

Research Article

Phytochemical screening and biological activities of *Aloe vera* (L.) Burm. F.

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Citation

Bibi Raad, Syed Shaukat Ali, Khushnood ur Rehman, Naveed Akhtar, Barkat Ullah and Sher Wali. Phytochemical screening and biological activities of *Aloe vera* (L.) Burm. F. Pure and Applied Biology. Vol. 10, Issue 1, pp360-367. <http://dx.doi.org/10.19045/bspab.2021.100039>

Received: 20/11/2019

Revised: 07/07/2020

Accepted: 12/08/2020

Online First: 29/09/2020

Abstract

In the present work phytonutrients including tannins, phlobatannins, flavonoids, saponins, cardiac glycosides and terpenoids were investigated in *Aloe vera* plant. Leaves of *Aloe vera* were chosen for this investigation. The plants were collected from the botanical garden of Islamia College, Peshawar. The phytochemicals were determined quantitatively using different solvents. The leaves were also investigated to find its antibacterial and antifungal activities. All the phytochemicals excepting Phlobotannins were found in all the extracts. Results revealed very profound activities of the plant extracts against the tested gram positive strains including *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus atrophoeus* and gram negative bacterial strains i.e. *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*. The extracts also showed considerable activity against the tested fungal strains *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans*.

Keywords: Alkaloids; *Aloe vera*; Cardiac Glycosides; *Escherichia coli*; Phlobatannins; *Staphylococcus aureus*; Steroids

Introduction

Aloe is described to be a very useful in curing infections, laxative and skin problems in the form of Mesopotamian tablets and ancient Egyptians papyrus [1]. *Aloe* cream is said to be included in the beauty items of Cleopatra [2]. It has been used by the Arabian physicians and Hippocrates. It was carried by the Spanish globetrotters to the western hemisphere. It is also on record that the Socotra Island in Indian Ocean was captured by Alexander for securing the supplies of *Aloe* which were used for the treatment of injured soldiers [3]. Ayurveda and traditional Chinese medicine have given

Aloe great popularity. In Chinese medicine its skin and inner layer of leaves is described as a bitter cold recipe which is used to cure constipation because of heat accumulation; while its gel is moist and cold [4]. According to India's traditional medicine, Ayurveda, it is internally used as antihelminthic, laxative, uterine stimulant and a remedy against hemorrhoid. Externally it is used for the treatment of psoriasis and eczema, often in combination with licorice roots. Its gel is used to relieve headache, coolant, laxative, disinfectant, anti-conjunctivitis and wood healing agent in Arabian medicine [5]. Nowadays the gel

obtained from *Aloe vera* plant serves to be the active component in cosmetics, sun-blocks and skin lotions [6]. Its uses have been enormously increased in cosmetics based on the arguments that it has anti-aging properties like derivatives of vitamin A [7]. In the United States *Aloe* got popularity for the first time in 1930's based on its capability to treat the burns of X-rays [8-10]. In the recent years its extracts have been used for the treatment of stomach ulcers, canker sores and as cleansing juice by some natural health curers [11]. In the practice of naturopathy, kidney stones are prevented and treated by its juice [12]. Its plant is kept in kitchen by many mothers where it requires a little care to readily thrive in bright sunlight [13]. The gel of freshly cut inner leaf is directly applied to minor burns immediately [14]. The inner lining of leaf works as a natural and potential laxative. In a survey in 1990 *Aloe vera* was reported to be used by 64% population. It was found to be useful by 91% of users [15]. In the Benzoic tincture it is also one of the ingredients [16]. The genus *Aloe* is comprised of more than 300 species. These species are mostly native to Arabia, Madagascar and South Africa. Among these *Aloe Arborescens*, *A. barbadensis*, *A. ferox*, *A. perryi* and *A. vera* have high medicinal values [17]. Active ingredients are present in different concentrations in different species [18]. This genus belongs to family Xanthorrhoeaceae. *A. vera* is a pea-green xerophytic, arborescent or shrubby perennial plant with succulent leaves. Its leaves are long fleshy and triangular having spikes on edges. The parenchymal fleshy gel taken from the leaf centre is transparent. It is sometimes dried for the formation of *Aloe vera* concentrate and in other cases diluted into *Aloe* juicy preparations [19, 20]. The yellow coloured flowers of the plant are not used medicinally. Laxative anthraquinones are derived from the tubules of pericycle

