

## REVIEW ARTICLE

# Biopesticides and its *Ayurvedic* Approach: A Review

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### ABSTRACT

Agricultural crops have to face lots of destruction due to invasion of micro-organisms, which can be bacteria, fungi, viruses, or pests. These invasions lead to a decrease in yield. Chemical pesticides used to control these destructions are quite effective but simultaneously responsible for severe health problems to mankind and other biodiversities. Biopesticides along with Ayurveda are potential tools used to overcome both of the above problems. Using advanced technologies (nanoparticles, extract preparations) along with Ayurvedic drugs (single or in combination) can replace these chemical pesticides efficiently, playing their integrated role in micro-organisms and pest management.

## 1. INTRODUCTION

Biopesticides are naturally occurring pesticides that come from living things like plants, animals, microorganisms (bacteria, fungus, viruses, and protozoa), or naturally existing chemicals. They are employed in an eco-friendly way to control weeds, nematodes, insects, plant diseases, and other agricultural pests. Unlike conventional chemical pesticides, biopesticides are often target-specific, biodegradable, and less damaging to humans, beneficial creatures, and the environment.

Biopesticides are crucial to integrated pest management (IPM), organic farming, and sustainable agriculture. They limit pesticide residues in food, lessen reliance on synthetic chemical pesticides, and reduce environmental pollution while preserving crop yield. Growing worries about food safety, ecological imbalance, and pesticide resistance have led to an increase in their use globally.

## 2. AIMS AND OBJECTIVES

Instead of being classified as general chemical pesticides, the word “biopesticides” refers to substances that are employed to control agricultural pests through unique biological effects. Biologist

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Shigetane Ishiwata from Japan discovered bio-pesticide in a sick silkworm in 1901. Then, 8 years later, Ernst Berliner in Thuringen, Germany, detected it in a sick flour moth caterpillar.

Present agricultural practices include the wide production and extensive use of chemicals known for their ability to cause negative health effects in humans and wildlife and to degrade the natural environment. Therefore, an urgent strategic approach is needed for a reduction in the use of agrochemicals and for the implementation of sustainable practices.

There were 390 registered active ingredients for biopesticides as of August 31, 2020.

### 2.1. Biopesticides and it's Classification<sup>[1]</sup>

Classification of bio-pesticides

- Microbial bio-pesticides: this contains living microorganisms like bacteria, fungi, viruses, protozoans to control pests.
- Some of the common examples are :*Bacillus thuringiensis*, *Trichoderma* fungi etc/biochemical biopesticides: occurs naturally having properties to control pests like plant extracts, essential oils, etc
- Plant incorporated protectants: it includes genetically modified plants produce pesticidal properties because of change in their genetics. Eg: Bt cotton Table 1.

### 3. MATERIAL AND METHODS

An alternate classification of biopesticides is of three types

1. Bio-fungicide: This type prevents fungal growth in and around plants. *Trichoderma* is one of the good examples
2. Bio-herbicide: It prevents the growth of herbs and provides plants competition free environment. *Phytophthora* is one of the bio-herbicides
3. Bio-insecticide: It helps in protecting the plants from insects. For example, *Bacillus thuringiensis*

With over 70% of Indians working in occupations related to agriculture, the Indian economy is mostly centered on agriculture. Following the Green Revolution in the 1970s, the use of artificial fertilizers and pesticides sky rocketed. The assessment notes that there is now a growing awareness of the threats that “rampant and continuous use of pesticides and fertilizers” bring to ecosystems, human health, and their “catastrophic” effects on soil microorganisms; over 970 bio-pesticide products are registered with the Central Insecticides Board and Registration Committee. Bio-insecticides would be the fastest-growing form in the Indian Biopesticides Market by 2023.

#### 3.1. Biopesticides Over Various Chemical Pesticides

Chemical pesticides have a range of outcomes. Two hundred deaths are thought to be caused each year in the USA by insecticide poisoning. According to the World Health Organization, there are 25 million incidents of pesticide poisoning each year, and 20,000 people die as a result in underdeveloped nations.<sup>[2]</sup>

1. Exposure to pesticides can cause various health disorders, such as Parkinsonism, Hodgkin’s disease, endocrine disruption, respiratory and reproductive disorders
2. Glyphosate is associated with breast cancer. The pesticides that contain alkyl urea and amines are found to be associated with brain tumors<sup>[3]</sup>
3. Overuse of pesticides can result in biodiversity loss. Due to the processes of bioaccumulation and biomagnification, numerous birds, aquatic species, and animals are in danger of dying from dangerous pesticides
4. Reduces the quality of soil; every year required of pesticide includes for crop production in the same area of land.

