



REVIEW ARTICLE

ANTIMICROBIAL ACTIVITY OF SIX AYURVEDA HERBS USED FOR WOUND WRAPPING EXPLAINED BY CHARAKA W.S.R. TO VRANA CHIKITSA - A REVIEW

AMIT A. GAJARMAL¹ SUDIPT KUMAR RATH²

ABSTRACT:

Ayurveda literature have emphasized at various places to take care of *Vrana* (wound), which occur due to vitiated *Doshas* or any trauma. *Sushruta*, an eminent surgeon of his era, was much ahead of his time in expounding and practicing the beautiful concept of *Vrana Ropana* (wound healing). The *Shalya Chikitsa* brings out very clearly that *Vrana* (wound) is the most significant surgical entity and the knowledge of its effective management is the basic skill required on which the outcome of surgery revolves. There is a constant need to deal with the infection and problems of healing in different surgical disorders. The term *Patradana* was coined by *Sushruta* for the prevention of wound from various infections (*Rakshoghna Karma*). In this relation, *Acharya Charaka* in *Dvivraniya Chikitsa Adhyaya* explain a *Sutra* (verse) in which the *Patra* (leaves) of the six ayurvedic herbs, *Kadamba*, *Arjuna*, *Nimba*, *Patala*, *Ashvattha* and *Arka* are mentioned for *Vrana Rakshoghna Karma* (for wrapping after applying wound healing medicine over a wound). So in this article, in spite of explaining well established wound healing and anti-inflammatory property, we try to evaluate the antimicrobial activity on this six herbs which successfully proves its *Rakshoghna Karma* in the treatment in wound.

Key words: Antimicrobial, *Vrana*, *Patradana*, *Arjuna*, *Nimba*

¹ PhD Scholar, PG dept. of *Dravyagunavigyana*, National Institute of Ayurveda, Jaipur, Rajasthan, India

² Assistant Professor, PG dept. of *Dravyagunavigyana*, National Institute of Ayurveda, Jaipur

Corresponding Email id: dgalaxy78@gmail.com

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INTRODUCTION

Exposure to infection and prolongation of inflammatory phase plays the prime role to delay the healing process, whereas creating the favourable conditions that allow the wound to heal (*Ropana*) properly is the ultimate aim of any surgeon. There are many measures to create favourable conditions for wound healing, such as use of antiseptic solutions and antibiotics to combat the infection, inflammation and many agents to remove slough/dead tissues to shorten the inflammatory phase and thus promote the healing process.

But these measures may have failed to achieve good antimicrobial effects by not producing minimal and fine scar formation. This is why the search is still on to find out a drug or drug combinations which can fulfil the optimal requirement. The healing activities have been attributed partially by very few active chemical constituents like *berberine* in *Daruharidra* (*Berberis aristata* DC), *anthraquinones* found in *Manjishtha* (*Rubia cordifolia* Linn.), etc. and studies reveal that it is difficult to achieve the complete aim of wound management with a single drug. Hence, there is a need to find out a rational and optimal antimicrobial as well as wound healing compound for the wound

management in a better way. Here, it is important to find out an effective formulation which possesses both wound healing property (*Vrana Ropana*) and prevention of wound from infection (*Rakshoghna Karma*). Many studies have been carried out regarding *Vrana Ropana*. Though the herbal drugs are prescribed with high appreciation for healing purpose, they need scientific validation.

The term *Patradana*^[1] was coined by *Acharya Sushruta* for the prevention of wound from various infections (*Rakshoghna Karma*). It is one of the most important *Karma* among the *Vranasya Shashti Upakramas*. *Rakshoghna Karma* is important in wound healing process. Keeping all these factors in view, *Acharya Charaka* in *Dvivraniya Chikitsa Adhyaya* explain a *Sutra* (verse) in which six ayurvedic herbs [Table No.1] named, *Kadamba*, *Arjuna*, *Nimba*, *Patala*, *Ashvattha* and *Arka* are mentioned for *Vrana Achadana Karma*^[2] (for wrapping after applying wound healing medicine over a wound). For this purpose *Acharya Charaka* says to used leaves of this six *Dravyas*. To explain that why only this six *Dravyas* are stated in *sutra* for wound wrapping, we go through the online available research literature of this six *Dravyas* and instead of reviewing its well established wound healing and anti-inflammatory

property, we try to explain it's the antimicrobial activity.

