wound healing activity and pharmacological effects of Albizia lebbeck stem bark. The bark contains triterpenoidal saponins (albizia saponins A-C, acacic acid type saponins), flavonoids and flavan-3-ols (catechin, epicatechin, procyanidins, luteolin, geraldone, isookanin), phenolic glycosides, alkaloids, sterols and fatty acids. Application of methanol extract in ointment form was effective in increasing wound contraction, decreasing time taken for epithelisation, increasing tensile strength and granuloma weight in mice. Root extracts have been found to increase collagen related markers, antioxidant enzymes and decrease lipid peroxidation. Antimicrobial, antioxidant, anti-inflammatory, analgesic, antidiabetic (inhibitory enzymes), antimalarial and cytotoxic activities have been demonstrated using extracts of the bark. Ethanol extract did not cause any deaths in mice at 5g/kg. However, almost all of the evidence is preclinical and varies greatly in terms of extraction methods. Wound healing constituents have not been isolated. Further work is needed using standardised extracts, bioassay guided fractionation, mechanistic research and well designed clinical trials to recommend Albizia lebbeck as an evidence based wound healing agent.

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INTRODUCTION

Plants have been used for treating injuries, infections and inflammatory ailments for thousands of years. Many traditional preparations are still used within rural communities, due to the lack of access to modern wound healing treatments. This has led to interest in wound healing agents derived from ethnomedicinal sources [1]. Within the Indian subcontinent, one of the popular species used is Albizia lebbeck (L.) Benth.

Albizia contains approximately 150 recognised species found globally in Asia, Africa, Australia and tropical/ subtropical America [2]. A. lebbeck belonging to the family Fabaceae is commonly known as siris, shirish or lebbeck tree. This species is predominantly grown within the Indian subcontinent and Myanmar [2]. It is a large, quick growing, versatile tree that has the ability to fix nitrogen from the atmosphere. Furthermore, it is also used as an ornamental plant [3].

All parts of this tree including root, leaves, flowers, bark and seeds are used within traditional Indian medicine. The plant has been documented to treat a range of ailments including respiratory, dermatological, gastro-intestinal, oral, ocular, urological, genital, anorectal, inflammatory, neurological and venereal ailments [2]. Experimental research has shown extracts, fractions and isolated compounds from this plant to have antiallergenic, anticancerous, anticonvulsant, antidiabetic, anti-inflammatory, antimicrobial, antinociceptive, antioxidant, antiparasitic, antivenom, neuroprotective, nootropic and wound healing properties [2].

One of the most commonly used parts of this plant is the stem bark. Traditionally the plant is recommended for treating inflammation, toothache and leprosy [4]. It has also been used to treat bronchitis, paralysis and helminth infections [5]. Previous reviews have discussed the botany, ethnomedicinal uses, chemical constituents and pharmacological properties of the whole plant [2,3,5,6]. However, information regarding the stem bark specifically and wound healing properties are found within various journals.

This review aims to collate the evidence surrounding the phytochemical constituents, wound healing potential and other pharmacological properties of A. lebbeck stem bark. This will highlight the current knowledge gaps which would be required to develop this traditional remedy into a standardised product.

2. Botany, Distribution and Traditional Uses

2.1 Botanical Description and Distribution

A. lebbeck is a deciduous shrub/tree growing to approximately 18m. The plant has a straight trunk and grey/ brown bark. The leaves are bipinnate and alternate along smooth green stems [2]. The plant is widespread across India, Andaman Islands and Myanmar. It can also be found in tropical Africa, other areas of Asia and north Australia [3]. The plant belongs to the Fabaceae family and the Albizia genus. It is known by several names; in hindi siris/shiris, in english the lebbeck tree and in sanskrit Bhandi, Sitapuspa, Sukapriya and Mrdupuspa [3].