#### 3.2. Advantages of Using Biopesticides

1. Typically, biopesticides are less hazardous by nature than traditional pesticides
2. Contrary to broad-spectrum, conventional pesticides, which may harm a variety of organisms, including birds, insects, and mammals, biopesticides often only impact the target pest and closely related organisms
3. Biopesticides frequently work in extremely small doses and frequently degrade quickly, reducing exposure levels and mainly avoiding the pollution issues brought on by conventional pesticides
4. Biopesticides have the potential to significantly reduce the use of traditional pesticides while maintaining excellent crop yields when employed as a part of Integrated Pest Management (IPM) programs
5. Costlier but reduced number of applications
6. Biopesticides are readily biodegradable and break down after use, posing little threat to people, food, or the environment. This avoids bioaccumulation and, thus, issues with environmental pollution brought on by synthetic pesticides.<sup>[4]</sup>

In spite of all these benefits of biopesticides still their penetration remains slow in developing countries due to some very common issues, such as

- High cost
- Poor efficacy
- Inconsistent field performance associated with bio-pesticidal utilization
- Lack of knowledge.

When compared to conventional pesticides, which often take more than 3 years to get registered, innovative biopesticides are frequently approved in under a year Table 2.

#### 3.3. Biopesticidal Market and Policies Regarding Bio-pesticide

Around 100,000 hectares of land in India are thought to be used for organic crop growing. In addition, thousands of hectares of forest land have received organic certification. In addition, certain states, including Sikkim and Uttaranchal, have declared themselves to be organic states. In addition, as people’s awareness of their own health increases, there may be a growth in the area dedicated to the cultivation of organic crops. This suggests that India’s bio-pesticide industry has a lot of room to grow. Dependence is a significant problem even though organic farming can feed an expanding population.

The government has also made the necessary actions in the previous 5 years to encourage the use of biopesticides on a national scale. The “Zero-Budget Farming” method, which has had great success in southern India, is already being used in a few other states. When compared to conventional pesticides, which often take more than 3 years to get registered, innovative biopesticides are frequently approved in under a year.

The Food and Agriculture Organization of the United Nations refers to this method of farming as Zero Budget Natural Farming and places emphasis on avoiding the unnecessary expense of farming inputs, such as the purchase of expensive seed, chemical fertilizers, and pesticides.

The United States, Canada, and Mexico, which are members of the North American Free Trade Agreement, consume the majority of the biopesticides sold internationally (around 45%), followed by the European Union (20%).

At present, it is estimated that the bio-pesticide market will be worth between USD 3 and USD 4 billion in the USD 56 billion global pesticide market. It is predicted that the development of biopesticides will surpass that of chemical pesticides, with compound annual growth rates of 14.1%.

Over 3,000 tons of biopesticides are produced globally each year, and this number is rising quickly.

At present, valued at around USD 205 million, the US biopesticides industry is expected to reach over USD 300 million by the end of the decade. About 40% of the biopesticides produced in the world are consumed in North America. In 2005, it was estimated that the market for European biopesticides would be valued at over USD 135 million, and by 2010, it would have grown to over USD 270 million, with Oceanic and European nations accounting for 20% of global sales, respectively.

#### 3.4. Bio-pesticidal Market—in Billions

Sales of conventional pesticides are forecast to decline, whilst sales of biopesticides, which collectively account for 10% of the worldwide bio-pesticide market, are predicted to moderately increase in South

and Latin America. Although currently quite modest, the Asian market offers a huge opportunity for biopesticides as the mega-economies of China and India boost their use of biopesticides. At present, just 2.89% of the 100,000 metric tons of pesticides sold worldwide are biopesticides, but that percentage is predicted to rise by an estimated 2.3% yearly, according to India's agricultural ministry Figure 1.<sup>[5]</sup>

The National Agriculture Policy (2000) was introduced in India for the 1<sup>st</sup> time on July 28, 2000. The program placed a strong emphasis on providing farmers with an adequate and prompt supply of approved seeds, fertilizers, plant protection agents, and biopesticides.