Kadamba (*Anthocephalus indicus*)

In one of the study, the antimicrobial activity of the various extracts of the leaves of *Anthocephalus cadamba* has been studied by agar cup plate diffusion method. The organisms selected for antimicrobial activity were *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger*, *Candida albicans*, *Pseudomonas aeruginosa* and *Salmonella typhi* using the *Gentamycin* and *Ketoconazole* as standard. It was found that Chloroform and acetone extracts exhibited strong activity against bacteria and fungi and the zone of inhibition was comparable with the standard drug^[3].

Arjuna (*Terminalia Arjuna*)

One of the study reveals that, the *Arjuna* bark extract was used as a biological tool to resolve the antibiotic resistant *Vibrio cholerae* problem. The in vitro sensitivity of the 12 selected *Vibrio cholerae* isolates to the crude extracts of *Terminalia Arjuna* was determined by disc diffusion method. The concentrations of crude extract used were 1 mg/disc, 2 mg/disc, 3 mg/disc, 4 mg/disc and 5 mg/disc. These plates were kept for 4-6 hours at low temperature and the test materials diffuse from disc to the surrounding medium by this time. The plates were then incubated

at 37⁰ C for 24 hours and zone diameter was measured in mm. contains *tannins*, *triterpenoids* *saponins*, *flavonoids*, *sterols*, *calcium salts*, *alkaloidal* and *glycosidal substances*, *arjunine* and *arjunglyciside* etc. It stops bleeding and pus formation in the gums and is useful in asthma, dysentery, menstrual problems, pains, leucorrhoea, wounds and skin eruptions. Arjun bark extract was used as a biological tool to resolve the antibiotic resistant *Vibrio cholerae* problem. Arjun extract showed promising effect against the isolated *Vibrio cholerae* at different concentrations (1 mg/L, 2 mg/L, 3mg/L, 4mg/L, 5 mg/L). It was found that *Arjuna* extract inhibits the growth of *V. cholerae* at all concentrations and zone diameter increases with the increase of concentrations. The regression coefficient of the relationship between concentration and zone diameter varies from 0.75 to 0.984 for most of the isolates which indicates that there exists a linear relationship^[4].

To explore bark of *Terminalia Arjuna* for some bioactive compounds, free flavonoid extracts were screened for antimicrobial activity using Disc Diffusion Assay. Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and Total activity (TA) determination of each extract was also calculated. Free flavonoid showed activity

against *Escherichia coli*, *Pseudomonas aeruginosa*, *Raoultella planticola*, *Enterobacter aerogenes*, *Bacillus subtilis* and *Agrobacterium tumefaciens*. All the extracts showed significant antimicrobial potential against test microbes but the maximum inhibition zone was observed against *Agrobacterium tumefaciens* (IZ= 19mm, AI=1.461±0.010) & *Bacillus subtilis* (IZ= 16mm, AI= 1.230±0.098) by the bound and free flavonoid extract of the plant respectively. Almost all parts of plants (roots, stem, leaves, flowers, fruits and seeds) have been reported to have one or the other important bioactive compound^[5].

Nimba (Azadirachta indica)

Antimicrobial activity in leaf extract of *Nimba* against human pathogenic bacteria was studied. The purpose of the present study was to investigate the antimicrobial activity of *Nimba* leaves against human pathogenic bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Bacillus pumilus*. For this alcoholic extracts of *Nimba Patra* were used. Varying concentration of each extracts 200mg/ml, 150 mg/ml, 100mg/ml, 50mg/ml, 25mg/ml prepared by using disc diffusion method. When compared with *gentamycin* 200mg and *gentamycin* 10mg, the methanol and ethanol extract shows maximum inhibition on *Bacillus pumillus*, *Pseudomonas aeruginosa* and

Staphylococcus aureus in an ascending order. *Nimba* leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premise^[6]. Supports the use of the *Nimba* seeds in traditional medicine to treat infections conditions especially those involving the eye and ear^[7].

Patala (Stereospermum suaveolens)

One study was done to evaluated in vitro antibacterial activity and their minimum inhibitory concentration of five plants. Out of five, one of the test drug for this study was stem of *Patala (Stereospermum suaveolens)* which was used in traditional and folk medicine of Sri Lanka for treatment of various bacteria induced infectious diseases. This was performed using aqueous extracts against two gram positive [*Staphylococcus aureus* & *Bacillus cereus*] and three gram negative [*Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhimurium*] clinically important bacterial pathogens, using Kirby-Bauer disk diffusion test and broth microdilution technique. But unfortunately, the results showed, that the extracts of *Patala (Stereospermum suaveolens)* possessed no antibacterial activity against any of the bacterial strains tested^[8].