2.2 Traditional Uses (Stem Bark)

Classically, the bark was described as bitter, cold, alexiteric and anthelmintic. It is used to treat disorders caused by vata and blood diseases. It can be used to treat leucoderma, itching, skin diseases, excessive sweating and inflammation. It has also been described as useful in the treatment of rat bites and ophthalmia. The bark has also been used to treat toothache and gum disease [1]. In Bangladesh the bark is used to treat inflammation, toothache and leprosy [4]. The bark has been reported to be effective when used as a decoction to treat experimentally induced bronchospasm, which supports its use in respiratory ailments for many years [5]. The stem bark has also been used to treat diarrhoea, poisoning, oedema, asthma and bronchitis. In the Siddha system it is used to treat arthritis with both the bark and flowers [7].

The bark has been used within India to treat many skin diseases and inflammatory ailments. Villagers have used extracts of the plant to treat wounds [1]. The prolonged use of the bark despite lack of scientific evidence is the main reason why experiments into wound healing have been carried out (see section 5).

2.3 Evidence Supporting Traditional Uses

Many traditional uses of the bark can be correlated with experimental evidence. The use of the bark to treat

inflammation and pain relates to the anti-inflammatory and analgesic effects seen in rodent models [8]. The treatment of infections and skin diseases correlates with the antibacterial effects seen with extracts of the bark [9,10]. An ethanolic extract of the bark has also been found to have antiplasmodial effects [11]. This adds to the reputation of the plant as an anthelmintic [1], however it introduces antiparasitic effects as it is effective against malaria parasites. Whilst helminths and malaria parasites are different organisms they have both been treated using this plant. The treatment of gum and tooth problems could relate to the astringent and antimicrobial properties of the phenolic compounds in the plant, however this has not been tested. This does not prove that the bark will be effective in humans but provides a logical reason to prioritise the bark for further research.

3. Scope and Approach

A narrative review was conducted using peer reviewed articles, review articles and journal documentation relating to A. lebbeck. Search terms included plant name and any of the following: stem bark, phytochemical, wound healing, antimicrobial, anti-inflammatory, antidiabetic, antimalarial or cytotoxic. 20 publications were cited due to their relevance to the stem bark and traceable documentation. If other parts of the plant were used (roots, leaves and seeds) this will be mentioned. This information will be included in order to gain context from the research. Due to the limited number of wound healing studies specifically looking at the stem bark, some conclusions have been made using the data from the roots. These will be marked as indirect.

4. Phytochemistry of the Stem Bark

Class	Representative constituents	Ref.
Triterpenoid saponins:	Albiziasaponins A, B and C as well as acacic acid type saponins (lebbeckoside C), Echinocystic acid glycosides, Albiziahexoside	[3,5,12,13]
Flavonoids & flavan-3-ols:	D-catechin, (-)epicatechin, Procyanidins B-2, B-5, C-1, Leucocyanidin isomers, Melacacidin, Geraldone (5-deoxyflavone), Luteolin, Isookanin	[3,5,7,10]
Phenolic glycosides:	Albizinin isolated from an acetone extract of bark.	[5]
Alkaloids:	The stem bark contains macrocyclic alkaloids as major components. Alkaloids were found in an ethanolic extract of bark.	[10,11]
Sterols & Terpenoid:	Phytosteroids and terpenoids were detected in ethanolic extracts of bark. β-Sitosterol and Betulinic acid have been reported.	[3,11]
Fatty acids & esters:	Long chain saturated fatty acids and esters were detected using GC-MS analysis on the stem bark extract.	[10]

The above table indicates the different compound classes reported in the studies. The exact solvents, techniques and plant material used vary.