which bound to the rind of leaf having yellowish green colour and contain liquid sticky latex [21]. This plant is indigenous to South America and South Africa. However it is under cultivation practice worldwide except rain forests, deserts and tundra. *Aloe* is commercially cultivated in the southern Texas, USA. This plant matures in four years and thrives for approximately twelve years [22]. Keeping in view the folk medicinal uses of the this important plant, *A. vera*, the current research work was designed to find out its phytochemical composition and its potential against the selected bacterial and fungal strains.

Materials and methods

Collection and identification of *Aloe vera* plant

The plant was collected from the Botanical Garden of the Department of Botany, Islamia College, Peshawar, Pakistan. It was identified by the Professors of Department of Botany and confirmed with flora of Pakistan available online at Tropicos [23]. The plants were dried at room temperature and then used it further for extraction.

Extraction

0.3 grams of *Aloe vera* leaves grinded and added with 50 ml of chloroform, n-hexane, ethyl acetate and double distilled water in separate flasks for phytonutrients extraction for 48 hours. These solutions were filtered by using Whattman filter paper of forty two size and the solutions were extracted. The extracts were further used for determination of various phytonutrients.

Qualitative tests for phytochemicals

Qualitative tests for Phytochemical were carried out on the different extracts and on the powdered samples. The standard methods for the identification of the components of the samples described by, Ogbuewu [24] and Trease [25] and Sofowar [26] were used.

Phlobatannins test

The presence of phlobatannins was confirmed by the formation of red precipitate by boiling the chloroform extract of each sample with 1% HCl.

Flavonoid test

Ammonia solution (5ml) was added to a small portion of filtrate of chloroform extract followed by addition of conc H₂SO₄. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappears on standing.

Steroids test

In this test 2ml of acetic anhydride and 2ml of H₂SO₄ were added to 5ml extract from each sample. Change of colour blue from violet would indicate the presence of steroids.

Cardiac glycosides test

Cardiac glycosides: Five milliliter of each sample juice was treated with two milliliter of glacial acetic acid containing one drop of FeCl₃ solution, this was under layered with 1 milliliter of conc. H₂SO₄. A brown ring shows the deoxy sugar

characteristics of cardenolides. Sometime a violet ring may form indicating cardiac glycoside.

Tannins test

Five milliliter of extract were taken and boiled with 20ml of CHCl₃. On addition of 0.1 % FeCl₃ solution to the filtrate and appearance of brownish colour shows the presence of tannin.

Saponins test

About five milliliter of citrus sample was taken and boiled in 20 milliliter of CHCl₃ and filtered. To the 10 milliliter filtrate added five milliliter of double distilled water with 3 drops of olive oil, emulsion formation shows the presence of saponins.

Terpenoids test

Five milliliter of the sample was added to 2 milliliter of CHCl₃ and three milliliter of conc. H₂SO₄. The formation of reddish brown colour would show the presence of terpenoids.

Results and discussion

Sample A

Finding of these experiments are shown in (Table 1) and described as follows.

Table 1. Phytochemicals screening tests for sample A

Extracts	Tannins	Saponins	Phlobotannins	Flavonoids	Steroids	Terpenoids	Cardiac glycosides
Chloroform	+	+	-	+	+	+	+
d. distilled water	+	+	-	+	-	+	-
Ethyl acetate	+	+	-	+	+	+	+
n-Hexane	+	+	-	-	+	+	+

Chloroform extract tests

The test was performed on chloroform extract; the results for tannins, saponins, terpenoids, flavonoids, cardiac glycosides and steroids were positive and for phlobotannins were negative.

n-Hexane extracts test

This test was performed on n-hexane and the results for tannins, saponins, cardiac glycosides, steroids and terpenoids were positive and for phlobotannins and flavonoids were negative.

Ethyl acetate extract tests

Ethyl acetate extract was used for this test and the results for tannins, flavonoids, saponins, steroids, terpenoids, and cardiac glycosides were positive and for phlobotannins were negative.