Moreover, barks, flowers, roots and leaves of *Patala* are used by traditional

healers, rural communities and pharmaceutical companies for remedies of diseases like heating, vomiting, eructation, piles, acidity etc. One of the study indicates the anti-inflammatory effect of the ethanol extract of *Patala* bark given orally at the dose of 200 and 400mg/kg body weight was studied in rats using the *carrageenan*-, *dextran*-, and *histamine*-induced hind paw oedema, and cotton pellet-induced granuloma formation models. *Indomethacin* at the dose of 10mg/kg body weight was used as a standard drug. The extract (400mg/kg body weight per os) showed maximum inhibition of oedema at the end of 3h with *carrageenan*-, *dextran*-, and *histamine*-induced rat paw oedema, respectively. The extract (400mg/kg) exhibited significant reduction (34.77%) in granuloma weight in the cotton pellet-induced granuloma model^[9].

Similar way one study was done to evaluation of anti-inflammatory potential of aqueous extract of root bark of *Patala* (*Stereospermum suaveolens* DC) by using *carrageenan*-induced paw oedema method in Wistar adult rats. The aqueous extract at 125mg/kg was showed significantly inhibited oedema and produced the significant ($p < 0.05$) anti-inflammatory effects. *Carrageenan* induced oedema model is biphasic and significant activity in dose 125mg/kg seen as

compared to 250mg/kg, 500mg/kg of aqueous extract^[10].

Ashvattha (Ficus religiosa)

Ficus religiosa L., commonly known as *Ashvattha* or *Peepal* is a medicinally important tree species. In one study, the scholar investigated the antimicrobial activity of diethyl ether and methanol extraction of bark and leaves of *Ficus religiosa* plant against three bacteria (*E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) and one fungi (*Aspergillus niger*). The result shows that the methanolic extract of both leaves and bark shows antimicrobial activity on three tested bacteria and no effect on *A. niger*. In methanol extracts *S. aureus* showed maximum sensitivity (inhibition zone 28mm) followed by *E. coli* (inhibition zone 16mm) and *P. aeruginosa* (inhibition zone 12mm). Diethyl ether extracts of leaves were also showed maximum inhibition on *S. aureus* followed by *E. coli* and *P. aeruginosa*. Both methanol and diethyl ether extract of bark showed antimicrobial activity on three types of tested bacteria and very less inhibition activity on *A. niger*. But comparatively bark extract of both the solvents were showed less antimicrobial activity than leaves extract on the tested microbes^[11].

Aqil and Ahmad in 2007 investigated the activity of 70% ethanol extracts of *F.*

religiosa leaf against nine bacterial species and found an inhibitory effect against six of those strains including *S.aureus* and *E.coli*. The same team also conducted studies in 2006 to show the activity of the ethanolic leaf extract against two other strains namely *B.cereus* and *S. agalactiae*^[12]. Ramakrishnaiah and Hariprasad in 2013 investigated the antimicrobial activity of *Ficus religiosa* by measuring the zone of inhibition (Zoi) produced by two types of solvent extracts namely methanol and diethyl ether extracts of bark and leaves, on three bacteria in which two Gram negative bacteria (*E.coli* and *Pseudomonas aeruginosa*), one Gram positive bacteria (*Staphylococcus aureus*) and one fungus (*Aspergillus niger*). Ethanolic leaves extract was successful to inhibit wide range of microorganisms which mainly includes *B.subtilis*, *S.aureus*, *P.aeruginosa*, and *E.coli* but failed to show any action against *C.albicans* and *A.niger*. The fruit extracts have high potential towards antibacterial activity but no antifungal activity. The antibacterial study was carried out by agar disc diffusion method. Leaves extracts were prepared with water, acetone, ethanol, and methanol. In case of *Ficus religiosa* aqueous and ethanolic leaves extracts showed antibacterial activity against *E.coli* and *P.vulgaris*^[13]. The chloroform, methanol and water extracts of leaves were observed to have activity against a

range of bacteria and fungus namely *S.typhi*, *S.typhimurium*, *P.vulgaris*, *K.pneumoniae*, *P.aeruginosa*, *A.niger*, *P.chrysogenum* using methanol extracts, *S.typhi*, *P.aeruginosa*, *K.pneumoniae*, *P.vulgaris*, *A.niger*, *P.chrysogenum* using chloroform extracts and *S.typhi*, *A.niger*, *P.chrysogenum* using water extracts. The method of well diffusion was used for the assay (Hemaiswarya et al., 2009)^[14].

