

Mutualistic Interaction of *Piriformospora indica* (*Serendipita indica*) with *Aloe vera*, the Wonder Plant for Modern Living

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Abstract

Aloe vera, a short-stemmed shrub is described as a “wonder plant”, due to its vast uses in various medical products. Since many decades, extensive research has revealed that the pharmacological active ingredients are distributed in both the gel and rind of the *Aloe vera* leaves. *A. vera* is very popular in cosmetic and pharmaceutical industries and it is needed in large quantities with higher fractions of important constituents. To satisfy the market demand, intervention of microbial community seems to be a promising approach, which helps to increase the growth and metabolites along with plant fitness. *Piriformospora indica* is a root colonizing endophytic fungus, having unique plant growth-promoting properties. It helps the plant to acquire more nutrients from soil even under extreme physical and nutrient stress conditions. It interacts with a wide range of hosts. Interaction of *P. indica* with *A. vera* resulted in overall increase in plant biomass and greater shoot and root length, as well as number of shoots and roots as compared to control under both *in vitro* and *in vivo* environment conditions. Apart from that, the photosynthetic pigments (*Chl a*, *Chl b* and total *Chl*) and aloin content were observed significantly higher in *A. vera* plantlets colonized with symbiotic endophyte. The antioxidant activities were also tested and found significantly higher as compared to control plants. This imparts the potential of *P. indica*, to resist the plants against phyto-pathogenic microbes. *P. indica* has been proved as a potential candidate to enhance the biomass production along with various value additions in the form of active ingredients in *A. vera*.

Keywords

Aloe vera, *Piriformospora indica*, Aloe Gel, Secondary Metabolites

1. Introduction

Aloe vera is a hardy, perennial, tropical succulent plant with properties like, drought resistant. It belongs to the family Liliaceae which, historically has been used for various medicinal purposes. Genus *Aloe* contains more than 500 species of flowering succulent plants in the world [1]. The size of these plants varies from an inch to two feet or more in diameter. These plants are highly complex in shape and spread out colonies consisting of thousands of plants. Many *Aloe* species are scattered naturally in North Africa. Among 500 species, most commonly occurring species of *A. vera* is *Aloe barbadensis* [2].

A. vera is generally cultivated in moderate environments rather than chilling, such as tropical or sub-tropical regions. Another beauty of this plant is blooming; some older plants bloom and produce beautiful flowers with tall stock covered by bright color petals.

Aloe vera, a short-stemmed shrub is described as a “wonder plant”, due to its frequent use in various medical products. The gel obtained from the plant’s leaves has been used in many pharmaceutical products (Figure 1). The medicinal properties are well documented in various Indian indigenous system of medicine like Ayurveda, Siddha, Unani and Homeopathy [3]. Extensive research since last decades has revealed that the pharmacologically active ingredients are distributed in both the gel and rind of the *A. vera* leaves. *A. vera* is very popular in cosmetic and pharmaceutical industries [3] [4].

Different products from *A. vera* gel which are commercially available in the market are depicted in Figure 2.

A. vera is very popular in skin care markets due to its cooling effect, which protects skin from long time exposure of sun light [2] [4]. It helps in eliminating skin diseases like acne and eczema (Figure 3). Aloe is also marketed as a remedy for ulcer, diabetes, cancer, arthritis, cough, wound and immune disorders. It has been proven as an effective antiseptic, powerful detoxifier and tonic for the nervous system [5].

Aloe flowers are usually tubular or trumpet-shaped and are arranged in clusters and their fruit is capsular. There are more than 20 species of aloe and their number is growing steadily, as new hybrids are constantly being created. The acíbar, a yellowish liquid layer present between the skin of penca and the gel of *Aloe vera* is composed of aloin. It is bitter in taste and by boiling, it is transformed



Figure 1. *Aloe vera* plantlets and gel present in mature leaves.



Figure 2. List of products made from *A. vera* gel.