Research, development, and commercialization of biopesticides and bio-fertilizers have been supported by a number of government organizations, including the Department of Biotechnology, the Ministry of Science and Technology, and the Ministry of Agriculture and Farmers Welfare. There are 12 biopesticides registered under the Insecticides Act, 1968, mentioned below.

### 3.5. Office of Pesticide Programmes (OPP) and Biopesticides and Pollution Prevention Divisions (BPPD's) Role

The Environmental Protection Agency (EPA) is devoted to fostering the development and use of biopesticides, which it regards as naturally low-risk pesticides.

The EPA's OPP, in collaboration with the Office of Chemical Safety and Pollution Prevention, collaborates with 10 Regional Offices and other EPA programme offices on a variety of pesticide challenges and themes, including:

1. Assessing prospective new insecticides and their applications
2. Reviewing safety of older pesticides
3. Registering pesticide producing establishments
4. Pesticide field programs, such as the frontline implementation activities carried out by states, tribes, and EPA Regional pesticide experts.

The BPPD is in charge of all regulatory actions involving biological pesticides. The biochemical pesticides branch and microbiological pesticides branch of the BPPD are in charge of registering biochemical and microbiological pesticides, respectively.

BPPD is also supporting IPM activities and organising the Pesticide Environmental Stewardship Program to reduce pesticide risk. The objective of the BPPD is to be a global leader in bio-pesticide regulation and pollution control. BPPD's aim is to preserve human health and the environment by lowering pesticide hazards.<sup>[4]</sup>

## 4. DISCUSSION

### 4.1. Recent Research Work Done

By using the leaf-dip method, the antifeedant and insecticidal effects of *Pedaliium murex* (Linn) (Family: Pedaliaceae) root ethanol extract (0.1, 0.2, 0.4, 0.8%) were examined against third, fourth, and fifth instar *Spodoptera litura* (Fab.) larvae. In the ethanol root extract, larval mortality of greater than 50% was seen at higher concentrations (0.8%).

The ethanol extract of *P. murex* contained reducing sugars, phenolic compounds, saponins, xanthoprotein, alkaloids, triterpenoids, tannins, and flavonoids, according to a preliminary phytochemical investigation. The development and growth of larvae are inhibited by saponins and their derivatives. In addition, protein and tannin combined to lower the availability of protein in hemolymph insect enzyme activity.<sup>[6]</sup>

### 4.2. Nanotechnologies

Nanoparticles (NPs) with a size between 10 and 1,000 nm or <100 nm are used in nanotechnology. Metals, including gold, silver, copper, and platinum, are the most common materials used to create NPs. Several studies have reported that nanotechnology shows an enhancement in the efficacy of certain biological substances on pests, a decrease in toxicity toward humans and the environment.

*Neem* (*Azadirachta indica*) oil can be effectively protected from quick degradation by the use of NPs, providing a more sustained action on the intended pests. The biodegradable polymers utilized in this type of formulation enable continuous administration of the active ingredient with no harm to the environment. *Neem* (*A. indica*) oil can be effectively protected from quick degradation by the use of NPs, providing a more sustained action on the intended pests. This type of formulation achieves continuous delivery of the active ingredient with no harm to the environment because the polymers utilised are biodegradable.<sup>[7]</sup>

*Catharanthus roseus* solvent extracts were tested for biological activity against larvae of the gram pod borer *Helicoverpa armigera*. The extracts were evaluated for preliminary screening at a concentration of 1,000 ppm. After 24 h of exposure to the extracts, larval mortality was detected. The findings indicate that ethyl acetate fractions of *C. roseus* leaf extract have the potential to be employed as a bio-pesticide.<sup>[8]</sup>

Weeds and pests are the primary issue in agriculture. Weedicides and pesticides are widely used to eradicate or kill weeds and pests, which results in double spending at the expense of crop maintenance. A bio-pesticide was created using the extraction process from 19 different weeds. The created bio-pesticide was evaluated against tea, paddy, maize, and cabbage plants in 5 agricultural fields. The pesticide was highly effective against aphids, leaf folder disease, thrips, Japanese beetle, armyworm, and the cutworm caterpillar. The bio-pesticide made from agricultural weeds had an impact that was between 50% and 75% more than that of pesticides with a traditional chemical basis.<sup>[9]</sup>

### 4.3. Biopesticides and its Approach with Reference to Ayurveda

Drugs mentioned in Ayurveda have been found beneficiary as biopesticides; some of the same mentioned in the table below.<sup>[10]</sup>

Nimba (*A. indica*), widely used as bio pesticide has been mentioned by various *Acharyas* as *krmighna*, *vishaghna* property with *tikt*, *katu*, *kashyam* rasa, important for prevention of microbial growth since it can work as an effective bio-pesticide by its antimicrobial activity.