4.1 Triterpenoid saponins

A variety of secondary metabolites are present in A. lebbeck stem bark including saponins, macrocyclic alkaloids, phenolic glycosides and flavonoids [10]. Three major saponins have been isolated from A. lebbeck stem bark; albizia saponins A, B and C [5]. Other saponins include echinocystic acid glycosides and albiziahexoside [3]. A new acacic acid type saponin, lebbeckoside C has recently been isolated and its structure determined using 1D and 2D NMR techniques, high resolution mass spectroscopy and chemical evidence [12]. Other triterpenoid saponins have also been isolated from A. lebbeck and found to inhibit survival of high grade human brain tumour cells [13]. A review has been carried out on the secondary metabolites of five Albizzia species. Fifty compounds were described showing the diversity of metabolites in the genus [4].

4.2 Flavonoids and Phenolics

The bark contains a variety of flavonoid and phenolic compounds. Flavan-3-ols including D-catechin and isomers of leucocyanidin and melacacidin [3]. From an acetone extract of A. lebbeck stem bark, albizinin (phenolic glycoside) and flavan-3-ols including (-)-epicatechin, procyanidin B-2, procyanidin B-5 and procyanidin C-1 [5]. Three flavonoids were isolated from A. lebbeck stem bark. Geraldone (5-

deoxyflavone), luteolin and isookanin were isolated from the ethyl acetate fraction of a silica gel fractionated methanolic extract. Their structure was determined using 1HNMR, 13CNMR and mass spectroscopy [7]. Catechin, geraldone, luteolin and isookanin were isolated from A. lebbeck bark. After characterising the acetone extract using HPLC and GC-MS, flavonoids were identified [10]. A previous study showed that the extract contained a high concentration of phenolics (148.00mg/g gallic acid equivalents).

4.3 Alkaloids, Sterols, Fatty Acids and Other Compounds

Macrocyclic alkaloids are major metabolites in A. lebbeck stem bark [10]. Alkaloids were identified in the ethanolic extract [11]. Fatty acids and fatty acid esters were identified in the stem bark extract using GC-MS. Long chain saturated fatty acids have also been associated with antimicrobial activity in the bark [10]. Phytosterols and terpenes were also identified in the ethanolic extract [11]. β-sitosterol and betulinic acid have been identified as constituents in A. lebbeck [3]. Antimicrobial activity of flavonoid, alkaloid and steroid enriched bark extracts have been compared using different solvents [9]. The concentration of these compounds will vary depending on the solvent used, extraction method and origin of bark**.**

4.4 Lack of chemical knowledge

Many of the studies carried out on A. lebbeck bark use qualitative colour tests. Only a few studies have isolated fully characterised compounds [7,10,12]. No validated quality standards using markers have been described in the literature. For example no HPLC quantification of catechin or saponin levels have been described. There is no agreement on what compounds should be used as markers. This makes it difficult to replicate results from one lab to another.

5. Wound Healing Potential

5.1 Rationale

Wound healing begins immediately after injury [1]. It goes through a number of phases from haemostasis and inflammation, through to proliferation where granulation tissue is formed, collagen is laid down, angiogenesis occurs and re-epithelisation takes place. Finally remodelling occurs which affects the strength of the scar. Infections, excessive oxidative stress and longer inflammation may cause delays in all healing phases. Therefore compounds with antimicrobial, antioxidant, anti-inflammatory and collagen promoting properties become interesting. Several of these properties have been reported for A. lebbeck extracts.

5.2 Evidence from stem bark

The most detailed A. lebbeck bark study was using a methanolic extract (prepared via Soxhlet) formulated into an ointment (5%). Excision, incision and dead space wounds were investigated in mice. The ointment was used as a control and 5% Betadine as a reference treatment [1]. The excision wounds showed a high level of contraction, 97.6% on day 13 compared to 73.8% in the control group and 99.6% in the reference treatment. Epithelisation occurred quicker in the extract group, taking only 13days compared to 22days in the control and 11days in the reference [1]. The extract also showed a high degree of early contraction. 55.34% on day 7 compared to 31.38% in the control [1].

