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Account of *Aloe barbadensis*: Emphasizing Phytochemistry and Antibacterial Potential in Darjeeling Himalayas

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ABSTRACT

Plants have been widely used throughout human history as a basis for medical treatment. Ethnobotany, the study of traditional human uses of plants, is recognized as an effective way to discover future medicines. The Darjeeling Himalayas are home to a huge number of medicinal plants used by the local people. *Aloe barbadensis* is a plant that grows in this area and has a long history of being used as a medicinal plant with diverse therapeutic applications. The study was conducted to determine the phytochemical properties of the leaves of the plant. Flavonoidsce or absence of alkaloids, tannin, glycosides, steroids, saponins, phenol, flavonoids, quinine, xanthoprotein, coumarin, anthraquinone were tested. The antimicrobial activity of the leaves was also examined against gram negative bacteria (*Klebsiella pneumonia*) and one-gram positive bacteria (*Bacillus subtilis*) by disc diffusion antibiotic sensitivity testing method. Water, ethanol, chloroform and acetone were used to prepare the extract from fresh and dry leaves. The *Aloe barbadensis* shows the maximum inhibition zone in acetone and ethanol extracts against gram positive bacteria (*Bacillus subtilis*). The *Aloe barbadensis* shows the maximum inhibition zone in chloroform extract and acetone extract of dry leaf against gram negative bacteria (*Klebsiella pneumonia*). In a nutshell, this study reveals the different phytochemicals present and antibacterial properties of different leaf extracts of *Aloe barbadensis* from Darjeeling Himalayas

Keywords: *Aloe barbadensis; Anti-bacterial Property; Phytochemicals; Darjeeling Himalayas*

Introduction

Plants have been widely used by man since time immemorial as a basis for medical treatment. Ethnobotany, the study of traditional human uses of plants, is recognized as an effective way to discover future medicines. Reports suggest that the first study of medicinal plants started about 5000 years ago in Sumerians. In India, a great culture of Ayurveda medicine has been found, possibly as early as 1900 BC. Ancient Indian herbalists Charaka and Sushruta during the 1st millennium BC, described different herbs used in Ayurveda. The World Health Organization (WHO) estimates that 80 percent of the population of some 3rd world countries (e.g. Asian and African countries) presently uses

herbal medicine for some aspect of primary health care, as Pharmaceuticals are costly and herbal medicine is very cost-effective.

Aloe vera (*Aloe barbadensis* miller) is a plant of the Asphodelaceae family. The name is derived from the Arabic word ‘alloeh’ which means ‘bitter’, referring to the taste of the liquid contained in the leaves. Aloe is a drought registrant perennial succulent plant. The thick leaves are capable of retaining water. Aloe has been reported to have different types of beneficial effects (Aida *et al*., 2021).

Medicinal Importance of *Aloe barbadensis*

Anti inflammatory action

A number of *in-vitro* and *in-vivo* studies have revealed the anti-inflammatory activity of *Aloe sp*. (Sánchez *et al*., 2020). The sterols in *Aloe* have been reported to reduce croton oil induced oedima in mice. Out of all the sterols found in *Aloe sp.* Lupeol was the most effective and acted in a dose-dependent manner (Haller Jr, 1990). A report suggests that in a rat adjuvant-induced arthritic in- flammatory model, the *Aloe sp*. extracts were really effective (Davis *et al*., 1991). *Aloe* contains bradykinase, which is capable of breaking down bradykinin, a pain causing inflammatory substance (Ito *et al*., 1993). The production of prostaglandin E2 from arachidonic acid is reduced by Aloe extracts through the inhibition of the cyclo-oxygenase pathway (Sahu *et al*., 2013). Anti-inflammatory effects of *Aloe* have been reported in human colorectal mucosa *in vitro*.

Anti-tumor activity

In rat hepatocytes, the binding of benzopyrene to form a cancer-initiating benzopyrene-DNA adduct has been reported to be inhibited by *Aloe* (Sánchez *et al*., 2020). In another study, the authors reported the anticancerous activity of *Aloe* in DMBA/croton oil-induced skin papillomagenesis in Swiss albino mice. The tumor promoting effects of phorbol myristic acetate and glutathione S-transferase induction by *Aloe* gel suggest some role of Aloe in cancer treatment (Kim & Lee, 1997) Apart from these studies, a number of glycoproteins present in *Aloe* have been reported to have antitumor activity (Sánchez *et al*., 2020). Reports suggest that aloe juice have the ability to heal the body from cancer and chemotherapy-induced damage. Emodin, an anthraquinone from *Aloe* can inhibit malignant cancer cell growth (Mathieson & Thomson, 1971). However, it is to be mentioned that statistically significant studies on the efficacy of *Aloe* on human health are limited (Eshun & He, 2004).