Acharya charak has mentioned *mahakashyam*, such as *krimighna* and *vishaghna mahakashyam*.

Many other drugs with *tikshna* guna and *katu tikta kashaya* rasa have the property to kill micro-organisms and prevent plant by acting as biopesticides since it has been written in Ayurveda that the drugs with *katukorasa krimina hinasti*<sup>[33]</sup> *tikta rasa vishaghna krimighna*<sup>[34]</sup>.

The technologies mentioned above, such as the preparation of extracts through alcohol or using nano-particle technologies, can be used to increase the potency of Ayurvedic drugs helps in reducing their size and work on much deeper levels. The combination of these modern technologies combined with Ayurveda can make more and more effective biopesticides, which can work as potentially as any other chemical pesticides Tables 3-5.

## 5. CONCLUSION

The preliminary aim of this article was to establish the importance of Ayurvedic drugs and plants in the field of agriculture as how efficiently these can work as biopesticides and replace chemical biopesticides and their toxic effects.

These drugs, when incorporated with modern technologies, can give much prominent results. Technologies should be used as per the modern era with Ayurveda to make them work faster and more efficiently. Hundreds of *Ayurvedic* drugs itself and with various combinations can act as excellent biopesticides with no side effects and sometimes may be beneficial for the cellular body.

All the drugs with *katu–tikta* rasa have somewhere *vishaghna* property and *krimighna* property, killing microbes, destroying plants beneficial for human beings, and prevent effect of chemical pesticides.

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## 9. ETHICAL STATEMENT

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## 10. CONFLICT OF INTERESTS

The authors declare no conflicts of interest regarding the publication of this paper.

## 11. DATA AVAILABILITY STATEMENT

The data analyzed in this review were obtained from publicly available sources, including peer-reviewed articles, observational studies, and surveys accessible through databases.

## 12. DISCLAIMER/VIEW AND OPINIONS

The opinions expressed in this article are solely those of the authors and do not reflect the views of the International Research Journal of Ayurveda and Yoga or its editorial board.

## 13. AI-USE DECLARATION

The authors declare that no generative AI tools were used to create scientific content, interpret data, or draft any sections of this manuscript. AI-based tools were used solely for minor language and grammar refinements to improve clarity and readability. All scientific content, analysis, and conclusions remain the sole responsibility of the author.

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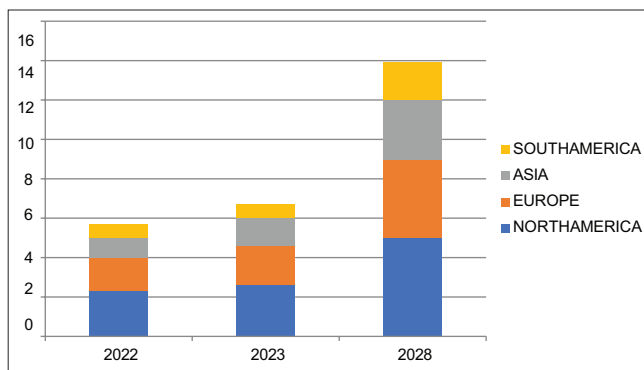
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**Figure 1:** Increased bio-pesticidal use in upcoming years for various countries (in million hectares of area)

**Table 1: Biopesticides are broadly classified into four categories**

| Types                               | Source                                                                                                |
|-------------------------------------|-------------------------------------------------------------------------------------------------------|
| 1. Biochemical pesticides           | Substances that interfere with mating, such as insects expheromones, as well as various scented plant |
| 2. Microbial pesticides             | Bacterium, fungus, virus, or protozoan as the active ingredient                                       |
| 3. Plant- incorporated- protectants | Pesticidal substances that plants produce from genetic material that has been added to the plant.     |