The incision wounds had a higher tensile strength in the extract compared to the control. 254.87g/cm on day 8 compared to 169.62g/cm. However the reference had a higher tensile strength. 304.87g/cm [1]. Dead space wounds showed a decrease in dry granuloma weight. 34.12mg/100g compared to 17.12mg/100g in the control and 43.62mg/100g in the reference [1]. Histology was also carried out and showed signs of significant wound healing potential [1]. Overall all parameters showed an improvement in the extract compared to the control. The extract didn't reach the level of the antiseptic reference.

Table 2: Time Course of Wound Contraction and Epithelialisation in the Excision Wound Model in Mice (Methanolic Stem Bark Extract, 5% Ointment)

Group	Day 4 (%)	Day 7 (%)	Day 10 (%)	Day 13 (%)	Epithelialisation period
Control (ointment base)	26.55	31.38	62.49	73.80	22 days
Standard (5% Betadine)	47.80	73.28	97.48	99.60	11 days
Test (5%Methanolic extract)	41.70	55.34	86.88	97.60	13 days

Values are mean percentage wound contraction. Data taken from [1]. On day 13 both treated groups differed from control at P<0.001.

These end points indicate various aspects of wound healing. Wound contraction and epithelisation times indicate the rate of wound surface closure. Tensile strength of incision wounds indicates the quantity and quality of collagen present within the tissue. Dry granuloma weight in the dead space model indicates granulation formation [1]. A good extract, such as the bark ointment, will improve all three end points. However, the extract was behind the standard in all cases of the excision model. There is the suggestion that the bark may be less potent than a powerful antiseptic since there was a large difference in the initial stages of healing (55.34% compared to 73.28% on day 7). The potency of the extract may be dependent on the concentration and formulation. Another study investigated the effects of aqueous and ethanolic extracts of stem bark on rats. The extracts were applied topically to wounds in the form of 5% and 10% concentrations (w/v) and also taken orally. This was done to confirm the traditional uses of wound healing [14]. The two bark studies are very different. Different species of animal were used, different types of extracts and different routes of administration.

Table 3: Experimental Studies on the Wound Healing Activity of Albizia Lebbeck.

Plant part and extract	Model and treatment	Main findings	Ref.
Stem bark, methanolic extract (Soxhlet)	Excision, incision and dead space wounds in mice; 5% ointment; Betadine as reference	Day 13 contraction 97.6% (control 73.8%); epithelialisation 13 days (control 22); day 8 tensile strength 254.87 g/cm (control 169.62); dry granuloma weight 34.12 mg/100 g (control 17.12)	[1]
Stem bark, aqueous and ethanolic extracts	Rats; topical 5% and 10% w/v and oral administration	Designed to substantiate the traditional wound healing claim	[14]
Root, ethanolic extract (indirect evidence)	Incision, excision and dead space wounds in rats; 500 mg/kg orally gave optimum effect	Contraction optimal on day 18 and complete by day 22; higher breaking strength, hydroxyproline, hexosamine, hexuronic acid, SOD and glutathione; lower lipid peroxidation and nitric oxide; antibacterial activity	[15]

The root study is included only as indirect evidence because the plant part differs.

5.3 Indirect evidence from the root extract

The root part of the same plant has been investigated in more detail. The root was found to contain high amounts of flavonoids, saponins, phenolics and tannins after extraction with ethanol. The extract also contained 4.66% w/w rutin and was investigated in the incision, excision and dead space models in rats [15]. The extract increased wound breaking strength and had a maximum effect at 500 mg/kg oral dosage. The extract also produced optimum wound contraction at 500 mg/kg on day 18 and complete wound contraction by day 22 [15]. The extract also significantly increased dry tissue weight, total protein, hydroxyproline, hexosamine, hexuronic acid, superoxide dismutase and reduced glutathione. However, lipid peroxidation and nitric oxide were reduced [15]. Histological results showed normal epithelialisation and an increase in collagen density. The extract contained antibacterial properties against strains involved in wound infections

[15]. This was due to increased collagen production and antioxidant activity [15]. Due to differences in the chemistry between the root and bark, this should be seen as supporting evidence.

