

Morphological, phytochemical and antifungal analysis of *Aloe vera* L. leaf extracts

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Abstract

This study was undertaken with the aim of analysing some morphological parameters, screening of important phytochemical compounds and the activity of *Aloe vera* leaf extracts against two fungal species viz; *Aspergillus niger* and *Rhizopus*. Morphological assessment of *A. vera* plant showed significant differences in leaf and root attributes including its color, length and weight under both dried and fresh form. Pendulous and yellow to whitish color flowers were found. Phytochemical analysis of glycosides, steroids, flavonoids, reducing sugar, phenolic compounds, terpenoids, carbohydrates, amino acid, tannins and saponin gave positive consequences and negative for cyanogenic glycosides in both the methanol and ethanol leaf extracts. Alkaloids were found only in the methanol extract but not in that of ethanol extract. Glycosides, reducing sugar, phenolic compounds, steroids, terpenoids, carbohydrates, flavonoids, saponins and tannins were present in the aqueous extract. Both methanolic and ethanolic extracts of *A. vera* showed significantly greater inhibitory effect whereas aqueous extracts were found with lesser inhibitory effect against *Rhizopus* and *A. niger*. This investigation strongly suggests *A. vera* plant leaf extracts with ethanol and methanol as better antifungal candidates.

Keywords: *Aloe vera* L., Morphology, Phytochemical screening, Antifungal action.

Introduction

Since the past decades, plants have been proven to be a crucial foundation of medicine. Even these days, up to 75 to 80% people around the world still depend on traditional remedies for their health purpose directly or indirectly obtained from herbal sources (WHO, 2001). *A. vera* plant is an important member of the *Liliaceae* family used to treat certain kind of ailments (Davis and Robson, 1999). It is a succulent plant and most frequently cited because of its use in herbal medicine since the first century AD (Ernst, 2000). It is a sessile herb with perennial growth habit. Its leaves are pea-green in color and 30-36 cm long from the

lower region and 10 cm broader. The flowers are yellow bright with tubular appearance, 25-35 cm in length having extended slim loose spike arrangement. The stamens of flower are present ahead of the perianth tube in a regular way (Burger, 1980).

The genus *Aloe* contains about 250 species and only two of them grown commercially are *Aloe arborescens* and *Aloe barbadensis* (Raphael, 2012). *A. vera* plant holds nearly 200 active phytochemicals along with 70-75 different kind of nutrients including vitamins, sugars, enzymes, minerals, lignin, saponins, anthraquinones, salicylic acid and amino acids (Park and Jo, 2006). Extracts from this important plant are commonly employed in pharmaceutical and



cosmetics industries. The efficiency or safety of *A. vera* extracts for either cosmetic or medicinal purposes is important consideration and there is nevertheless, little scientific evidence about its safety and effectiveness (Sharma, 2013). The positive evidence obtained from this succulent plant may be frequently contradicted by other investigation (Marshall, 1990; Vogler and Ernst, 1999; Boudreau & Beland, 2006).

Besides the presence of phytochemicals, *A. Vera* leaves also contain a kind of gel which is used to treat stomach related problems. This gel is also important in the stimulation of immune system of body (Davis, 1997) and also showed its better effects against diabetes, skin diseases, inflammation, constipations, wounds healing and ulcer (Johnson et al., 2012). The most commonly found vitamins in this plant are A, B1, B2, B6, B12, C and E which are not prepared inside the human body. Vitamins from this plant like vitamin C and B complex play a crucial role in reducing stress and acting an antiinflammation (Coats, 1979).

On the other hand, different types of enzymes are also present in this plant such as lipase, amylase and carboxypeptidase. Lipase is an important enzyme that holds tendency in digestion by breaking down fats and sugars. Another enzyme amylase has potency to hydrolyse starch and ultimately releases dextrin. The pancreatic carboxypeptidase is metalloenzyme that deactivates bradykinin and generates an anti-inflammatory influence. During the progression of inflammation, analgesic conditions arise by bradykinin which is linked to the vasodilation and its hydrolysis that ultimately produces pain (Obata, 1993; Shelton, 1991).

In spite of its therapeutic potentials, there have been limited reports on the antimicrobial effects of different isolated compounds from *A. vera*. In one investigation, Ferro et al., (2003) corroborated that gel from leaves of *A. vera* plant holds a potential to restrict progression of two Gram-positive bacteria namely, *Streptococcus progenies* and *Shigella flexneri*. Particular compounds form this plant like dihydroxyanthraquinones (Wu et al., 2006), anthraquinones (Dabai et al., 2007) and saponins (Reynolds & Dweck, 1999) are better antimicrobial candidates.

There are some antibiotic agents that have been used presently and failed to control the devastating possessions of microbial contagions. One reason behind this problem is the capability of an organism to fight against these antibiotic agents. In order to grab this problem, researchers are looking for new antimicrobial agents. They may do this either by

creating new agents or by patrolling natural sources especially from plant origin, because the undiscovered antimicrobial agents still existing in different plant sources. Their curiosity is primarily towards the extraction and characterization of vigorous elements for succeeding expansion of drugs, use of herbal products as supplement (Khan et al., 2008), tropical products and varieties of surfactants for internal use (Hamman, 2008).

Keeping these views in mind, the present study was accompanied with the aim of morphological assessment, screening of important phytochemicals, and revealing the antifungal activity of leaf extracts (methanolic, ethanolic and aqueous) of *A. vera* plant against two fungal strains.