Arka (*Calotropis procera*)

One of the study reveals the antimicrobial effect of ethanol, aqueous and chloroform extracts of leaf and latex of *Calotropis procera* on six bacteria namely, *E.coli*, *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* and three fungi: *Aspergillus niger*, *Aspergillus flavus*, *Microsporium boulardii* and one yeast *Candida albicans* using agar well diffusion and paper disk methods. The results revealed that ethanol was the best extractive solvent for antimicrobial properties of leaf of *Calotropis procera* followed in order by Chloroform and aqueous (P<0.05). The growth of six bacterial isolates were inhibited by the three extracts except *P.aeruginosa* and *S.pyogenes* that were not inhibited by the aqueous extracts of both leaf and latex of *Calotropis procera*. The best antifungal activity was recorded in ethanol extract of *Calotropis procera* latex against *Candida albicans*. The

minimum inhibitory concentration (MIC) for the ethanol extract was between 5.0 and 20.0 mg/ml for fungi^[15]. Leaf extracts, chopped leaves and latex of *Calotropis procera* have shown great promise as a nematicide in vitro and in vivo^[16]. The potentials of *Calotropis procera* leaves in water treatment and its ability to reduce total viable count have also been reported (Shittu, et al., 2004).

DISCUSSION

Kadamba has been reported to possess wound healing, antioxidant, antimalarial and hepatoprotective activity^[3]. The chemical constituents of *Kadamba* are *Cadambin*, *Pentosan*, *Benzoic acid*, *Cincotannic acid*, *N-Nonacosane*, *Chryin*, *Gossypetin*, *Umbelliferone* and *Adicardin*^[17]. The study should be done to explore the exact mode of antimicrobial action according to modern point of view. That's why, *Kadamba* might be prove as one of the potent source to avoid various infections. The flavonoids in the *Arjuna* was majorly responsible for its antimicrobial activity. This study revealed that *Terminalia Arjuna* would be a good antibacterial drug.

Azadirachta indica leaves possessed good anti-bacterial activity, confirming the great potential of bioactive compounds and is useful for rationalizing the use of this plant in primary health care. The extracts of *Nimba* when used as medicinal plant, could be useful

for the growth inhibition of the *carcinogenic bacterium*, *S. sobrinus*. (Md Mohashine Bhuiyan et al., 1997). The phytoconstituents alkaloids, glycosides, flavanoids and saponins are antibiotic principles of plants. These antibiotic principles are actually the defensive mechanism of the plants against different pathogens (Hafiza, 2000). The result was also supported by (Faiza aslamet al., 2009)^[6].

After online search, we found the important article on *Patala* bark and root bark for its anti-inflammatory action instead of finding its antimicrobial action. One study reveals no used of *Patala* stem in antimicrobial activity. That's why one should try to evaluate the same study by using *Patala* leaves. In spite of that, the ethanol extract of *Patala* (*Stereospermum suaveolens*) stem and root stem possesses maximum anti-inflammatory activity in a dose-dependent manner, in various experimental model. It was found that the *Stereospermum suaveolens* revealed the presence of alkaloids, steroids, flavonoids, and phenolic compounds. The Anti-inflammatory activity may be attributed to the presence of different phytoconstituents present in the plant root extract, especially steroids and flavonoids, which are found to act by reducing the release of inflammatory substance like prostaglandin there by reducing exaggeration^[9]. Similar way we should try the experimental study to evaluate the anti-

inflammatory as well as antimicrobial study of *Patala* leaves also.

Leaves of the *Ficus religiosa* were found to yield *campesterol*, *stigmasterol*, *isofucosterol* etc^[18-20]. The *Calotropis procera* latex demonstrated strong inhibitory effect on the test organisms than *Calotropis procera* leaf. It was observed that the leaf extract could be said to be bacteriostatic while the latex extract exhibited bactericidal effects.

After overlooking the literature of *Charaka Chikitsa Sthana*, in *Dvivraniya Chikitsa*

Adhyaya, the above *sutra* comes at the successive last part of this chapter. After explaining wound types, various wound healing medicine etc. *Acharya Charaka* explain this six *Dravyas* [Table No. 1] typically for *Vrana Rakshoghna Karma*^[2]. From this we assumed that *Charaka* would also be expect the *Rakshoghna Karma*, prevention of wound from various infections, instead of wound healing and anti-inflammatory property from this six *Dravyas*.