5.4 Possible mechanisms

There is some evidence from different sources that the bark may have healing properties. The evidence suggesting collagen deposition comes in the form of an increase in granuloma weight and tensile strength when using the bark ointment [1], and the increased hydroxyproline and hexosamine content observed in the root study [15]. Antimicrobial properties were also observed in the use of the bark, inhibiting several pathogenic bacteria such as S. aureus and P. aeruginosa in disc diffusion assays [9]. The stem bark extract was also effective against both Gram positive and Gram negative strains [10]. Antioxidant activity may help reduce oxidative damage at the wound site. Flavonoids isolated from the bark inhibited DPPH radicals in vitro [7]. Anti-inflammatory and analgesic properties were observed in a rodent study using an extract of the bark [8]. The root extract also had an inhibitory effect on lipid peroxidation and nitric oxide [15].

This is logical evidence but has been found through different experiments. To determine what components of the bark contribute to these properties, bioassay guided fractionation can be used to isolate components of the bark. The saponin rich, flavonoid rich and phenolic rich extracts can be tested in wound models.

6. Other Pharmacological Properties of the Stem Bark

The wound healing activity of the bark was supported by a variety of biological activities. These have been investigated in various studies using different extracts and models (see table 4). These biological activities are outlined below. Due to differences in solvents, concentrations and models used in these studies it is best to see them as an overall indication of bioactivities found within the bark.

Table 4: Summary of Other Pharmacological Activities Reported for Albizia Lebbeck Bark

Activity	Extract	Model key	Findings	Ref.
Antimicrobial	Bark extract using 7 different solvents	Bark extract using 7 different solvents Disc Method, MIC/MBC	Methanolic extract inhibited growth by 20mm for R. planticola. MIC 0.625-0.078mg/mL. MBC 0.312-0.039mg/mL	[9]
Antimicrobial &cytotoxic	Acetone extract stem bark	Bacterial/fungal strains Antiproliferative activity HPLC/GC-MS	Inhibited Gram positive and Gram negative bacteria	[10]
Antioxidant &cytotoxic	Petroleum, ethylacetate and methanol extract Stem bark	In vitro antioxidant activity. Cytotoxicity (LC50).	Ethylacetate extract had highest antioxidant activity. LC50 42.36 µg/mL	[16]
Analgesic & anti-inflammatory	Cold extracted bark extract	Carrageenan paw oedema, writhing test and Tail flick test. In rodents.	Inhibited 36.68% oedema and 52.4% writhing at 400mg/kg	[8]
Anti-inflammatory	Petroleum, chloroform and ethanol extracts	Carrageenan, dextran, cotton pellet and Freund's adjuvant models in rats	Inhibited 59.57% (carrageenan model) and 68.57% (adjuvant model) with ethanol extract (400mg/kg).	[17]
Antidiabetic (*in-vitro*)	Geraldone, luteolin and isookanin isolated from bark.	Inhibition of α-glucosidase and α-amylase. Docking study.	All 3 flavonoids inhibited both enzymes.	[7]
Antimalarial	Ethanolic extract	P.falciparum and P.berghei In-vitro and in mice	IC50 8.2 and 5.1 µg/mL. Chemosuppression up to 84.7% (Schizonticidal).	[11]
Cytotoxic (saponins)	Lebbeckoside C and other saponins	Human glioblastoma and brain tumour cells	IC50 1.69µM (U-87MG), IC50 1.44µM (TG1).	[12,13]

Activity	Extract	Model key	Findings	Ref.
Cytotoxic	Bark extract	Human cell panel. MTT assay.	Cytotoxicity are examined, including in MCF-7 cells	[18]

MIC - Minimum Inhibitory Concentration. MBC - Minimum Bactericidal Concentration. IC50 - Half maximal inhibitory concentration. LC50 - Median Lethal concentration.

