International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Phytochemical Extraction and Characterization of the Leaves of *Aloe vera barbadensis* for its Anti-Bacterial and Anti-Oxidant Activity

Priyanka Das[1], Alok Kumar Srivastav[2]

[1, 2] Master of Technology, Department of Biotechnology, Bengal College of Engineering & Technology, Durgapur, West Bengal, India

Abstract: ***The aim of the study was to investigate the phytochemical compounds, anti-bacterial and anti-oxidant activity of different extracts of Aloe vera. The phytochemical compounds were screened by qualitative analysis method. The detected phytochemical constituents were Tannins, Saponins, Alkaloids, Phenols, Terpenoids, Flavonoids. The different solvents such as methanol, petroleum ether, chloroform and aqueous were used to extract the bioactive compounds from the leaves of Aloe vera to screen the anti-bacterial and anti-oxidant activity. The anti-bacterial activity were demonstrated against the bacterial strains like Bacillus subtilis, Bacillus cereus, Staphylococcus aureus and Escherichia coli by disc diffusion method. Methanolic extract showed maximum inhibitory action against the bacterial strains followed by the aqueous extract. The anti-bacterial activity is attributed to anthraquinones and saponins. The anti-oxidant activity was evaluated by DPPH radical scavenging method. The highest radical scavenging activity was of methanolic extract followed by petroleum ether and aqueous extract.***

Keywords: *Aloe vera*, Phytochemicals, Anti-Bacterial, Anti-Oxidant, DPPH, Disc Diffusion.

1. Introduction

Aloe vera (Syn. *Aloe barbadensis* Mill). a monocotyledonous plant, belonging to family Asphodelaceae (Ali & Qaiser, 2005) and is indigenous to the Eastern and Southern Africa, the Canary Island and Spain. The genus comprises about 300 perennial species (Reynolds 1985). *Aloe vera*, a monocotyledonous, is a member of the family liliaceae. The genus Aloe has more than 500 species but only a few are medicinally important (Deng et al., 1999). *Aloe vera* is well known for its marvelous medicinal properties. These plants are one of the richest sources of health for human beings coming from nature. It has been grown as an ornamental plant widely. Products of the plant are used in the treatment of various ailments.

Aloe vera is a unique plant which is a rich source of many chemical compounds and plays an important role in the international market. Chemistry of the plant revealed the presence of more than 200 different biologically active substances including vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid (Chauhan *et al*. 2007).

Prof. Tom D. Rowe (1941) was probably first to take vital steps in the chemical analysis of the plant. With his efforts, *A. vera* achieved its first detailed evaluation. *Aloe vera* now reported to contains as many as 75 nutrients and 200 active compounds including sugar, anthraquinones, saponins, vitamins, enzymes, minerals, lignin, salicylic acid and amino acids (Vogler and Ernst 1999, Dureja *et al.* 2005 and Park and Jo 2006).

Aloe vera contains many vitamins including A, B1, B2, B6, C, E and F excluding vit. D (Chauhan *et al.* 2007). Among these, *Aloe vera* is the plant of greatest interest. Its leaves have been found to contain over 200 bioactive constituents (Waller et al., 1978). *Aloe vera* contain different bioactive matherials such as saponins, anthraquinones, mucopolysaccharides, steroids, vitamins and glucomannans (Liu et al., 2006, 2007). The juice from the leaves of different species yields a medicinal substance called the 'Aloe' drug. The drug contains anthracene derivatives occurring either free or in the form of glycosides, usually containing glucose (Fairbrain 1949; Capasso and Donatelli 1982).

Figure 1: Represents Active Constituents of *Aloe vera* Plant

2. Review of Literature

Aloe vera extracts are utilized in the development of anti-bacterial and anti-fungal products (Farnsworth 1984). Scientific studies support anti-bacterial and anti-fungal effect for substances in *Aloe vera* (Klein and Penneys 1988). The anti-microbial activity of *Aloe vera* juice was investigated by Cock Ian Edwin (2008) by agar disc diffusion against a panel of bacteria, fungi and yeast.

The anti-bacterial activity of leaves is attributed to anthraquinones (Boateng 2000, Garcia-Sosa *et al*. 2006 and

Dabai *et al*. 2007) and saponins (Reynolds and Dweck 1999 and Urch 1999).

Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. They exert their effect by scavenging reactive oxygen species, activating a buttery of detoxifying proteins or preventing the generation of reactive oxygen species (B. Halliwell and J. M. C. Gutteridge, 1992). In recent years, there has been an increasing interest in finding natural antioxidants, which can protect the human body from free radicals and retard the progress of many chronic diseases (C. Kaur and H. C. Kapoor, 2001) .The plant contains a large amount of phenolic compounds (T. Reynolds, Jr. J. S. Haller and B. Sultana, 2008) . It also has a high content of 1,8-dihydroxyanthraquinone derivatives (aloe emodin) and their glycosides (aloins), which are used as cathartic (Y. Ishii, H. Tanizawa and Y. Takino, 1994).

3. Materials and Methods

3.1 Preparation of Plant Extract

Fresh leaves of *Aloe vera* were collected from a local farm in Burdwan city, West Bengal state, India in the month of August, 2014 and was identified and authenticated by the Botany Department University of Burdwan. The fresh leaves were washed and dried under shade paper towel in laboratory for two weeks. Then they were homogenized into fine powder using a Mortar and Pestle. They were then stored in air tight bottles and were used for all the extraction process. Twenty grams of powdered plant material was mixed with 100 ml of various solvents such as Methanol, Petroleum Ether, Distilled water and Chloroform. The plant extracts were prepared by soxhlet apparatus collected and stored in vials for further studies.

3.2 Screening of Phytochemical Components

The freshly prepared extracts were subjected to standard phytochemical analyses for different constituents such as tannins, alkaloids, flavonoids, anthraquinones, glycosides, saponins, terpenoids and reducing sugars as described by Jigna *et al*. (2006) and Harbourne (1998).

3.3 Anti-Bacterial Assay

Antibacterial activity of the aqueous, chloroform, petroleum ether and methanolic extracts were evaluated by the agar diffusion method (Aida et al., 2001). The bacterial cultures were inoculated onto Nutrient Agar Medium. A sterile cork borer was used to make a well (6 mm in diameter) on the Nutrient Agar plates. 100 µl of each of the aqueous, chloroform, petroleum ether and methonolic extracts at concentrations of 25, 50, 75 and 100µg/ml, were applied separately in each of the wells in the culture plates previously seeded with the test organisms. The cultures were incubated at 37ºC for 24 h. Controls were set up in parallel using the solvents that were used to reconstitute the extract. The effects were compared with streptomycin at a concentration of 10µg/ml. Antibacterial activity was determined by measuring the zone of inhibition around each well (excluding the diameter of the well). For each extract, three replicate trials were conducted against each organism.

3.4 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The estimation of MIC of the crude extracts was carried out using the method of Akinpelu and Kolawole (2004). The MIC was taken as the lowest concentration that prevented the growth of the test microorganism. To 0.5 ml of varying concentrations of the extracts (25, 50, 75 and 100 µg/ml) 2ml of Nutrient Broth was added in the test tubes and then a loopful of the test organism was introduced. The procedure was repeated on the test organisms using the standard antibiotic streptomycin. A tube containing Nutrient broth only was seeded with the test organisms as described above to serve as controls. The culture tubes were then incubated at 37ºC for 24hrs. After incubation the tubes were then examined for microbial growth by observing for turbidity. To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any growth and inoculated onto sterile Nutrient agar plates by streaking. Nutrient agar plates were also streaked with the respective test organisms to serve as controls. All the plates were then incubated at 37ºC for 24 h. After incubation, the concentration at which no visible growth was seen was noted as the Minimum Bactericidal Concentration (MBC) (Spencer and Spencer) (2004).