Double distilled water extract tests

Double distilled water was used to perform this test and the results for tannins, saponins, flavonoids and terpenoids were positive and for phlobotannins, steroids was negative.

Sample B

Finding of these experiments are shown in (Table 2) and described as follows.

Table 2. Phytochemicals screening tests for sample B

Extracts	Tannins	Saponins	Phlobotannins	Flavonoids	Steroids	Terpenoids	Cardiac glycosides
Chloroform	+	+	-	+	+	+	-
d. distilled water	+	+	-	-	-	+	-
Ethyl acetate	+	+	-	-	-	+	+
n-Hexane	+	+	-	-	-	-	+

Chloroform extract tests

In these tests chloroform extract was used and the results for tannins, saponins, flavonoids, steroids, terpenoids, were positive and for phlobotannins and cardiac glycosides were negative.

n-Hexane extract tests

n-hexane was employed in these tests and the results for tannins, saponins, and cardiac glycosides were positive and for phlobotannins, flavonoids, steroids, terpenoids were negative.

Ethyl acetate extracts tests

Ethyl acetate extract was used to get results for tannins, saponins, terpenoids, cardiac glycosides were positive, and for phlobotannins, flavonoids and steroids were negative.

Double distilled water extract tests

Double distilled water was used and the results for tannins, saponins, and terpenoids were positive and for flavonoids, phlobotannins, steroids and cardiac glycosides were negative.

Anti-microbial activities

For the antimicrobial activity determination the solvents methanol and ethanol were used. The extract was prepared from leaves which were cleaned with water and after that dried in shade. An electric blender was used to grind the material into powder form. All this process was carried out in PCSIR lab Peshawar.

For filtration the extracts were filtered using Whatman filter paper. The filtrate after collection was shifted to rotary evaporator. In rotary evaporator the extract solution was collected less than 45⁰C of temperature.

The water contents of the residue were further dried on water bath at less than 60⁰C temperature. Sterile bottles were used for storage of crude extract of plants under refrigerator for further use of antimicrobial tests.

The microorganism for antimicrobial tests included four gram negative bacterial strains, three gram positive bacterial strains and one fungal strain effects were investigated.

Gram +ive strains: *Staphylococcus aureus*, *Bacillus atrophoeus* and *Bacillus subtilis*.

Gram -ive strains: *Escherichia coli*, *Kleibsiella pneumoniae*, *Salmonila typhi*.

Fungal Strains: *Aspergillus flavus*, *Aspergillus Niger* and *Candida albicans*.

Nutrient agar culturing media was utilized. Such media was utilized for the shaking, inoculation, incubation as well as for standardization and proper growth of microorganism.

The antibiotic Azithromycin 50Micro grams /six µl for gram positive bacteria, Ciprofloxacin 30 Micro grams /six µl for Gram negative bacteria and Ciprofloxacin and erythromycin 50 Micro grams /six µl for fungal strain of *Candida albicans*, *Staphylococcus aureus*, and *Bacillus subtilis* were utilized.

Disc diffusion method

Disc diffusion method was utilized for antimicrobial activity. The 0.5 Mc farland standards were for bacterial culture (Aida *et al.*, 2001). Discs prepared from filter paper impregnated with plant extracts of six and twelve micro liters samples were applied on plate's discs.

Antimicrobial activity determination

On the first day nutrient dissolved agar medium (2.8 gram /100 milliliter) plus nutrient broth (1.3 gram /100 milliliter) in double distilled water were added in flasks. The broth media of 20 milliliter of was added in test tubes. For avoiding contamination agar media were poured into plates placed in incubator under laminar flow. All the apparatus were sterilized at 121°C. On next day the stock microbial culture were freshed and were streaked on the nutrient agar plates under laminar flow, and further incubated at 37°C for other twenty four hours.

On third day the fresh streak were further reinoculated and again incubated the culture plates. On 4th day the cultures were inoculated to flask media and shaken at 200rpm overnight. The 5th day the flasks contained cultures were diluted and spread out on plates and kept in refractor for twenty minutes [27, 28]. After 20minutes Whattman filter paper disc were located on plates through forceps under luminar flow and plants parts extracted with dimethyl sulfoxide solvent were spotted applied on every plats disc. At last day of experiment the zone of inhibition were marked around the disc and make a snapshot the photos for zone of inhibition.