Table No.1: Showing the Details of Six Anti-Microbial herbs

Sr.No.	Dravya	Family	Latin Name	Part Use
1.	<i>Kadamba</i>	<i>Rubiaceae</i>	<i>Anthocephalus indicus</i> Miq.	<i>Patra</i> (Leaf)
2.	<i>Arjuna</i>	<i>Combretaceae</i>	<i>Terminalia Arjuna</i> W.&A	<i>Patra</i> (Leaf)
3.	<i>Nimba</i>	<i>Meliaceae</i>	<i>Azadirachta indica</i> A. Juss.	<i>Patra</i> (Leaf)
4.	<i>Patala</i>	<i>Bignoniaceae</i>	<i>Stereospermum suaveolens</i> DC.	<i>Patra</i> (Leaf)
5.	<i>Ashvattha</i>	<i>Moraceae</i>	<i>Ficus religiosa</i> Linn.	<i>Patra</i> (Leaf)
6.	<i>Arka</i>	<i>Asclepiadaceae</i>	<i>Calotropis procera</i> (Ait) R.Br.	<i>Patra</i> (Leaf)

According to *Dhanvantari Nighantu*, *Kadamba* has property of *Vrana Samrohana* (wound healing)^[21]. *Arjuna* has property of *Vrana Nashana* (wound healing) explain by *Dhanvantari Nighantu*, *Bhavaprakasha Nighantu* and *Kaiyyadeva Nighantu*^[22]. *Nimba Patra* has *Vranaghna Karma*, explained in *Bhavaprakasha Nighantu* along with *Vrana*

Nashana property also explained by *Dhanvantari Nighantu* and *Shodhala Nighantu*. It has *Krimighna* property (antimicrobial) explained in *Bhavaprakasha Nighantu* and *Raj Nighantu*^[23]. *Acharya Bhavaprakasha* explain the *Vrana-Astra Jita* (wound healing along with blood purifier) *Guna* of *Ashvattha*^[24]. *Arka* has *Krimihara* and *Shophahara* (anti-

inflammatory) *Karma* explained by *Dhanvantari Nighantu*, *Kaiyyadeva Nighantu*, *Bhavprakasha Nighantu* and *Raj Nighantu*^[25]. Along with this, though *Patala* has been not explain directly for its *Vrana Ropana* activity or for its *Rakshoghna Karma* but it shows the

properties like *Tridosahara*, *Astra Jita* explain by *Shodhala*, *Kaiyyadeva* and *Bhavprakasha Nighantu*. Also it is explained for its *Shophahara* and *Dahaghna Karma* explained by *Kaiyyadeva Nighantu* and *Raj Nighantu*^[26].

Table No.2: Showing the Rasadi Panchaka of Six Anti-Microbial herbs

Sr. No.	Dravya	Rasa	Vipaka	Virya	Guna
1.	<i>Kadamba</i>	<i>Madhur, Kashaya lavana</i>	<i>Katu</i>	<i>Shita</i>	<i>Guru, Ruksha</i>
2.	<i>Arjuna</i>	<i>Kashaya</i>	<i>Katu</i>	<i>Shita</i>	<i>Laghu, Ruksha</i>
3.	<i>Nimba</i>	<i>Tikta, Kashaya</i>	<i>Katu</i>	<i>Shita</i>	<i>Laghu,</i>
4.	<i>Patala</i>	<i>Tikta, Kashaya</i>	<i>Katu</i>	<i>Ushna</i>	<i>Laghu, Ruksha</i>
5.	<i>Ashvattha</i>	<i>Kashaya, Madhur,</i>	<i>Katu</i>	<i>Shita</i>	<i>Guru, Ruksha</i>
6.	<i>Arka</i>	<i>Katu, Tikta</i>	<i>Katu</i>	<i>Ushna</i>	<i>Laghu, Ruksha, Tikshna</i>

According to *Guna* [Table no.2] & *Karma*, *Kadamba*, *Arjuna*, *Nimba*, *Patala*, *Ashvattha*, and *Arka* all are *Vrana Ropaka* and have tendency to inhibit the growth or to avoid microbes (*Rakshoghna Karma*). All the six *Dravyas* are *Kashaya Rasa Pradhan* (Astringent) and *Shita Virya* (Cold in Nature with endothermic property). Due to *Panchamahabhuta* (five primitive factors) *Siddhanta*, *Kashaya Rasa* and *Shita Virya* both are important in *Vrana Ropana*. Along with this, *Katu Vipaka*, *Laghu* (light) and *Ruksha* (dry) *Guna* are *Kapha Shamaka* and *Kleda*

Nashaka. *Arka* is *Tiksha* in *Guna*^[25]. That's why we conclude that the growth of microbes may not be possible under the influence of this *Dravyas*.

CONCLUSION

The results of this review article are very encouraging. The study revealed that *Kadamba*, *Arjuna*, *Nimba*, *Patala*, *Ashvattha* and *Arka* possess antimicrobial activity. As now, little work has been done on above plant leaves for the antimicrobial activity and hence extensive investigation is needed, to exploit,

to take this herbs successfully under various preparations like ointment, jelly, liniment, oils etc. as antiseptic medicine.

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