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VILWADI AGADA AND ITS ANTIMICROBIAL PROPERTY WITH SPECIAL REFERENCE AS ANTI-VIRULENCE AGENT

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ABSTRACT

The discovery of antibiotics is one of the greatest medical breakthroughs in the treatment of bacterial infections. Although the antibiotics are highly effective, inappropriate, or overuse has led to bacterial antibiotic resistance. In the present era of multiple antibiotic resistance generated by various strains of bacteria, sort an alternative ways of tackling this global public threat from naturally occurring antimicrobial agents especially from medicinal plants. In this regard, formulations with specific indication against infectious diseases may find useful in tackling multiple drug resistance. This article review the role of Vilwadi Agada, an antitoxic formulation in bacterial infections as a remedial measure from the context of *Agadtantra* by establishing its antibacterial activity in a conceptual way.

KEYWORDS: Vilwadi Agada, Antibiotic resistance, Agadtantra, Antimicrobial property.

INTRODUCTION

The discovery of antibiotics is one of the greatest medical breakthroughs in the treatment of bacterial infections. Although the antibiotics are highly effective, inappropriate, or overuse has led to bacterial antibiotic resistance. With every passing year, the overall number of antibiotics effective against bacteremia is found declining which predisposes us towards a future of antibiotics that are ineffective.[1] Present guidelines revealed that many antibiotics suggested against bacterial pathogens have been deleted with the addition of relatively few antibiotics and antibiotic combinations.[1] Furthermore, there are incidences of resistance against some of these newly added antibiotics. It is therefore imperative to find alternative ways to treat infections especially those caused by bacterial pathogens.[1] An alternative

approach is to develop anti-virulence therapies that interfere with bacterial virulence determinants (e.g. toxins, enzymes, or surface proteins) and/or pathways that mediate virulence (e.g. two-component virulence regulatory systems) that would less likely promote the development of antibiotic resistance. One of the pivotal characteristics of an anti-toxin or anti-virulence compound is that the compound does not affect bacterial viability or growth. Hence, this strategy ensures the preservation of the host endogenous micro flora population. That is by inhibiting bacterial virulence traits, the bacteria are less able to colonize the host and this may allow the host natural immunity to eradicate the attenuated pathogen.

Many studies showed that a number of bioactive components isolated from natural products have the ability to significantly reduce bacterial growth. Although, the underlying molecular mechanism of these chemical entities has yet to be established, these compounds represent potential new scaffolds for the development of viable leads from natural products as anti-virulence agents in a bacterial infection. Hence, Bacterial toxins or virulence factors appears to be a key approach for future drug design.

Bacteria causes infections by the secretion of bacterial toxins similar to the release of poison by *viṣa dravya* such as *jangama*, *sthavara*, *gara* etc. Hence, the bacterial toxin can be considered as a *viṣa* and it possess the qualities what *viṣa* carries, even though to a lesser extent. So, the antitoxic formulation which are having *viṣahara prabhava* mentioned in *Agadtantra* can be a good choice of intervention against bacteremia. Even though the antitoxic formulations have the inherent nature of neutralizing the *viṣa*, it mainly act by improving the immunity of the host to fight against the invasion or further spread of *viṣa* by its property of *hrdayavarana*. Hence, the effect of antitoxic formulation against bacterial pathogenesis can be studied under the anti-virulence therapies. These conceptualization signifies the importance of ayurvedic formulation particularly antitoxic drugs to combat the infectious disease with its specific action against bacterial toxin- a major virulence factor.

Extensive knowledge on how bacterial pathogens establish infection and increased understanding of genomic determinants of pathogen virulence have opened a path to tailor robust novel anti-virulence therapeutics. Even though the effect of ayurvedic formulation against bacterial infection was studied by numerous researchers, its mechanism of action with special reference to genomic determinants of virulence factor was still unknown.