Antibacterial Activity: A total of four bacterial strains of were tested for plant extract which are *Streptococcus aureus*, *B. subtilis*, *B. cereus*, *E. coli*, *P. aeruginosa* and *S. typhi*. The reference fungal strain was *C. albicans*. Antibacterial activity and antibacterial activity results of *Aloe vera* are shown in (Table 3; Fig. 1 & Table 4; Fig. 2) respectively. The activities of *Aloe vera* extract against gram positive bacteria are very effective with respect to standard drug of Ciprofloxacin and erythromycin. Similarly gram negative microbes were tested but were not very susceptible except *K. pneumonia*.

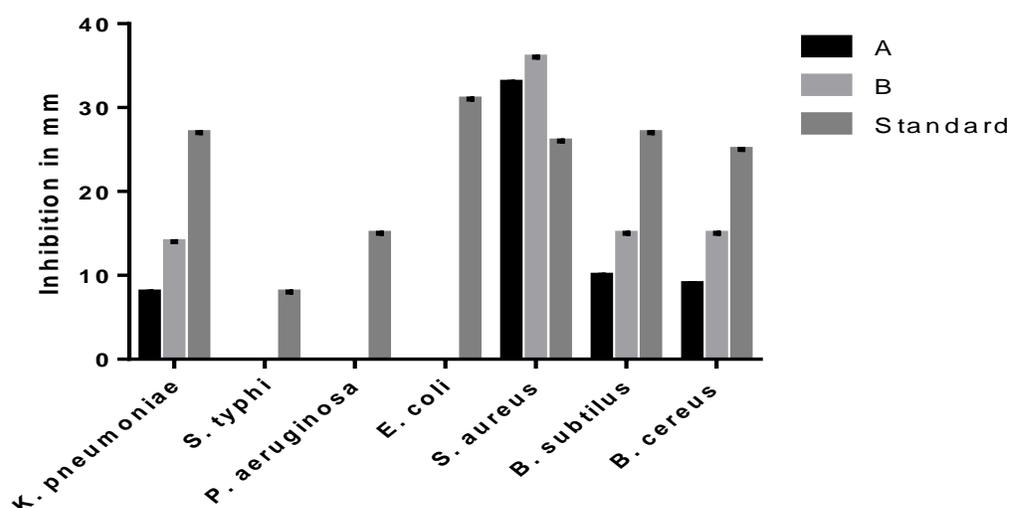
Antifungal activity

Antifungal activities for *Aloe vera* extract were tested against two fungal strains which were *Aspergillus flavus* and *Aspergillus niger*. The most prominent activity was shown in ethanolic (11±0.53 and 10±0.32 and acetonic extract disc of plant extract. The maximum antifungal activities were observed in acetone extract disc (15±0.73 and 8±0.37) other than aqueous extract (0.00 and 0.00) and for ethanol extract was (11±0.53 and 10±0.32).

Table 3. Antibacterial activity of decoction of *Aloe vera* in paper disc method [28]

Bacteria	Zone of Inhibition (mm)				
	Sample concentration in ml				
	A	B	DMSO	Ciprofloxacin	Erythromycin
<i>K. pneumoniae</i>	8.0±0.2	14.5±0.2	–	27±0.02	–
<i>S. typhi</i>	0	0	–	8±0.01	–
<i>P. aeruginosa</i>	0	0	–	15±0.02	–
<i>E. coli</i>	0	0	–	31±0.02	–
<i>S. aureus</i>	33.5±0.1	36.5±0.1	–	–	26.9±0.03
<i>B. subtilus</i>	10.00±0.1	15.5±0.3	–	–	27±0.02
<i>B. cereus</i>	9.0±0.2	15.2±0.1	–	–	25±0.01

Data represent mean of three individual studies

**Figure 1. Antibacterial activity of decoction of *Aloe vera* in paper disc method****Table 4. Antibacterial activity of decoction of *Aloe vera* in agar well method [29, 30]**