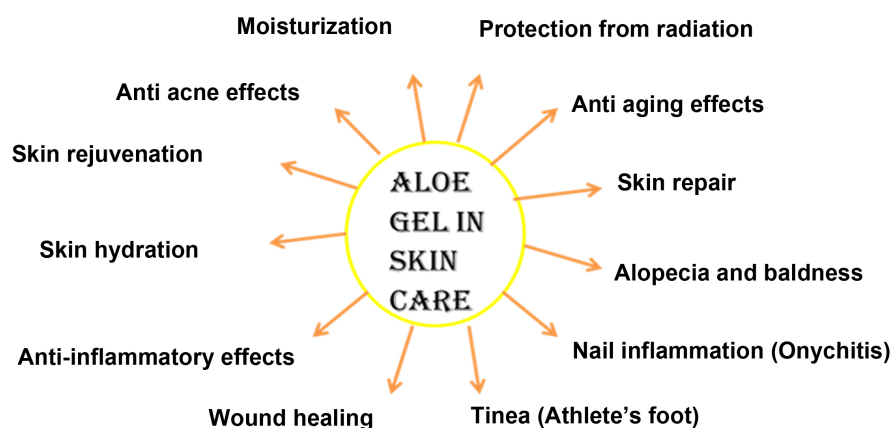


Figure 3. The pharmacological attributes which can be cured by *A. vera* gel products.

into a dark brown amorphous mass. Garcia *et al.* (2019) explained that acibar is composed of 40% to 80% of resin, and up to 20% of aloin, anthraquinone glucoside [3] [6]. The bark, green or bluish green exhibits 20% to 30% of the total weight of the plant. Color of the bark depends on various factors such as temperature, nutrition, climate and physiology of the plants. Pulp or gel is basically parenchyma tissues located at the central part of the leaf and exhibits 65% to 80% of the total weight [4] [7]. After fertilization, the flower matures and turns into a small fruit which later releases the seeds into atmosphere through wind. Since *A. vera* grows in difficult environmental conditions, so, apart from seed dispersal phenomenon it also reproduces by means of stolon or shoots, which are born at the basal part of stem. This new plant grows together with the mother plant and plant density increases with greater biomass [2] [3] [4] [7].

2. Chemical Constituents of *A. vera*

A. vera is a rich source of different chemical compounds which play a very important role in commercial production of medicines. Chemical composition of *A. vera* revealed the presence of <200 different biologically active molecules including carbohydrates, essential amino acids, enzymes, vitamins, minerals, secondary metabolites like; anthraquinones or phenolic compounds, saponins, sterols, and salicylic acid [4] [8]. Studies have revealed that there are 75 ingredients present in the Aloe leaf and have many nutraceutical properties. These chemical compounds are divided into following categories:

Anthraquinones: Almost 12 varieties of anthraquinones; Aloin, Isobarbaloin, Anthracene, Emodin, Ester of Cinnamonic acid, Chrysophanic acid, Barbaloin, Anthranol, Aloetic acid, Aloe Emodin, Ethereal oil and Resistannol are present in *A. vera* sap. They perform the functions of natural laxatives, painkillers and analgesics [6] [7]. **Figure 4** showing various metabolic components present in different *A. vera* leaves and tissues which are useful for pharmaceutical and beauty products. They contain powerful antibacterial, antifungal and virucidal properties. Two compounds, Aloin and Emodin have analgesic properties, and also work as potent antibacterials and antivirals [2] [6] [7] [8].

Enzymes: Following pharmacologically important enzymes are identified from various parts of *A. vera* plants: bradykinase, peroxidase, lipase, amylase, cellulase, alkaline phosphatase, carboxypeptidase, and catalase. Bradykinase facilitates to lessen the excessive inflammation of skin. Other enzymes help in the breakdown of fats and sugars, which help in fitness of our body by reducing the blood pressure and controls the sugar level in the blood [6] [8].