***Vilvādi agada*[2]**

It is one of the widely practised and potent antitoxic formulation, mentioned in *Aṣtānga Hridaya, Aṣtānga samgraha, Kriyākoumudi, Viṣavaidya Jyōtsnika, Sahasrayoga* under the context of *Sarpaviṣa* chikitsa. It has got wide range of indication such as in conditions like *bhujanga (snake poisoning), lūtha (spider), unduru (rat poisoning), vrischika (scorpion), Viṣucika (cholera), Ajīrna (indigestion), gara (artificial poisoning), jvara (fever)* and in infectious conditions. Practically, it gained popularity among the traditional and ayurvedic practioners of Kerala owing to its extensive utility in acute infectious diseases and other emerging diseases. It has been used in waterborne diseases, viral exanthemas and other infectious epidemics and pandemics occurred in this locality.

Constituents of *Vilvādi Agada*

Table 1: Constituents of *Vilvādi Agada.*

Sl.no	Drug	Botanical name	Family	Common name	Parts used	Proportion
1	*Vilva*	*Aegle marmelos* (L.) Corrêa	Rutacea Juss	Bael tree Golden apple	Root bark	1 part
2	Surasaḥ	*Ocimum tenuiflorum* L	Lamiaceae Martinov	Holy basil	Inflorescence	1 part
3	*Karañja*	*Pongamia pinnata* (L.) Pierre	Fabaceae Lindl	Indian beech	Seed	1 part
4	*Nataṃ*	*Valeriana jatamansi* Jones ex Roxb.	Valerianaceae Batsch	Indian valerian	Root	1 part
5	*Surāhva*	*Cedrus deodara* (Roxb. ex D.Don) G.Don	Pinacea	Deodar	Heart wood	1 part
6	*Harītakī*	*Terminalia chebula* Retz.	Combretaceae R.Br	Chebulic myrobalan	Fruit rind	1/3 part
7	*Āmalakī*	*Phyllanthus emblica* L.	Phyllanthaceae Martinov	Emblic myrobalan	Fruit rind	1/3 part
8	*Vibhītakī*	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae R.Br	Belleric myrobalan	Fruit rind	1/3 part
9	*Pippalī*	*Piper longum* L.	Piperaceae Giseke	Indian long pepper	Dried spikes	1/3 part
10	*Nāgaram*	*Zingiber officinale* Roscoe.	Zingiberaceae	Dry Ginger	Rhizome	1/3 part
11	*Maricam*	*Piper nigrum* L.	Piperaceae	Black pepper	Fruit	1/3 part
12	*Haridrā*	*Curcuma longa* L.	Zingiberaceae Martinov	Indian saffron	Rhizome	1/2 part
13	*Dāruharidrā*	*Berberis aristata* DC	Berberidaceae Juss	Tree turmeric	Stem bark	1/2 part

14	*Basta mūtra*	*Capra aegAgrus hircus*	Bovidae	-	Urine	Sufficient quantity

Pharmacodynamics of *Vilvādi Agada*[3]

Sl.no	Drug	Rasa	Guna	Veerya	Vipaka	Karma
1	*Vilva*	*Kaṣāya Tikta*	*Laghu Rūksha*	*Uṣna*	*Katu*	*Kapha-vata śāmaka*
2	*Surasa*	*Katu Tikta*	*Laghu Rūksha Tīkṣna*	*Uṣna*	*Katu*	*Kaphavātajit viṣāpaha*
3	*Karañja*	*Katu Tikta*	*Laghu Tīkṣna*	*Uṣna*	*Katu*	*Kapha-vātajit bhūtagna*
5	*Nataṃ*	*Katu*	*Laghu Snigdha*	*Uṣna*	*Katu*	*Kaphavātahara viṣāpaha*
6	*Surāhva*	*Tikta*	*Laghu Snigdha*	*Uṣna*	*Katu*	*Kaphavātahara*
7	*Harītakī*	*Madhura Amla Katu Tikta Kaṣāya*	*Laghu Rūksha*	*Uṣna*	*Madhuram*	*Tridoṣasamana*
8	*Āmalakī*	*Amla Kaṣāya Katu Tikta Madhura*	*Sara Rūksha*	*Sēta (as per Cakrapāni Mruduvērya)*	*Madhuram*	*Tridoṣasamana*
9	*Vibhītakī*	*Kaṣāya*	*Laghu Rūksha Sara*	*Sēta*	*Madhuram*	*Kaphapithajit*
10	*Pippalī*	*Katu*	*Snigdha Sara Laghu Tīkṣna*	*Anuṣnasēta*	*Katu*	*Vātakaphanāsana rasāyana*
11	*Nāgaram*	*Katu*	*Guru Tīkṣna Rūksha*	*Uṣna*	*Madhuram*	*Vātakaphahara*
12	*Maricam*	*Katu*	*Laghu Tīkṣna Rūksha*	*Uṣna*	*Katu*	*Kaphagna*
13	*Haridrā*	*Katu Tikta*	*Rūksha*	*Uṣna*	*Katu*	*Kaphapithaśamana viṣanāsana*
14	*Dāruharidrā*	*Tikta*	*Rūksha*	*Uṣna*	*Katu*	*Kaphanāsana Viṣanāsana*