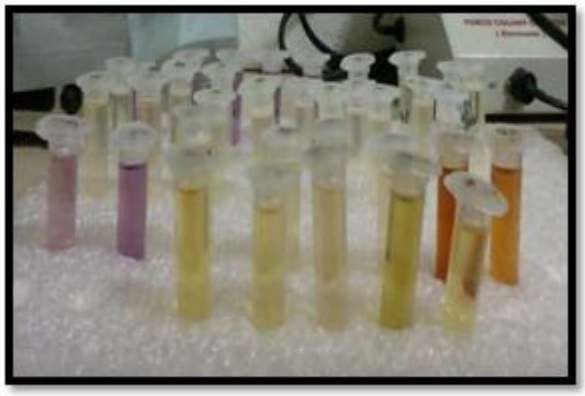

Figure 2: Nutrient Agar Plate showing Zone of Inhibition

3.5 Anti-Oxidant Activity Assay

The antioxidant activity of *Aloe vera* methanolic, aqueous, choloroform and petroleum ether extract and the standard antioxidant ascorbic acid was assessed on the basis of the radical scavenging effect of the stable 2, 2- diphenyl-1-picrylhydrazyl (DPPH) free radical activity according to the method described by Brand- William et al. (1995). The methanolic, choloroform, aqueous and petroleum ether extract with different concentrations (10, 50, 100, 200, 400, 600, µg/ml) were prepared. Ascorbic acid was used as the standard in 1- 100 µg/ml solution. 0.004 % of DPPH solution was prepared in ethanol and 5 ml of this solution was mixed with 5 ml of extract solution and standard solution distinctly. These solution mixtures were kept in dark for 30 min. The degree of DPPH purple decolourization to DPPH yellow indicated the scavenging effectiveness of the extract. The absorbance of the combination was determined at517 nm using UV-Visible Spectrophotometer and ascorbic acid was

served as positive control. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

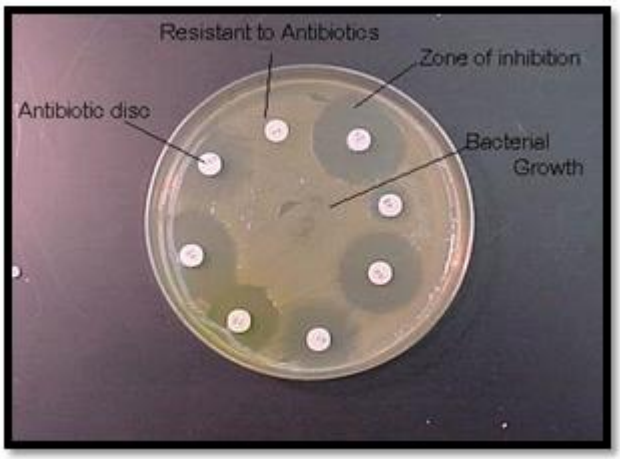

Figure 3: Procedure of Anti-Oxidant Activity

4. Results and Discussion

4.1 Phytochemical Screening

The result revealed the presence of medicinal active constituents of *Aloe vera* . In analysis of tannin compounds brownish green colour was developed to indicate the presence of tannin. Similarly based on the presence or absence of colour change indicate positive or negative results. In the screening process Tannins, Saponins, Alkaloids, Flavonoids, Glycosides gave positive results while steroids and phlobatannins gave negative results. Phytoconstituents have been found to inhibit bacteria, fungi, viruses and pests. The presence of phytoconstituents in the root extracts may be responsible for the antibacterial and antioxidant activity of the plant (Marjorie, 1999).

4.2 Anti-Bacterial Activity

All the extracts of the plant showed varying degree of antibacterial activities against the test bacterial species. The antibacterial activities of each extracts was compared favourably with standard antibiotic streptomycin and chloramphenicol. The methanol extract exerted highest activity on bacterial agents tested compared to the other extracts. The methanol extract at the concentration of 100 µg / ml showed 25 mm diameter zone of inhibition against *E. coli.* This was followed by 20, 18 and 15.5 mm zone of inhibition against *B. subtilis, B. cereus* and *S. aureus.*

The results of this study showed that the methanolic extract was more effective than aqueous extract. This may be due to the better solubility of the active components in organic solvents (de Boer *et al*., 2005).

4.3 Anti-Oxidant Activity

Phytochemical screening reveals that the major constituents of *Aloe vera* extract are phenolic compound, glycosides, alkaloid and flavanoid. Among these phenolic compounds which may be responsible for the activities of antioxidant.

Scavenging activity of DPPH radical was found to rise with increasing concentration of the extracts. Additionally, it has been determined that the antioxidant effect of plant products is mainly due to radicalscavenging activity of phenolic compounds such as flavonoids, polyphenols and tannins [Rahman et al, 2007]. The antioxidant activity of phenolic compounds is mainly due to their oxidation reduction properties, which can play an important role in adsorbing and neutralising free radicals, reducing singlet and triplet oxygen, or decomposing peroxides [Hasan *et al.*, 2008].
The decrease in absorbance of the DPPH radical due to the scavenging capability of 80% of ethanol (v/v) extracts of *Aloe vera*

All the extracts showed a rapid decrease in absorbance with methanolic extracts of *Aloe vera* exhibiting the fastest scavenging rate, while the aqueous extracts of *Aloe vera* showed the slowest rate over the first 10 mins.