Sugars: *A. vera* produces many important monosaccharides and polysaccharides. These are derived from the leaf's mucilaginous layer. One of the most

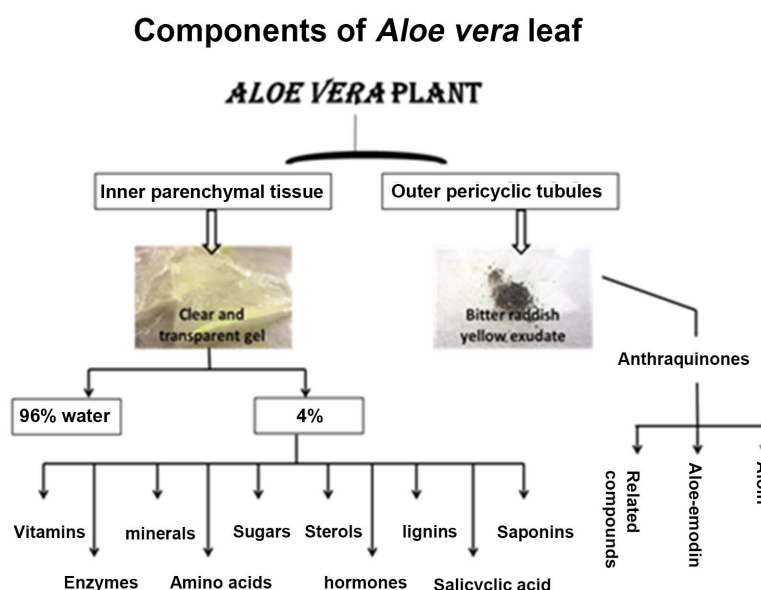


Figure 4. Metabolic components present in different *A. vera* leaf tissues.

important monosaccharide is mannose-6-phosphate, and polysaccharide is glucomannans [beta-(1,4)-acetylated mannan]. In recent studies, a glycoprotein with antiallergic properties, known as alprogen and novel anti-inflammatory compound, C-glucosylchromone, has been identified in *A. vera* gel [4] [7] [8].

Essential fatty Acids: There are 4 kinds of plant steroids present in *A. vera*; lupeol, LDL cholesterol, β -sisosterol and campesterol. All of these have anti-inflammatory properties and helps in wound cure. Another fatty acid constituent lupeol also possesses antiseptic and analgesic properties and is used in various medicinal products [4] [7].

Minerals: Essential minerals required for the proper functioning of various physiological and metabolic pathways are significantly present in *A. vera*. These minerals include; calcium, chromium, copper, selenium, magnesium, manganese, potassium, sodium and zinc. These minerals are essential cofactors of various enzymes which are being used in different metabolic processes [3] [6].

Vitamins: Vitamins are essential supplements to neutralize the adverse effect of free radicals and act as antioxidants. Vitamins protect the cells and tissues from toxic effects of these free radicals. A fair amount of Vitamin A (beta-carotene), vitamin C, vitamin E, vitamin B12, folic acid, and choline are identified from various parts of *A. vera* plantlets [2] [6].

Others constituents: Out of 22 amino acids, 20 amino acids and out of 08 essential amino acids (important for human beings), 07 essential amino acids were identified in *A. vera* plants. Two important plant hormones, Auxins and Gibberellins are present in *A. vera* which assist in wound recuperation and reduce inflammation. Additionally, salicylic acid holds antibacterial and anti-inflammatory properties. Saponins, which is a major component of *A. vera* gel (about 3%), possesses cleansing and antiseptic properties [6]. An inert polysaccharide lignin from *A. vera* is also used in skin medicines to enhance the penetrative effect of the other ingredients inside the skin tissues [2].

3. Mechanism of Actions

Wound healing: A mannose-rich polysaccharide, Glucomannan and a growth hormone, gibberellin, interacts with growth factor receptors at the fibroblast. Then it stimulates its activity and proliferation, which in turn notably increases collagen synthesis after oral intake. Aloe gel helps in increasing the collagen content on the wound which facilitates the penetration of other constituents inside the skin tissue [2] [4] [7]. It speeds up the wound contraction and accelerated the process of ensuing scar tissue. An increased synthesis of dermatan sulphate and hyaluronic acid in the granulation tissue of a healing wound accelerated the process of curing the wound [6] [9].

Anti-aging effects: Mucopolysaccharides present in skin tissue helps in binding moisture. *A. vera* sap/gel stimulates fibroblast cells which produces the elastin fibers and collagen. These two makes the skin pores less elastic and makes the skin tight and less wrinkled. Additionally, Aloe has cohesive properties which binds with the superficial epidermal tissue and stick them together and

softens the skin. Another important mineral Zinc, an astringent helps to tighten the skin pores. Amino acids help in softening the hardened skin cells and nourish the skin cells. Due to its moisturizing effect *A. vera* has been extensively studied in the field of treatment of dry skin. It has been proven *Aloe* gel improved the skin integrity, reduces wrinkles and decreases erythema. Apart from these effects aloe gel also shows anti-acne impact [2] [4] [9] [10].