Bhavana dravya : Ajamootra[4]

Rasa – Katu, Tikta, Lavana

Guna – Uṣna, Tīkṣna, Snigdha

Veerya – Uṣna

Vipaka – Katu

Karma – kapha samana, vāta anulomana, pithāvirodhi

Phytoconstituents and its pharmacological properties

Table 3: Phytoconstituents & pharmacological properties of constituents of *Vilvādi Agada.*

Sl.no	Drug	Phytoconstituents	Pharmacological property
1	*Vilva*	marmenol, marmin, marmelosin, marmelide, psoralen, alloimperatorin, rutaretin, scopoletin, aegelin[5]	Antidiarrhoeal, Antimicrobial, Radioprotective, Anticancer, Anti-inflammatory, Ulcer healing potential[6]
2	*Surasa*	Linalool, Eugenol, methyl eugenol, carvacrol, five fatty acids – stearic, palmitic, oleic, linoleic, linolenic acids	Antibacterial, antioxidant, anti-inflammatory, analgesic, immunomodulatory[7]
3	*Karañja*	Demethoxy-kanugin, gamatay, kaempferol, kankone, kanugin, karangin, pinnatin, pongamol, pongapin, quercitin, saponin.	Antimicrobial, antioxidant, anti-inflammatory, anti-diabetic, anthelminthic, and insecticidal activities[8]
4	*Nataṃ*	Valepotriates, flavones, sesquiterpenoids, terpenoids, phenolic compounds[9]	anti-oxidant, neuroprotective anti-inflammatory, anti-viral, antidepressant, antispasmodic, analgesic
5	*Surāhva*	sterols, β-himachalene, sesquiterpene, Deodarin, Himachalol, Cedeodarin, β-sterol, shikimic acid[10]	Anticancer, Antimicrobial, anti-inflammatory, analgesic, Antiarthritic
6	*Harītakī*	5-methylindolo-quinoline, gallic acid, ellagic acid, tannic acid, chebulic acid, chebulagic acid, corilagin, mannitol[11]	Antibacterial, antioxidant, anti-inflammatory, anti-cancer, hypoglycemic
7	*Āmalakī*	apigenin, gallic acid, ellagic acid, chebulinic acid, quercetin, chebulagic acid, Emblicanin A, corilagin[12]	Anti-inflammatory, anti-pyretic, antineutrophil and antiplatelet properties, anti-bacterial, anti-viral[13]
8	*Vibhītakī*	gallic acid, chebulic, chebulagic, chebulinic acids ellagitannins, corilagin, ellagic acid, triterpenes and triterpenoidal glycosides[14]	antidiabetic, antiulcer, analgesic, antifungal, antibacterial, anti-hypertensive activity
9	*Pippalī*	piperine, methyl piperine, pipernonaline, asarinine, pellitorine, piperlongumine[15]	Hepatoprotective, cardioprotective, antimicrobial, anti-tumour, antiapoptosis
10	*Nāgaram*	gingerols, shogaols, 3-dihydroshogaols, paradols, dihydroparadols, acetyl derivatives of gingerols, gingerdiols[16]	antimicrobial, anticancer, antioxidant, antidiabetic, nephroprotective, hepatoprotective, immunomodulatory activity
11	*Maricam*	Piperamide, Pipericide, Piperine, B, Sarmentine, Sarmentosine, Brachyamide	antioxidant, antitumor, antipyretic, analgesic, anti-