6.1 Antimicrobial activity

Antimicrobial activity is one of the best reported properties of the bark. A study of different solvent extractions showed that the highest inhibition zone (20mm) was obtained with the methanol extract. P. aeruginosa and S. aureus were some of the most susceptible organisms whilst B. subtilis, E. aerogenes and E. coli were less susceptible to the extract [9]. The bound flavonoid and methanolic extracts had the highest activity with MIC values ranging from 0.625-0.078 mg/mL and MBC values ranging from 0.312-0.039 mg/mL. The lowest MIC value was recorded against R. planticola at 0.078 mg/mL [9]. The acetone extract of the stem bark was active against many Gram positive and Gram negative bacteria. This extract was also tested on fungal strains [10]. Long chain saturated fatty acids present in the bark may play a role in this activity [10]. An antimicrobial study was carried out comparing A. lebbeck to Acacia nilotica using stem bark. Antioxidant and cytotoxicity studies were also carried out [16].

6.2 Antioxidant activity

Flavonoids in the bark were investigated using a DPPH radical scavenging assay, as well as enzyme inhibition assays [7]. The ethyl acetate fraction of the stem bark had the greatest antioxidant and cytotoxic activity when compared to petroleum ether and ethyl acetate fractions [16]. Hydrogen peroxide was also scavenged in vitro by zinc oxide nanoparticles prepared from the stem bark extract [19]. This is supported by the high levels of different phenolics including catechins found in the bark [3,5]. However, this is only in vitro.

6.3 Anti-inflammatory and analgesic activity

A cold extracted bark extract (petroleum ether:ethyl acetate:methanol-1:1:1) reduced oedema induced by carrageenan (400mg/kg) by 36.68% after 4 hours [8]. The extract also showed 39.9% and 52.4% reduction in writhing induced by acetic acid when administered at 200mg/kg and 400mg/kg respectively. An increased latency period was observed in the tail flick assay (40.74% and 61.48%) at the same concentrations [8]. Petroleum ether, chloroform and ethanol extracts of A. lebbeck (100, 200 and 400mg/kg) were tested using various models including carrageenan, dextran, cotton pellet and Freund's complete adjuvant in rats [17]. The highest effects were recorded using the 400mg/kg petroleum ether and ethanol extracts. The ethanol extract inhibited carrageenan oedema by 59.57%, dextran oedema by 52.93%, cotton pellet granuloma by 53.57% and inflammation induced by adjuvant by 68.57% [17]. These results therefore support the use of the plant in traditional medicine to treat inflammatory diseases [17]. The abstract did not specify what part of the plant was used.

[Note: This reference has been included as it refers to the plant in general.]

6.4 Antidiabetic activity

The isolation of three flavonoids from the ethyl acetate fraction of the methanolic bark extracts (geraldone, luteolin and isookanin) showed inhibition of both α-glucosidase and α-amylase in vitro. Molecular docking confirmed the interaction between the flavonoids and the two enzymes [7]. This results in slowing down the digestion of carbohydrates which would in turn reduce glucose levels after a meal. However, the data is purely based on enzyme inhibition in vitro and molecular docking.

6.5 Antimalarial potential

The ethanolic extract of the stem bark displayed good inhibition towards chloroquine sensitive Plasmodium falciparum strains (MRC2; IC50: 8.2 µg/mL). It also had strong inhibitory effects towards the chloroquine resistant strain (RKL9; IC50: 5.1 µg/mL). Selectivity indices were >121.9 and >196.07 respectively [11]. In vivo tests in mice infected with P. berghei showed the chemosuppression increased from 69.4% in mice administered the extract (100mg/kg/day) to 84.7% (1000mg/kg/day) compared to chloroquine (96.8%).