Anti diabeic property of *Aloe*

Reports suggest that *Aloe* contains polysaccharides capable of exhibiting hypoglycemic properties. Phytosterols like 24-ethyl-lophenol, 24-methyl- lophenol, 24-methy-lenecycloartanol, cycloartanol and lophenol present in *Aloe* have shown anti diabetic properties in mice models of type 2 diabetes (Aida *et al*., 2021). In a streptozotocin induced rat model of diabetes, Aloe gel has been reported to exhibit a hypoglycemic effect as well as a beneficial effect on the lipid profile (Rajasekaran *et al*., 2004, Rajasekaran *et al*., 2006).

In general, *Aloe* extract reportedly increases glucose tolerance in both normal and diabetic rats (Mathieson & Thomson, 1971).

Anti aging and moisturizing property of *Aloe*

Due to the presence of biogenic materials in *Aloe*, it exhibits gerontology and rejuvenation of aging skin. Mucopolysaccharide which helps in moisture binding to the skin, is present in *Aloe* in high quantities. The formation of collagen and elasin from fibroblast is stimulated by *Aloe*. This, in turn, helps make the skin elastic. *Aloe* gel has reportedly improved the condition of dry skin associated with occupational exposure by having a moisturizing effect. The hardened skin is softened by the amino acids present in *Aloe*. The Zinc present in *Aloe* acts as an astringent and tightens the pores (Lanka, 2018).

Figure:1 Aloe sp.

Antibacterial property of Aloe barbadensis from Darjeeling Himalayas

Darjeeling is a beautiful hill town located in the northern part of West Bengal. It is a Himalayan region that houses different types of medicinal plants (Das, 1995). Many of the plants are used by the locals, however, proper scientific documentation is scanty.

Aloe barbadensis is commonly found in Darjeeling area and is used by the locals. The aim of the present study was to evaluate the phytochemicals present in Aloe and study the antibacterial properties of *Aloe* from Darjeeling Himalayas. *Bacillus subtilis* and *Klebsiella pneumonia* were taken as representative species of gram positive and gram-negative

bacteria, respectively for studying the antibacterial properties of *Aloe. Bacillus subtilis* is a rod shaped, endospore forming free living soil bacteria. *Klebsiella pneumonia* on the other hand, is a nonmotile encapsulated rod-shaped bacteria capable of causing destructive changes to the lungs of humans and animals if inhaled.

Materials and methods

Materials

All the chemicals were purchased from SRL and Glaxo, India. All plastic wares were purchase from Tarsons.

Plant Collection

Leaves of *Aloe barbadensis* were collected from different areas of Darjeeling town mostly near Mall Road, Hooker Road, Lebong Cart Road.

Preparation of Plant Extracts

The leaf extracts were prepared as described earlier (Al-Manhel & Niamah 2015, Harborn, 1998). Briefly, 5gm of dried leaf were mixed with 50ml of the different solvents in a conical flask. The mixture was kept in a shaker for 24 hours. Next, the mixture was centrifuged at 5000 rpm for 10 mins. The supernatant was used for the treatment.

Test for Tannin:

Tanin was detected by diluting the test sample with water and adding 2-3 drops of ferric chloride solution. Presence of tannin was confirmed by appearance of blue or green colour.

Test for Flavonoids:

Test for flavonoids was performed as described earlier (Mandal & Sanphui, 2023). In short, a few drops of Sodium hydroxide was added to the test sample. In presence of flavonoids, an intense yellow colour appears, which turns colourless on adding few drops of dilute sulfuric acid

Test for Alkaloids:

Mayer's test was performed to test the presence of alkaloids as described earlier (Mandal & Sanphui, 2023). In brief 3ml of Ammonium solution was added in1 ml of test sample. After 10 mins 10 ml of chloroform and 2 ml of mayer's reagent was added. Appearance of cream coloured precipitate confirms the presence of alkaloids.

Test for Glycosides:

The test sample was mixed with few drops of ferric chloride solution & glacial acetic acid. Two layers were formed on adding concentrated sulfuric acid. Lower radish brown layer and upper acetic acid layer turns bluish green indicating presence of glycosides.

Test for Saponin:

Presence of Saponin was tested as described earlier (Mandal & Sanphui, 2023). The test sample was diluted with water and shaken for 10-15 minutes. Presence of saponin was confirmed by formation of a foam layer on the top.

Test for Coumarin:

On mixing equal volume of test sample with alcoholic sodium hydroxide, appearance of yellow colour indicates presence of coumarin (Mandal & Sanphui, 2023).