Bacteria	Zone of Inhibition (mm)				
	Sample concentration in ml				
	A	B	DMSO	Ciprofloxacin	Azthromycin
<i>K. pneumoniae</i>	11.0±0.01	16.5±0.2	–	27±0.02	–
<i>S. typhi</i>	0	0	–	8±0.01	–
<i>P. aeruginosa</i>	0	10	–	15±0.02	–
<i>E. coli</i>	0	0	–	31±0.02	–
<i>S. aureus</i>	38.5±0.01	40.5±0.02	–	–	26.9±0.03
<i>B. subtilus</i>	15.5±0.02	17.5±0.01	–	–	27±0.02
<i>B. cerus</i>	12.5±0.02	14.5±0.03	–	–	25±0.01

Data represent mean of three individual studies

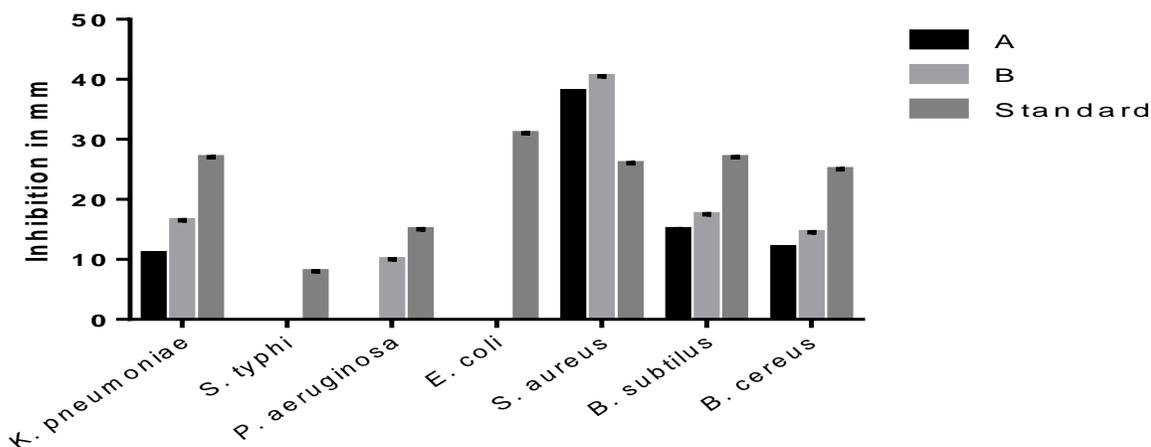


Figure 2. Antibacterial activity of decoction of *Aloe vera* in agar well method

Conclusion

The present study of phytochemical was carried out on two species of *Aloe vera* leaves obtained locally from garden of Islamia College, Peshawar, Pakistan. The sample revealed the presence of medicinally active constituents like tannins, saponins, phlobbasstannis, steroid, terpenoids, flavonoids, and cardiac glycosides. These phytochemicals were investigated. For the investigation of each phytochemical, specific tests were conducted which confirm the presence or absence of phytochemical. All the tests were carried out with different solvents extracts such as water, ethanol, chloroform, methanol etc. Then the tests were carried out with all solvents extracts for the confirmation of phytochemicals. Similarly the leaves of two species of *Aloe vera* antimicrobial studies effects were investigated which shows potent effects against some gram positive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus atrophoeus* some gram negative bacterial strains such as *Escherichia coli*, *Kleibsiella pneumonia* and *Salmonila typhi*. similarly its ethanolic and acetonic extract were also very susceptible to fungal strains like *Aspergillus flavus* and *Aspergillus niger* and *Candida albicans*.

Authors' contributions

Conceived and designed the experiments: B Raad & SS Ali, Performed the experiments: B Raad, SS Ali & KU Rehman, Analyzed the data: N Akhtar, B Ullah & S Wali, Contributed materials/ analysis/ tools: SS Ali, KU Rehman & N Akhtar, Wrote the paper: B Raad, B Ullah & S Wali.

Acknowledgment

The authors are thankful to Higher Education Commission of Pakistan for providing financial support to carry out this research work.

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