		B, isobutyl-eicosadienamide, Tricholein	inflammatory, hepatoprotective, immuno-modulatory, antibacterial, antifungal
12	*Haridrā*	Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tumerone, diferuloylmethane[17]	Antimicrobial, anticancer, neuroprotective, anti-inflammatory, antioxidant[17]
13	*Dāruharidrā*	Protoberberine, berbamine, Berberine, oxycanthine, palmatine, dehydrocaroline, jatrorhizine and columbamine[18]	Hepatoprotective, antimalarial, anticancer, anti-inflammatory, antimicrobial[18]
14	*Ajamootra*	Nitrogenous constituents: nitrogen, urea, uric acid, allantoin, creatinine, creatine, ammonia. Non nitrogenous constituents: carbonates, bicarbonates, phosphates, sulphates, chlorides, calcium, magnesium.[19]	Antimicrobial activity

Antimicrobial activity of individual constituents of *Vilvādi Agada*

Vilva

The essential oil isolated from the leaves of *A. marmelos* has showed antifungal activity against animal and human fungi like *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum gypseum*, *Epidermophyton floccosum*, *Aspergillus niger*, *Aspergillus flavus.*[20] Its leaf extracts have fungicidal activity against various clinical isolates of dermatophytic fungi.[21] Studies showed that essential oil may interfere with the Ca^{2+} dipicolinic acid metabolism pathway and possibly inhibit spore germination. Thus it act as antifungal agent by lowering the vegetative fungal body inside the host or in solid medium.[22]

Various extracts of *A. marmelos* leaves, roots and fruits have been reported to be active against many bacterial strains. In a study conducted by Venkatesan *et al.* in 2009 showed that aqueous and ethanolic extract has activity against *E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*; in which ethanolic extract showed considerably more activity than the aqueous extract. Maximum activity was shown against *Bacillus subtilis* followed by *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa.*[23]

It shows activity in the early stages of viral replication with minimum host cytotoxicity in contrast to modern virucidal chemotherapeutic agents which usually act in the later stages of viral replication and have potent side effect.[24]

Surasa

Essential oils of Ocimum have antibacterial, antifungal and antiviral properties. Studies show that aqueous, alcoholic, chloroform extract of leaves of *Ocimum sanctum* (OS) shows significant activity against *E.coli*, *P.aeruginosa*, *S. typhimurium* and *S.aureus*. Extract obtained from OS were observed equally effective against pathogenic gram-positive and gram- negative bacteria.[25] A study conducted Viviane.et.al. (2016) observed the effects of combinations of Ocimum essential oil with standard antibiotics used in clinical practice against *S.aureus* and *P. aeruginosa* strains.[26] This study also proved that *O. basilicum* essential oil, either alone, or in combination with antibiotic imipenem displayed significant antibacterial activity against *S.aureus* strains. Various studies in the literature suggest that linalool, a monoterpene, is the main ingredient responsible for the antibacterial activity.[27]

Karañja

A study conducted by Punitha R, et al., 2006 showed that the methanolic extract of seed have the highest antimicrobial activity among all the plant parts and the possible reason behind these may be the presence of several essential oils trapped in its seeds. In this study the highest zone of inhibition was shown in *Klebsiella pneumoniae* (26 mm) at a concentration of 400 µg/ml and *Escherichia coli* (25 mm) at a concentration of 400 µg/ml.[28]

Various extracts of the plant exhibited antibacterial activity against a broad spectrum of gram-negative and gram-positive bacteria, such as *Proteus vulgaris, Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Salmonella typhimurium*, *Escherichia coli* etc.[29]

Seed oil showed maximum antifungal activity against *Aspergillus niger* followed by *Aspergillus terreus* and *Candida albicans.*[30]