Test for Anthraquinone:

Presence of anthraquinone was tested as described earlier(Mandal & Sanphui, 2023). Briefly, a few drops of magnesium acetate was added to the test sampleand mixed well. Presence of anthraquinone was confirmed by appearance of light pink colour

Test for Quinone:

Presence of Quinone was detected following the method described earlier (Mandal & Sanphui, 2023). Briefly, few drops of concentrated sulfuric acid or aqueous sodium hydroxide solution was added to the test sample. Change of colour confirms the presence of quinine.

Test for Phenol:

Presence of phenol was detected by adding 5% ferric chloride to the test sample. Appearance of dark green colour confirms presence of phenol (Mandal & Sanphui, 2023)

Test for Steroids:

Presence of steroids was tested as described earlier (Mandal & Sanphui, 2023). In short 10 ml chloroform was added to 1ml of the test sample. 1 ml concentrated sulfuric acid was added slowly by the wall. The presence of steroid was confirmed by the appearance of upper red colouredlayer and lower yellow coloured layer with green fluorescence.

Evaluation of Anti-Bacterial Property

The antibacterial activity of the extracts was tested by the disc diffusion method. In brief, a small round piece of Whatman filter paper was made using a punching machine. The paper discs were dipped in plant extract, dried in air and placed on the agar plate containing bacteria. For control, solvent dipped paper discs were used. The plates were incubated at 37^0 C for 24 hours. After 24 hours, the zone of inhibition was measured.

Results

Phytochemical Analysis of the Leaf Extract of *Aloe barbadensis*

The phytochemical analysis of the leaf extracts of *Aloe barbadensis*from Darjeeling Himalayas was performed following the standard methods mentioned in the Materials and

Methods section. Tests were performed to check the presence of different phytochemicals like alkaloids, tannin, glycosides, steroids, saponins, phenol, flavonoids, quinine, xanthoprotein, coumarin, anthraquinone. Test was performed using Aqueous, Ethanolic, Chloroform and acetone extracts of *A. barbadensis* leaf. This study revealed the presence of different types of phytochemicals in the leaf extracts. The result is shown in Table 1. The result indicates the presence of Alkaloids and Saponin in the Aqueous, Chloroform and acetone extracts. Tanin, Flavonoids and coumarin were found to be present in all four extracts tested. On the other hand Glycosides and Anthraquinone were absent in all four extracts tested. Steroids, Phenols and Quinone could be detected only in the Aqueous and Ethanolic extracts. Xanthoprotein was detected only in the Chloroform extract. Therefore, the result of this study indicates that *A. barbadensis* leaf is a rich source of phytochemicals.

Table 1: Phytochemical Analysis of Aloe barbadensis from Darjeeling Himalayas

Phytochemicals	LEAF			
	Aqueous extracts	Ethanol Extract	Chloroform extract	Acetone Extract
Alkaloid	+	-	+	+
Glycosides	-	-	-	-
Saponin	+	-	+	+
Tanin	+	+	+	+
Flavonoids	+	+	+	+
Steroids	-	-	+	+
Phenols	-	-	+	+
Coumarin	+	+	+	+
Quinone	-	-	+	+
Anthraquinone	-	-	-	-
Xanthoprotein	-	-	+	-

Anti Bacterial Property of Leaf Extract of *Aloe barbadensis*

The antibacterial properties of different leaf extracts of *Aloe barbadensis* from Darjeeling Himalayas were tested using the disc diffusion method. Two concentrations of each extract were used. Anti bacterial property was tested against one gram positive and one gram negative bacteria. *Bacillus subtilis* and *Klebsiella pneumonie*was were the gram positive and gram negative bacteria used, respectively. Result clearly indicates the antibacterial property of leaf extracts of *Aloe barbadensis* (Fig. 2). The chloroform and acetone extract exhibits the highest antibacterial effect against gram positive *Bacillus subtilis.* However, the aqueous and acetone extracts were more effective against gram negative *Klebsiella pneumonie.* The aqueous and acetone leaf extracts were more effective against gram

negative bacteria compared to the gram positive bacteria tested. Interestingly, no such preference was seen in the case of ethanolic and chloroform extracts.