Immunity booster: Calcium influx into mast cells due to inhibition of Al-progen, by inhibiting the antigen-antibody-mediated launch of histamine and leukotriene from mast cells. Anthraquinones stimulates the host's immune system. These actions are also act as antitumor activity. The anthraquinone and aloin inactivates various enveloped viruses like herpes simplex, varicella zoster and influenza and inhibit the progression inside the host cell [2] [8].

Laxative effects: A molecule anthraquinone present in *A. vera* shows laxative effects. It stimulates mucus secretion by restoring intestinal water content thus increases intestinal peristalsis [6] [8].

UV protection: It has been reported that *A. vera* gel have a defensive effect against radiation damage to the skin [2] [6].

Anti-inflammatory properties: *A. vera* reduces prostaglandin E2 manufacturing from arachidonic acid by inhibiting the cyclooxygenase pathway [2] [6].

Antiseptic properties: Six antiseptic agents; Lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols and sulfur has been identified in *A. vera*. These agents show inhibitory effect against microorganisms like fungi, bacteria and viruses [6] [10].

4. *Piriformospora indica*: A Mutualistic Plant Growth Promoter

P. indica is root colonizing endophytic fungus, extensively studied since last two decades due to its unique plant growth promoting properties. It helps plant to acquire more nutrients from soil even under extreme physical and nutrient stress conditions [11] [12]. It interacts with a wide range of hosts, including bryophytes, pteridophytes, gymnosperms and cruciferae and large number of mono and dicot plants [13]. The fungus promotes nutrient uptake, allows plants to survive under water, temperature and salt stresses, and confers systemic resistance to toxins, heavy metal ions, insects and pathogenic organisms. Further, it is shown to stimulate excessive production of biomass, early flowering, seed production and a potential microorganism imparting biological hardening to tissue culture-raised plants [11] [12] [14] [15] (Figure 5).

4.1. *P. indica* Promotes Growth of *Aloe vera* with Value Additions

The study had been undertaken to investigate the effect of endophyte *P. indica* on the physiological growth and the essential phytochemical contents of *A. vera*. The results showed overall increase in plant biomass and greater shoot and root length as well as number of shoots and roots as compared to control under both *in vitro* and *in vivo* environment conditions (Figure 6). Apart from that the photosynthetic pigment (*Chl a*, *Chl b* and total *Chl*) and gel content were

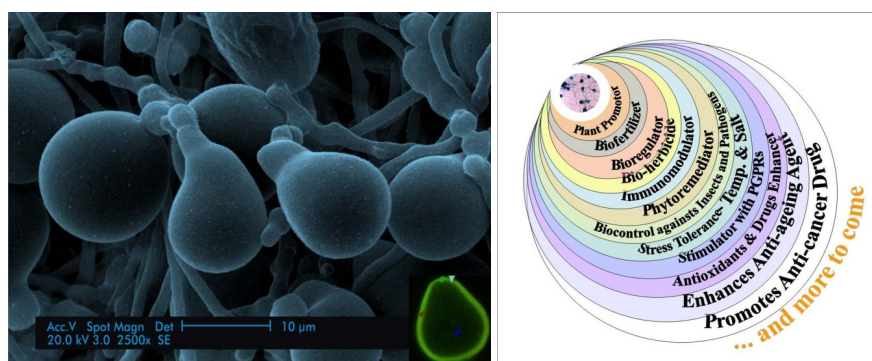


Figure 5. (a) Ultrastructure of *P. indica* Chlamydospore and (b) Globe showing important properties of *P. indica* since the year it has been discovered.