Crude aqueous extract of the seed completely inhibited the growth of herpes simplex virus type-1 (HSV-1) and HSV-2 at concentration of 1 and 20 mg/ml (w/v), respectively.[31]

Nataṃ

8-acetoxyl-pathchouli alcohol, a kind of sesquiterpenoid, showed slight antibacterial activity against *Pseudomonas aeruginosa* (64 µg/mL minimum inhibitory concentration, MIC) and *Staphylococcus aureus* (128 µg/mL MIC) compared to gentamicin (5 µg/mL MIC).[32] Its

different extracts were shown to exert adverse effects on *S.aureus* (chloroform fraction, 0.27 mg/mL MIC) compared to control imipenem (less than 0.0005 mg/mL MIC).[33] Powder and essential oil of *Valeriana jatamansi* Jones exhibited inhibitory effect on *S.aureus* (0.625 mg/mL and 6.25 mg/mL MIC).

Essential oil extracted from *Valeriana jatamansi* at the concentration of 400 µg/mL exhibit antifungal effect against *Microsporum canis* and *Aspergillus flavus* with 50% and 60% inhibition respectively and inhibit the growth of *Fusarum solani* to 70%.[34]

Surāhva

Many in vitro studies have been conducted on *C. deodara* to elucidate their antimicrobial potential. Among different tested microbial strains, Gram positive bacterial strains were found more sensitive towards *C. deodara* than Gram negative bacterial and fungal strains. A study conducted by Bai et al. (2015) speculated that its water extract exhibited maximum inhibition zone against *S.aureus* (26.5 mm) followed by *B. subtilis* (21.5 mm) and B. cereus (21.2 mm) respectively when compared with positive control (22.5–27.3 mm).[35] The compound 3-p-trans-coumaroyl-2-hydroxyquinic acid (CHQA) isolated from needles of *C. deodara* caused significant hyperpolarization, membrane protein conformation as well as complete cell membrane lysis of *S.aureus* cell membrane. It is considered as one of the reason behind its activity against *S.aureus* strains.[36] 3R-dihydromyrecetin (DMY), a flavonoid present in the *C. deodara* extract exhibited promising bactericidal potential against *S.aureus* with a minimum inhibitory concentration (MIC) of 0.25 mg/ml. DMY completely inhibited bacterial growth with increased nucleotide release in a concentration-dependent manner thereby indicating leakage of cytoplasmic components from bacterial cells.

Harītakī

Ponnusamy et al. studied the antibacterial activity of ethanolic extract of *T. chebula* fruit against clinically important strains of bacteria and found out that *T. chebula* extract was highly effective against *Salmonella typhi, Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa.* These results suggests that T. chebula dry fruit possesses a potential broad spectrum antimicrobial activity.[37] In a study conducted by Vemuri.et.al found out that methanolic extract has an excellent anti-microbial activity.[38] Gallic acid and its ethyl ester isolated from ethanolic extract of *T. chebula* showed antimicrobial effect against methicillin-resistant *Staphylococcus aureus.*[39] Ripe seeds of *T. chebula* also exhibited strong antibacterial activity against *S.aureus.*[40] It protects epithelial

cells against influenza A virus, supporting its traditional use for aiding in recovery from acute respiratory infections.[41] The methanol and aqueous extracts of *T. chebula* showed a significant inhibitory activity on human immunodeficiency virus-1 reverse transcriptase.[42] A study conducted by a team of Japanese researchers found out that *T. chebula* was effective in inhibiting the replication of human cytomegalovirus in vitro and in an AIDS model with immunosuppressed mice and concluded that it may be beneficial for the prevention of CMV diseases and immuno compromised patients.[43]

Āmalakī

A study by Hossain et al., 2012 found out that Fruit ethanol and acetone extracts have moderate activity against *Fusarium equiseti* and *Candida albicans* in which Grisofulvin was used as standard antibiotic.[44] Alcoholic and aqueous extracts of *Phyllanthus emblica* showed significant antibacterial effect against *Staphylococcus aureus* and *E. coli*. In this study the activity appears to be stronger against gram-positive bacteria, and have only limited efficacy against fungi.[45]