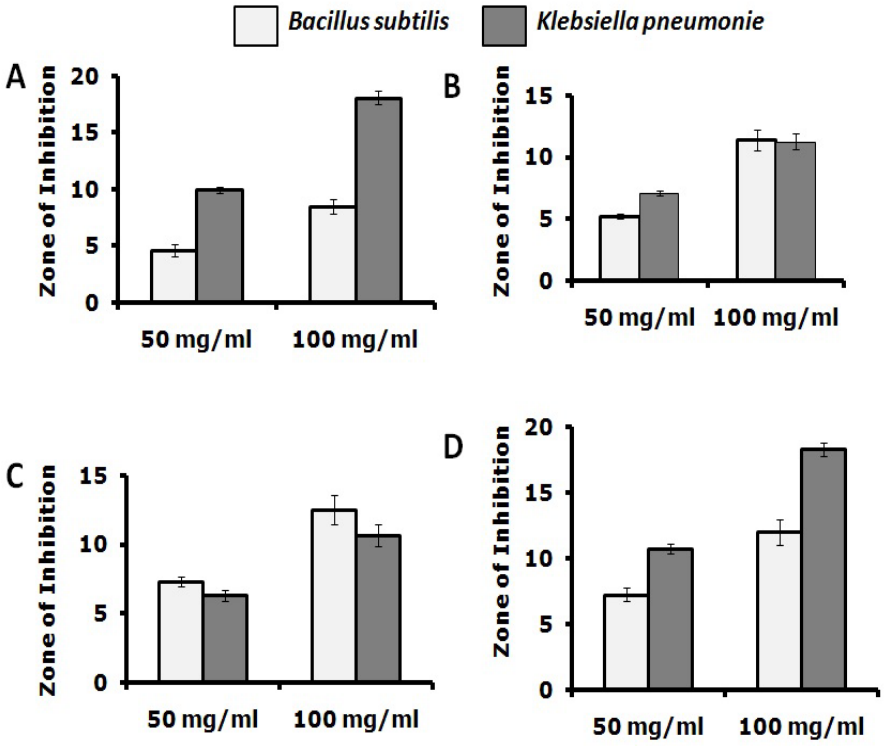

Figure 2: Antibacterial Activity of Leaf Extracts of Aloe barbadensis from Darjeeling Himalayas

The extracts were prepared at a concentration of 50mg/ml and 100 mg/ml. For testing the antibacterial activity, Disc diffusion method was used on Gram positive bacteria *Bacillus subtilis* and Gram-negative bacteria *Klebsiella pneumoniae*. The zone of inhibition (in mm) are represented graphically. Data represented as mean ± SEM of three independent experiments. A: Antibacterial property of Aqueous extract of leaf, B: Antibacterial property of Ethanolic extract of leaf, C: Antibacterial property of chloroform extract of leaf, D: Antibacterial property of Acetone extract of leaf. (Figure 2 graphs were plotted based on the result of experiments performed).

Discussion

The active components present in the leaves of *Aloe* have the power to make human life and health better in a number of ways. Aloe is a wonder plant, exhibiting its antiseptic, anti-inflammatory, anti-cancer, and anti-diabetic properties (Sahu *et al.,* 2013). This study on *Aloe barbadensis* from the Darjeeling Himalayas evaluates the phytochemistry and antibacterial properties of the plant. Results indicate the presence of various phytochemicals in four different solvent extracts of Aloe. The presence of different Phytochemicals contributes to the medicinal properties of the plants. The investigation revealed the antibacterial activity of *Aloe*. As the climatic and spatial conditions contribute to

the variation in the phytochemical property and, accordingly, the medicinal property of a plant, investigation of other medicinal properties of *Aloe barbadensis* from the Darjeeling Himalayas would be interesting.

The antibacterial property of *Aloe barbadensis* was tested on one gram positive bacteria *Bacillus subtilis* and one gram negative bacteria *Klebsiella pneumoniae.* All the extracts showed antibacterial potential against the tested bacteria. The aqueous and acetone extracts showed more antibacterial properties against the gram-negative bacteria *Klebsiella pneumonie* whereas acetone and chloroform extracts were most effective against the gram positive bacteria *Bacillus subtilis.*

Though an impressive number of in vitro and in vivo studies have been conducted on Aloe , the number of clinical trials has been limited. Moreover, all the clinical trials have been conducted with Aloe and not the active compound (Sánchez *et al*., 2020). It would therefore be very interesting to study the clinical effects of the relevant components in different human pathologies and conditions.

Conclusion

In conclusion, it can be said that the study indicates the presence of various phytochemicals in *Aloe barbadensis* from Darjeeling Himalayas and that the plant has antibacterial properties. The active ingredients present in succulent plants like Aloe have the power to benefit human life in a number of ways. The plant is used in everyday life in different ways. Aloe is sometimes referred to as a wonder plant due to its diverse medicinal activity. Though the plant is known for its medicinal values, detailed scientific study and clinical trials are needed to prove its acute efficacy. *Aloe barbadensis* is definitely nature’s gift to mankind and should be exploited scientifically in a more extensive way.

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