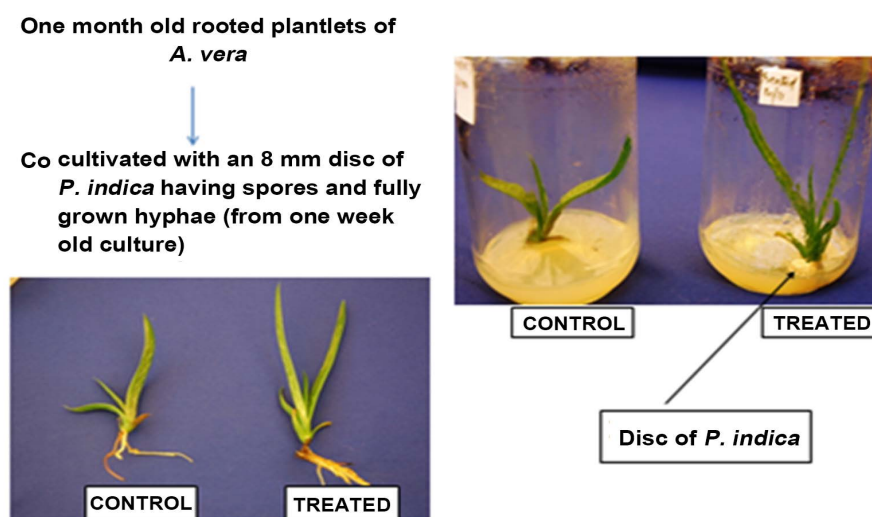


Figure 6. Interaction of *P. indica* with *A. vera* plantlets under *in vitro* conditions.

observed significantly higher (93.45%, 101.61%, and 60.46% respectively) in symbiotic fungus inoculated *A. vera* plantlets [16] [17].

The interaction study of medicinal plant with *P. indica* was conducted at large scale in bio hardening faculty at Amity University Noida, India (Figure 7).

The root colonization study with *P. indica* showed 67.5% colonization in *A. vera* roots. The percentage of colonization inside the root of plants showed the affinity of host-symbiont relationship. This colonization resulted in 16.5% increment in gel content along with 100% survival rate of colonized plants. This gel content has a great commercial importance to cosmetics and medicine industries as described earlier in this article. *P. indica* treated plantlets also had 52.53% higher aloin content as compared to the control plantlets. The aloin content as determined by HPLC was found to be 1.28 ± 0.057 mg aloin/g of leaf exudate for the treated and 0.844 ± 0.001 for the control set, marking an overall increase of 52.23%. This aloin is a potential anticancer agent and used as a laxative. These have various therapeutic properties and are used frequently in recovery from radiation damage and wound healing etc. [16].



Figure 7. The green house experiments of *A. vera* plants. Plants have been growing in bio-hardening facility of Amity University.

Further, the colonized host had higher phenolic content (41.73 ± 3.73 mg gallic acid equivalents/g dry wt.) as well as appreciable increase in radical scavenging capacity ranging from 20.05 ± 0.43 to 70.03 ± 0.98 (% antioxidant activity or % DPPH radicals inhibited). This confirms the ability of fungus to reduce oxidative damage associated with many phyto-pathogenic diseases and impart a greater tolerance to plants against pathogens. There could have been a shift in metabolic carbon flow towards antioxidant biosynthesis [17] [18].

4.2. *P. indica* Facilitates Nutrient Supply and Activates Various Biological Pathways

Our laboratory at Amity University, Noida first time successfully performed the interaction study of *P. indica* with *A. vera* under *in vitro* conditions. After successful colonization of *P. indica*, it facilitates the transfer/supply of nutrients like nitrogen and phosphorus to the roots of host plant which stimulates the cascade of biological reactions to synthesize various proteins, which participated in growth promotion of plant, enhancement of secondary metabolites, boosting immune system to trigger the defense mechanism against various biotic and abiotic stresses to increase the plant fitness [13] [19].

5. Conclusion

P. indica is considered as a potential candidate to enhance the biomass production along with various value additions in the form of active ingredients. It significantly increases the vegetative growth of plant and boost immunity to sustain in diverse environment as compared to control. The pharmaceutically important metabolites were also found increased many folds in fungal treated *A. vera* host.

Future Prospects

The study proves the future prospective of *P. indica* being used as biopriming agent for achieving better growth, better survival rate along with enhanced growth of *in vitro* raised plantlets along with substantial enhancement of secondary metabolites. The mechanism involved in interaction with mycobiont

with host needs to be elucidated to understand the physiology behind this phenomenon.

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Conflicts of Interest

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

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