**Table 2:** Registered biopesticides under Insecticides Act, 1968

| S. No. | Insecticide                                               |
|--------|-----------------------------------------------------------|
| 1.     | <i>Bacillus thuringiensis</i> var. <i>israelensis</i>     |
| 2.     | <i>Bacillus thuringiensis</i> var. <i>kurstaki</i>        |
| 3.     | <i>Bacillus thuringiensis</i> var. <i>galleriae</i>       |
| 4.     | <i>Bacillus sphaericus</i>                                |
| 5.     | <i>Trichoderma viride</i>                                 |
| 6.     | <i>Trichoderma harzianum</i>                              |
| 7.     | <i>Pseudomonas fluorescens</i>                            |
| 8.     | Nuclear polyhedrosis virus of <i>Helicoverpa armigera</i> |
| 9.     | <i>Beauveria bassiana</i>                                 |
| 10.    | NPV of <i>Spodoptera litura</i>                           |
| 11.    | Neem-based pesticides                                     |
| 12.    | Cymbopogon                                                |

**Table 3:** Drugs of Ayurveda found beneficiary as a biopesticides

| S. No. | Drug                                                 | Uses                                                                           |
|--------|------------------------------------------------------|--------------------------------------------------------------------------------|
| 1.     | Extract of <i>Adhatoda Vasa</i> (vasa)               | Leaf folder, bacterial leaf blight, <i>Helminthosporium</i> leaf spot          |
| 2.     | <i>Thriphalakashayam</i>                             | <i>Helminthosporium</i> leaf spot                                              |
| 3.     | <i>Andrographis kashayam</i> and <i>Sidakashayam</i> | Aphids and borers in brinjal, ladies finger                                    |
| 4.     | Cow's urine <i>arkam</i>                             | <i>Helminthosporium</i> leaf spot, vein clearing disease, <i>Fusarium Wilt</i> |
| 5.     | Garlic <i>arkam</i>                                  | Bacterial leaf blight, <i>Helminthosporium</i> leaf spot                       |
| 6.     | Neem seed extract (for all crops)                    | Leaf folder, aphids, Jassids, fruit borer and stem borer                       |

**Table 4:** Krimighana Mahakashyam by Acharya Charak<sup>[11]</sup>

| S. No. | Drug         | Latin Name                  | Ras Panchak (Ref)                                                   | Action                                                                                                                                                                 |
|--------|--------------|-----------------------------|---------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1.     | Akshiv       | <i>Moringa oleifera</i>     | Rasa-Katu, Madhura Guna-Laghu, Ruksa Virya-Usna Vipaka-Katu         | The antibacterial effect is attributed to the presence of saponine, tannin, and alkaloid phytoconstituents <sup>[12]</sup>                                             |
| 2.     | Marich       | <i>Piper nigrum</i>         | Rasa-Katu Guna-Laghu, Teekshna Virya-Usna Vipaka-Katu               | Piperine is the major constituent of maricha responsible for anti-microbial action <sup>[13]</sup>                                                                     |
| 3.     | Gandir       | <i>Euphorbia antiquorum</i> | Rasa-Katu Guna-Laghu, Snigdha Virya-Usna Vipaka-Katu                | Diterpenoids showed important antimicrobial activity <sup>[14]</sup>                                                                                                   |
| 4.     | Kebuk        | <i>Costusspeciosus</i>      | Rasa-Tikta Guna-Laghu Virya-Sheeta Vipaka-Katu                      | The high antibacterial activity may be due to the presence of diosgenin, an alkaloid which is the pre-cursor for the synthesis of steroidal hormones <sup>[15]</sup>   |
| 5.     | Vidang       | <i>Embeliaribes Burm.</i>   | Rasa-Katu, Kashaya Guna-Laghu, Ruksa Virya-Usna Vipaka-Katu         | The major bioactive constituent of Vidanga is embelin, found to have antimicrobial activity <sup>[16]</sup>                                                            |
| 6.     | Nirgundi     | <i>Vitex negundo</i>        | Rasa-Katu, Kashaya Guna-Laghu Virya-Usna Vipaka-Katu                | Flavonoids present in the leaves have antimicrobial action <sup>[17]</sup>                                                                                             |
| 7.     | Kinihi       | <i>Achyranthes aspera</i>   | Rasa-Katu, Tikta Guna-Laghu, Ruksa Virya-Usna Vipaka-Katu           | The antibacterial activity of Kinihi can be attributed to the alkaloids <sup>[18]</sup>                                                                                |
| 8.     | Shvadanshtra | <i>Tribulus terrestris</i>  | Rasa-Madhura Guna-Guru, Snigdha Virya-Sheeta Vipaka-Madhura         | Steroidal saponins are responsible for antimicrobial action <sup>[19]</sup>                                                                                            |
| 9.     | Vrishparnika | <i>Ipomea reniformis</i>    | Rasa-Kashaya, Katu, Tikta Guna-Laghu, Ruksa Virya-Usna Vipaka-Katu  | Scopoletin is one of the most prominent phytoconstituents present in the Ipomoea reniformis plant, which may be responsible for antimicrobial activity <sup>[20]</sup> |
| 10.    | Akhuparnika  | <i>Merremiae marginata</i>  | Rasa-Kashaya, Katu, Tikta Guna Laghu, Ruksa Virya-Usna Vipaka- Katu | The antibacterial activity of may be attributed to the presence of flavonoids, terpenoids, amino acids, and glycosides <sup>[21]</sup>                                 |