[11] In the prophylactic test, 94.9% chemosuppression was seen with the extract (500mg/kg) which was better than pyrimethamine (71.2%). The extract displayed dose dependent curative effects towards established infections [11]. It was suggested that the activity may be due to either alkaloids or flavonoids or both.

6.6 Cytotoxic and Anti-cancer potential

The acacic acid type saponins present in the Albizia species have been studied for their cytotoxic properties. The triterpenoid type saponins found in A. lebbeck showed inhibition of viability in high grade human brain tumour cells [13]. Lebbeckoside C isolated from the stem bark displayed excellent inhibitory effects against the U-87 MG glioblastoma cell line (IC50: 1.69µM) and the TG1 stem-like glioma cell line (IC50: 1.44µM) [12]. The extract of the bark was tested against a range of human cell lines (HeLa, MiaPaCa-2, HepG2, AGS, U373MG, A431, NIH3T3 and MCF-7) using the MTT assay [18]. In comparison to Acacia nilotica the ethyl acetate fraction of A. lebbeck stem bark displayed good LC50 values (42.36µg/mL) [16]. The acetone extract also showed anti-proliferative effects [10]. Although the results from these experiments are promising, there is a difference between cytotoxicity in vitro and anti-cancer properties. More investigation into the selectivity towards cancerous cells is needed.

6.7 Green Synthesised Nanoparticles

The extract of the stem bark has been used as a reducing and capping agent to produce zinc oxide nanoparticles using zinc nitrate (0.01, 0.05 and 0.1M). The nanoparticles displayed antimicrobial and antioxidant properties with cytotoxic effects shown against MDA-MB-231 and MCF-7 breast cancer cell lines [19]. A review on this plant reported that Zinc Oxide nanoparticles synthesised using the stem bark extract displayed organoprotective effects in animals in a concentration dependent manner. Changes in body weight, Alanine aminotransferase, Alkaline phosphatase, Urea and Creatinine were seen [2]. These systems could potentially be investigated to create wound dressings as there could be a synergistic effect between the zinc and the active plant extract. However, this has not been investigated.

6.8 Other Activities

Seed extracts of A. lebbeck have been investigated for their hepatoprotective potential. The methanolic seed extract showed the highest cell viability (50µg/mL) [20]. However, this was using the seeds of the plant rather than the bark.

7. Safety and Toxicity

There is little evidence regarding the safety profile of the bark. An ethanolic extract taken orally in mice did not cause any death at 5g/kg making the LD50 greater than 5g/kg [11]. The extract had a CC50 of >1000µg/mL on HeLa cells in vitro [11]. This suggests the extract is safe in rodents [11]. However, it has been highlighted in a wider review that there is limited research into the safety of A. lebbeck formulations [2]. There is no specific evidence detailing chronic toxicity, genotoxicty, reproductive toxicity, skin irritation and sensitisation. However, this information along with sterility and irritation testing would be necessary for a product to be used topically on open wounds**.**

8. Discussion and Future Perspectives

Overall, the evidence presented does support the traditional claims of the stem bark of A. lebbeck. The methanolic extract of the bark showed increased wound contraction, epithelisation, tensile strength and granuloma formation in mice when compared to a control [1]. The root extract data show increased collagens markers and antioxidant status [15] and the bark show antimicrobial, antioxidant and anti-inflammatory properties which are relevant to wound healing [7-10]. The chemical composition of the bark containing saponins, flavan-3-ols, flavonoids and phenolic glycosides [5,7,12] provides a logical rationale.

However, the evidence presented is limited. There are only two studies looking specifically at wound healing properties of the bark [1,14]. Of these, only one study provided in-depth data and only one concentration was used (5%) [1]. The control used in this study was Betadine which is an antiseptic ointment. This is very different to using a plant extract which could have multiple mechanisms of action. Using a control such as a conventional wound healing product or using just an ointment base along with a known antimicrobial could

provide more information. The extracts were made using various solvents. The best described wound healing study used methanol which is not an ideal solvent to use if a product was to be used on open skin. Therefore, aqueous, ethanolic or other suitable extracts would need to be tried. Overall, most of the pharmacological evidence comes from in vitro studies or small rodent studies. Many of the articles were based on qualitative phytochemical screening.