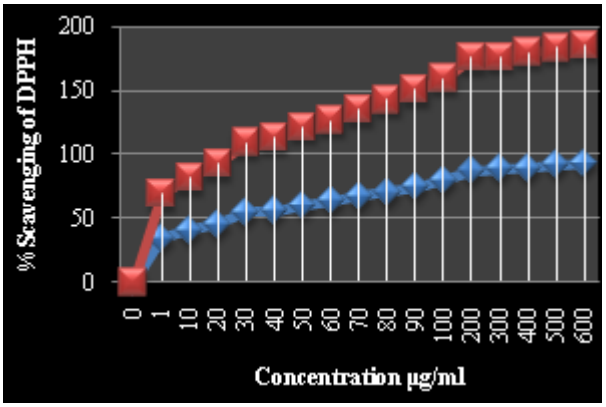

Figure 4: Radical Scavenging Activity of Methanolic Extract of *Aloe vera*

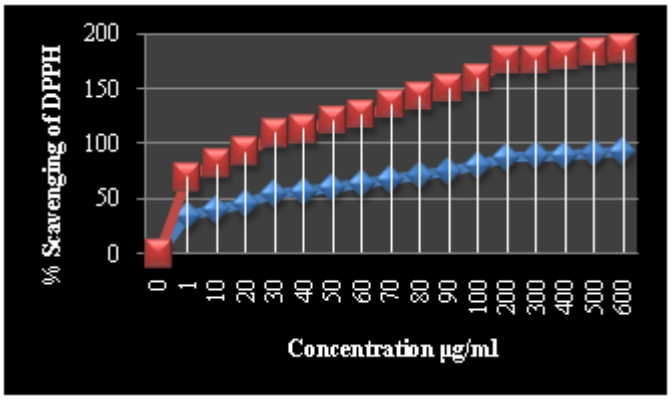

Figure 5: Radical Scavenging Activity of Petroleum Ether Extract of *Aloe vera*

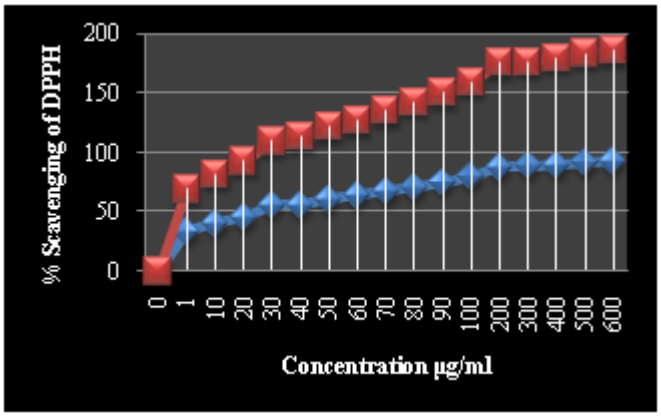

Figure 6: Radical Scavenging Activity of Chloroform Extract of *Aloe vera*

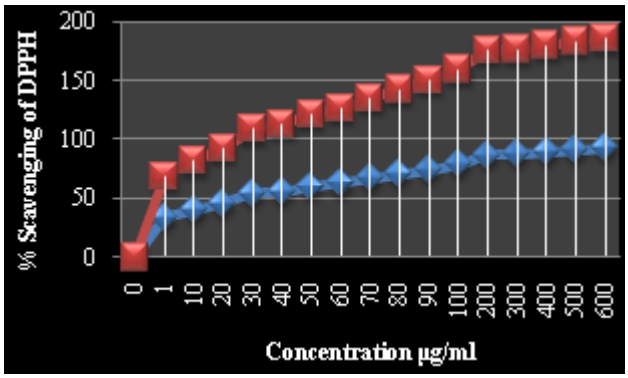

Figure 7: Radical Scavenging Activity of Aqueous Extract of *Aloe vera*

References

[1] Aida P, Rosa V, Blamea F, Tomas A, Salvador C. Paraguyan plants used in traditional medicine. J.Ethnopharm. 2001; 16: 93-98.
[2] Akinpelu DA, Kolawole DO. Phytochemical and antimicrobial activity of leaf extract of Piliostigma thonningii (Schum.). Sci. Focus J. 2004; 7: 64-70.
[3] Afolayan AJ, Meyer JJM. The antibacterial activity of 3, 5, 7- trihydroxyflavone isolated from the shoots of *Helichrysumau- reonitens*. J. Ethnopharmacol. 1997;57: 177-181.
[4] Bibitha B, Jisha VK, Salitha CV, Mohan S, Valsa AK. Antibacterial activity of different plant extracts. Indian J. Microbiol. 2002; 42: 361-363.
[5] Crosswhite FS, Crosswhite CD. *Aloe vera* plant symbolism at the threshing floor. Desert plants 1984; 6: 43-50.
[6] Doughari JH**,** El-mahmood AM, Manzara S. Studies on the antibacterial activity of root extracts of *Carica papaya* L. Afri. J. Microbiol. Res. 2007; 37- 41.
[7] Farnsworth NR. The role of medicinal plants in drug development. In: Krogsgaard-Larsen S, Brogger-Christensen S, Kofod H (Eds), Natural Products and Drug development, Munksgaard, Copenhagen.1994.
[8] Imanishi K, Ishiguro T, Saito H, Suzuki I. Pharmacological studies on Plant Lectin, Aloctin A. I. Growth inhibition of mouse methlcholanthrene-induced fibrosarcoma (Meth A). in ascites form by Aloctin A. Experientia, 1991; 37: 1186–1187.
[9] Jager AK, Hutchings A, van Staden J. Screening of Zulu Medicinal plants for prostaglandin-synthesis inhibitors. J. Ethanopharamacol. 1996; 52: 95–100.
[10] Jain SK, De Fillips A. Medicinal plants of India, Vol. 1. Algonac, Michigan: USA Reference Publication. 1991.
[11] Latha SP, Kannabiran K. Antimicrobial activity and phytochemicals of *Solanum trinobatum* Linn. Afr. J. Biotechnol. 2006; 5(23): 2402- 2404.