**Table 5:** Vishghna mahakashyam by Acharya Charak<sup>[22]</sup>

| S.No. | Drug        | Latin name                  | Ras                                                                               | Action                                                                                                                                                                                                                                          |
|-------|-------------|-----------------------------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1.    | Haridra     | <i>Curcuma longa</i>        | Rasa Tikta, Katu Guna-Laghu, Ruksa Virya-Usna Vipaka-Katu                         | An active principle curcumin, antimicrobial effect anti-inflammatory effect, immunomodulatory effect <sup>[23]</sup>                                                                                                                            |
| 2.    | Manjishtha  | <i>Rubia cordifolia</i>     | Rasa Tikta, Kashaya, Madhur Guna-Guru, Ruksa Virya-Usna Vipaka-Katu               | Saponins and some naphthene derivatives are also isolated blood purifier, antiseptic, inflammations <sup>[24]</sup>                                                                                                                             |
| 3.    | Suvaha      | <i>Pluchea lanceolata</i>   | Rasa Tikta, Guna -Guru, Virya-Usna, Vipaka-Katu                                   | It contains protein, quercetine, iso-rhamnetin, and pluchine. Allaying the pain caused by thesting of scorpions <sup>[25]</sup>                                                                                                                 |
| 4.    | Sukshmaela  | <i>Elettaria cardamomum</i> | Rasa Katu, Madhur Guna-Laghu, Ruksha Virya-Sheeta Vipaka-Madhur                   | Seeds contain fixed oil, essential oil, principal constituent of the oil are cineol, terpineol, terpinene, limonene, subinone and terpineol inthe formof formicand acetic acids. Antimicrobial effect anti-inflammatory effect. <sup>[26]</sup> |
| 5.    | Palindi     | <i>Operculina terpehum</i>  | Rasa Tikta, Katu Guna-Laghu, Ruksha, Tikshna Virya-Usna Vipaka-Katu               | Antimicrobial effect anti-inflammatory effect, anti-helminthic effect <sup>[27]</sup>                                                                                                                                                           |
| 6.    | Chandan     | <i>Santalum album</i>       | Rasa Tikta, Madhur Guna-Laghu, Ruksa Virya- Sheeta Vipaka-Katu                    | Disinfectant, blood purifier, skin diseases <sup>[28]</sup>                                                                                                                                                                                     |
| 7.    | Katak       | <i>Strychnos potatorum</i>  | Rasa Madhur, Kashaya, Tikta Guna-Laghu, Vishada, Ruksa Virya-Sheeta Vipaka-Madhur | Antimicrobial, antioxidant leprosy <sup>[29]</sup>                                                                                                                                                                                              |
| 8.    | Shirish     | <i>Albizia lebbeck</i>      | Rasa Tikta, Katu Guna-Laghu Ruksha, Tikshna, Virya- Usna Vipaka-Katu              | Saponins and tannins, skin diseases, inflammation, anti-itching, allergic disorders, leucoderma, treatment of snake bite <sup>[30]</sup>                                                                                                        |
| 9.    | Sindhuvarak | <i>Vitex negundo</i>        | Rasa Katu Tikta Guna-Laghu, Ruksha Virya-Usna Vipaka-Katu                         | Anti-bacterial, anti-inflammatory, anti-helminthic property <sup>[31]</sup>                                                                                                                                                                     |
| 10.   | Shleshmatak | <i>Cordia dichotoma</i>     | Rasa Madhur, Guna-Snigdha, Guru, Pischil, Virya-Sheeta, Vipaka-Madhur             | Astringent, anthelmintic, anti-inflammatory <sup>[32]</sup>                                                                                                                                                                                     |