Vibhītakī

The aqueous and methanol extracts of *T. bellirica* fruits have shown significant antibacterial activity against *S.aureus*, *Salmonella enterica* serovar Typhi, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica* and *Escherichia coli.*[46] In a study it have been concluded that Tannins present in *T. bellirica* fruit inhibited microbial proliferation by denaturation of proline rich proteins. Tannins have strong affinity towards proteins having higher proline content and hence form tannin-complex. The complexation of tannin with proteins and other compounds causes inhibition of extracellular enzyme as well as unavailability of substrates for bacterial nutrition, and this eventually leads to inactivation of microorganisms and eventually death.[47] In a study by Elizabeth suggested that the antimicrobial potential of fruit extracts can be attributed to the presence of chemical substances gallic acid and ethyl gallate in the *T. bellirica* fruits.[168] In this study further analysis of minimal inhibitory concentrations (MICs) of aqueous and methanol extracts indicated that methanolic fruit extract was highly effective against *S.aureus* with lower MIC values.[48]

Pippalī

Various extracts of *P. longum* were evaluated against bacterial pathogens such as *S. albus*, *S. typhi*, *P. aeruginosa*, *E. coli* and *B. megaterium* and one fungus, *A. niger* in comparison with

streptomycin. All the extracts except the aqueous extract exhibited a good antibacterial activity against the tested strains.[49] The studies conducted by Vaghasiya et al[50] and Aneja et al[51] investigated significant activity against bacterial strains like *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella aerogenes, Klebsiella pneumonia, Citrobacter freundii, Pseudomonas aeruginosa* etc. Khan et al concluded its antibacterial effect is due to the alkaloids and terpenoids present in crude drug.[49]

Nāgaram

Many in vitro studies proved the antimicrobial potential of *Z. officinale* extracts towards both gram positive and gram negative bacteria. Ginger extract (10 mg/kg) intra peritoneally exhibited a dose dependent anti-microbial activity against *Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli and Candida albicans.*[52] The study revealed that all the extracts except the water have antibacterial activity and among all ethanol extract showed maximum antimicrobial activity.[53] Antibacterial activity of crude polysaccharides, flavonoids, aqueous and ethanol extracts of Z. officinale were reported by the study conducted by Gao et al.[53] In this study the ethanol extracts and crude flavonoids exhibit antibacterial activities against *Escherichia coli, Staphylococcus aureus, Shigella fiexneri, Proteus vulgaris and Pseudomonas aeruginosa*, while polysaccharides and aqueous extracts did not showed any activity. Antifungal activity of the ethanol extract of *Z. officinale* was reported against two strains of *Candida albicans* and this results revealed the efficient antifungal activity of the extract.[54]

Maricam

The antibacterial activity of *P. nigrum* carried out against *B. subtilis, S.aureus, P. aeruginosa, E. coli, A. niger, A. alternate, A. flavus and F. oxysporum* showed zone of inhibition ranging from 8-18 mm. In this study, maximum inhibition is exhibited by gram positive bacteria *S.aureus* (18 mm) and minimum by gram negative bacteria *E. coli* (8 mm). Similarly, Piperine showed maximum antifungal activity towards *Fusarium oxysporum* (14mm), *Alternaria alternata* (17mm), minimum effect against *Aspergillus flavus* (30mm) and very least effect against *Aspergillus niger* (38mm).[55] The chloroform extract of black pepper showed significant damage to bacterial cell membrane of *E. coli*, and *S.aureus* followed by disruption of respiration.[56]