[12] Morton JF. Folk uses and commercial exploitation of aloe leaf pulp. Econ. Bot. 1961; 15: 311-319.
[13] Narayana Rao and K. Thammanna. Medicinal Plants of Ritual Hills, Department of Garden, Tirupati Devasthanams, Tirupati, India. 1997.
[14] Prescott L, Harley J, Klein DA. Microbiology. 5th edn. McGraw- Hill. London 2002; P. 820-950.
[15] Pretorius CJ, Watt E. Purification and identification of active components of *Carpobrotus edulis* L. J. Ethnopharm. 2001; 76: 87-91.
[16] Sharif MDM, Banik GR. Status and Utilization of Medicinal Plants in Rangamati of Bangladesh. Res. J. Agric. Biol. Sci. 2006; 2(6): 268-273.
[17] Spencer ALR, Spencer JFT. Public Health Microbiology: Methods and Protocols. Human Press Inc. New Jersey 2004; P. 325-327.
[18] Srinivasan D, Perumalsamy LP, Nathan, Sures T. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J Ethnopharm. 2001; 94: 217-222.
[19] Yates A. Yates Garden Guide. Harper Collins Australia, Australia 2002.
[20] Vogler BK, Enst E. *Aloe vera* a systematic review of its clinical effectiveness. British Journal of General Practice 1999; 49(447): 823-828.
[21] Vogler, B.K. and E. Enst. *Aloe vera* a systematic review of its clinical effectiveness. British Journal of General Practice, 1997; 49(447): 823-828.
[22] Rabe, T. and J. Van Staden. Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethnopharmacology, 1997; 56(1): 81-87.
[23] Yates, A., 2002. Yates Garden Guide. Harper Collins Australia, Australia.

Author Profile

Priyanka Das has completed her Master of Technology (M. Tech) in Biotechnology from West Bengal University of Technology, Kolkata, West Bengal, India and Master of Science (M.Sc) in Biotechnology from Devi Ahilya Vishwavidyalaya, Indore, Madhya Pradesh, India. Her field of interest is in Plant Tissue Culture, Microbiology and Pharmacological studies.

Alok Kumar Srivastav has completed his Master of Technology (M. Tech) in Biotechnology from West Bengal University of Technology, Kolkata, West Bengal, India and Master of Science (M.Sc) in Biotechnology from Devi Ahilya Vishwavidyalaya, Indore, Madhya Pradesh, India. His field of interest is in Plant Tissue Culture, Microbiology and Pharmacological studies.