Future research on this plant should include: i) Authenticating and standardising the plant material (using HPLC fingerprinting). ii) Bioassay guided fractionation using wound healing models to identify what fraction/compound is responsible and investigate alone and in combination. iii) Dose response studies, chronic wound models (diabetic and infected wounds), cytokine, growth factor, angiogenesis and collagen type data to understand the mechanism. iv) Suitable formulations should be developed and tested (e.g. gels, creams, films and nanoparticle dressings [19]). v) Toxicology and skin safety studies should be carried out. vi) Controlled clinical trials should be carried out. vii) As harvesting the stem bark can cause damage to the tree, sustainability should be considered or investigating alternative plant parts if the bark is shown to be clinically useful.

When formulating a product from this plant there are some practical considerations. A topical product needs to be able to deliver the active components to the wound bed at a consistent dose. This will depend on the extract, excipient and release profile. A product needs to be stable and not become contaminated with microbes. This is especially important as it is a natural product being applied to damaged skin. The extracts used in the studies were produced in the lab using solvents. This may not be suitable to produce at scale. A pharmaceutical grade extraction method would need to be determined.

This review is a narrative review and so only twenty publications were chosen to look at. This means some relevant studies may have been missed. Some publications could only be found as abstracts meaning I couldn't always verify the data (doses and statistical analysis). Overall there is a limited amount of literature available on wound healing properties of the bark. Much of the evidence comes from root data and other test systems. Those wanting to use this information should refer to the full articles listed in the reference list.

Table 5: Main Gaps in the Evidence and Suggested Research Priorities for Albizia Lebbeck Stem Bark.

Gap	Currently known	Proposed Strategy
Standardization	Most research uses non-standardized qualitative screening with varying solvent systems.	Bark authentication using HPLC fingerprinting. Quantification of key marker compounds (e.g. Catechin, Luteolin, Representative Saponin)
Active principles	Only found to have activity in wound healing with crude extracts.	Bioassay guided fractionation. Saponin, Flavonoid and Phenolic fractions tested.
Dose & formulation	One 5% methanolic ointment was detailed.	Dose response studies using acceptable pharmaceutical solvents. Gels, Creams, Films and Nanoparticle based dressings
Mechanism	Mechanism inferred via independent antimicrobial studies, antioxidant studies and anti-inflammatory studies	Determine levels of collagen types, cytokines, growth factors, angiogenesis and oxidative agents within wound model.
Wound models	Only normal excision, incision and dead space wounds used	Diabetic, infected and chronic wound models.
Toxicology	Acute Oral Toxicity and in vitro Cytotoxicity with Ethanol extract.	Skin Irritation and Sensitisation, Repeated Dose and Genotoxicity studies
Clinical Trails	No clinical trial were identified in the sources reviewed	Small scale controlled trials using standardised topical formulations. Once safety confirmed.

This table was created by the author of this review using the evidence outlined in sections 4-7.

CONCLUSION

The stem bark of A. lebbeck is chemically complex and has been traditionally used for skin disease, inflammation and wounds. Experimental studies have shown that extracts can increase rates of wound healing in rodents, improve healing tissue quality and have antimicrobial, antioxidant, anti-inflammatory, antidiabetic, antimalarial and cytotoxic activity which supports traditional uses. However, studies are pre-clinical, number of wound related studies specific to the bark are limited and the extracts are not standardized. In order for A. lebbeck stem bark to be considered a validated wound care agent, further studies on standardisation, bioassay guided isolation, mechanisms of action and safety data as well as clinical trials should be carried out**.**

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