Haridrā

Khattak et al. studied the antifungal and antibacterial an ethanolic extract of turmeric. The extract showed antifungal activity towards *Trichophyton longifusus* and *Microsporum canis* and weak antibacterial activity against *Staphylococcus aureus.*[57] The antibacterial study on aqueous extract of *C. longa* rhizome showed that the MIC (minimum inhibitory concentration) value of 4 to 16 g/L and MBC (minimum bactericidal concentration) value of 16 to 32 g/L against *S. epidermis*, *S.aureus*, *Klebsiella pneumoniae*, and *E. coli.*[58] A study on the methanolic extract of turmeric revealed MIC values of 16 µg/mL and 128 µg/mL against *Bacillus subtilis* and *S.aureus*, respectively.[59] Curcumin, the main ingredient of turmeric has a wide-ranging antiviral activity. In several studies it has been shown to inhibit (human immunodeficiency viruses) HIV-1 integrase. Moreover, it can also inhibit the infection and replication of viral genes.[60] There are a number of researches on anti-influenza activity of biological constituents of turmeric and its ability to fight influenza-A virus (IAV) via the inhibition of its adsorption and replication.[61]

Dāruharidrā

Three extracts of *B. aristata* (aqueous, alcoholic and powdered drug in distilled water) were tested for antifungal activity and all the three extracts showed antifungal activity against the Candida and Aspergillus species tested. Out of the three types of extracts, the best results were obtained by using the alcoholic extract and significant antifungal activity was found against Candida species and Aspergillus species.[62] The aqueous extract of *Berberis aristata* exhibited a broad spectrum antimicrobial potential ranging from 12 to 25 mm. In this study, *Klebsiella pneumoniae* was most susceptible organism (25 ± 0.889 mm) followed by *Staphylococcus aureus* > MRSA > *Salmonella typhimurium* > *Staphylococcus epidermidis. Enterococcus faecalis* was found to be the least sensitive organism (12.75 ± 0.322 mm) whereas *Klebsiella pneumoniae*, *Shigella flexneri* and *Salmonella typhimurium* were completely resistant to the extract. Among the two yeast cultures tested, *Candida albicans* exhibited an inhibition zone of 22.75 ± 0.595 mm while *Candida tropicalis* was completely resistant.[63]

Goat's urine

An experimental study on goat's urinary peptides depicted that cationic peptides present in the goat urine showed significant antimicrobial activity against *S.aureus* and E. coli with a zone of inhibition 23 mm and 26 mm and MIC value of 0.0199 µg/ µl and 0.039 µg/µl

respectively. While no antimicrobial activity was observed in all other fractions containing neutral/anionic peptides.[64]

In a study conducted by Sibel.et al[137] concluded that Goat's use exhibit significant antibacterial effect against the tested organism (*E.coli, V.cholera, P.aeruginosa, S.aureus, P.mirabilis*) in which *S.aureus* showed highest zone of inhibition(9.7mm) at a concentration of 7µg/disc while *E.coli* have a least inhibition zone of 6.7mm.

CONCLUSION

Vilvādi Agada consist of 13 herbal drugs in which Surasa, Karañja, Nataṃ, Haridrā, Dāruharidrā exhibit viṣanāsana property. While considering the pharmacodynamics of the ingredients, the main rasa of Vilvādi Agada is tikta katu and almost all the drugs possess Laghu, ruksha, teekshna guna and ushna veerya which may help the speedy action of the drug and may help to the fast spreading of the action of drug all over the body. Tikta rasa is raktaprasādhana in nature and it helps to detoxify the blood. Tikta rasa is krimighna and viṣaghna in nature. Due to its krimihara property it directly helps to act against microorganisms. Due to its viṣaghna property it helps to removes toxic substance from cellular level. Laghu Rūkṣa teekshna guṇa provides speedy action of the drug and made the drug capable of penetrating through the thick cell wall. Most of the ingredients have rasayana property and the drugs having rasayana property are particularly aimed at boosting the immunity of an individual by increasing the ojas. Hence, antimicrobial drugs also possess rasayana property to an extent. Besides all these facts, all viṣahara drugs mainly act due to their prabhāva which is unexplainable to some extent. Plants synthesize a diverse array of chemicals, known as secondary metabolites, as an adaptation for self-defense and communication with other organisms in their ecosystems. These secondary metabolites may be the reason behind the antibacterial activity of herbal drugs.

Compiling data of all its ingredients based on reported works showed that all the constituents possess antimicrobial property and among them most of them have antibacterial activity against S.aureus which need to be authenticated by experimental research works supporting this view.

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