Materials and Methods

The current research work was performed in the Department of Botany, Mirpur University of Science and Technology (Bhimber campus) Azad Kashmir, Pakistan. This research work was carried out on three major features i.e., assessing the morphology of healthy leaves and roots of *A. vera* plant, phytochemical analysis and the antifungal activity of leaf extracts of *A. vera* plant.

Collection of Sample

A. vera plant was collected from kitchen gardens of Bhimber city of Azad Kashmir. The fresh *A. vera* leaves were washed thoroughly 2-3 times with distilled water and air-dried. Few of these leaves were chopped into small pieces and grinded into fine powder with the help of grinder machine, mortar pestle and polyethene bags were used to store them. These bags were kept in the oven for further analysis.

Morphological characters of *A. vera*

Prior to grinding, the morphological characters of leaf and roots of *A. vera* plant were studied. Morphological characters of flower include color and type. Leaves morphology include shape, colour, length, width and weight while morphological characters of root includes, colour, length and diameter in both fresh and dried form.

Crude Extract Preparation

The grinded leaves powder of *A. vera* was separately extracted with cold methanol, ethanol and hot water. These were prepared using the method as described by



Oyagade, (1999). Briefly, 10 grams of grinded powder of leaves were suspended in 70 ml of distilled water, 95 % methanol or ethanol. The extraction using hot water was performed at 80°C for half an hour in water bath. The methanolic extraction was carried out for 120 hours at 28°C. After extraction, the obtained extracts were decanted and with the help of a Whatmann filter paper, the extracts were then filtered. The filtrate was then sterilized by means of membrane filter and evaporated to dehydration at 45° C. In 95 % ethanol, the residues attained were reconstituted at a concentration of 10 mg/mm. The extracted solutions were kept in refrigerator at the temperature of 4 to 2°C (Omojosola and Awe, 2004) for further analysis.

Chemicals and Reagents

The chemicals employed in this study includes Wagner's reagent, Drangendroff's reagent, Mayer's reagent, Benedict's solution, Ninhydrin reagent, 40% lead acetate solution, 10% α -naphthol, Sodium picrate solution, Ferric chloride solution, distilled water, Acetic anhydride, conc. H_2SO_4 , 10% lead acetate, Benzene $FeCl_3$ (or) conc. HCL, Mg turning and Bromo cresol green, Potato dextrose agar and ketoconazole antibiotics.

Phytochemical Investigation

The investigation of phytochemicals was performed by the methods given by Sofowara, (1993) and Harborne, (1973). The extracts were exposed to the screening of the presence of phytochemical like flavonoids, alkaloids, saponin, cardiac glycosides, phenolic compounds, steroids, terpenoids, carbohydrates, amino acid, tannins, cyanogenic glycosides and reducing sugar. The detailed methods for the detection of each compound are briefly described below.

Test for Alkaloids

To check the presence of alkaloids, few amount of *Aloe vera* leaf extract was mixed in 2ml of 1% HCl and then gently heated. Different reagents like Mayer's, Wagner's and Drangendroff's along with sodium picrate solution were added to the mixture. As a result, precipitation or turbidity with any of the subjected reagent was the confirmation for the presence of alkaloids. When the extract was treated with Drangendroff's reagent (1 ml), it gives orange precipitates and when the extract was subjected to sodium picrate (1 ml) then it gives yellow precipitates.

Whereas mixing of extract with Wagner's reagent (1 ml) do not give reddish brown precipitate.

Test for carbohydrates or reducing sugar

Fehling's test

Fehling A and Fehling B reagents in equal amount were mixed and 2 ml of their solution was added with crude extract and then boiled gently. This gives rise to brick red precipitate in the bottom of test tube which is the confirmation for the presence of reducing sugar.

Benedict's test

The extract in small amount was mixed with Benedict's reagent (2 ml) and boiled gently on burner. As a result, reddish brown precipitate forms which is considered to be the indicator of carbohydrates presence.

Molisch's test

Extract was mixed with Molisch's reagent (2 ml) and shaken appropriately. 2 ml of con. H_2SO_4 was dispensed in to the sides of test tube very carefully. This give rise to the formation of violet ring at the inter phase that ensures carbohydrates occurrence.

Phenolic Compounds Test

For the detection of phenolic compounds, 2ml of 2% solution of Ferric Chloride $FeCl_3$ was added to the crude extract. This gives rise to the appearance of blue-greenish or blackish colour which confirms the presence of phenolic compounds.

Steroids Test

Crude extract when mixed with conc. H_2SO_4 and chloroform (2ml) gives red or pink colour in the lower chloroform layer, which is indicator for the presence of steroids.

Terpenoids Test

The extract was dissolved in chloroform (2ml) and evaporated to dry. After that, conc. H_2SO_4 (2ml) was added. After adding H_2SO_4 the solution was heated for 2 minutes. This results in the formation of grayish colour which is evidence for the presence of terpenoids.

Amino Acids Test

0.2% solution of ninhydrin (2ml) was taken and mixed with the crude extract and boiling was done for few



minutes. As a result, violet or pink colour appeared that indicated the presence of amino acids.

Flavonoids Test

For the detection of flavonoids, two procedures were employed as given by (Harbrone, 1973; Sofowara, 1993) namely shinoda and alkaline reagent test.

Shinoda test

In this test, few portions of magnesium ribbon and conc. HCl were taken and added drop wise to the crude extract. As a result after few minutes, pink scarlet colour was seen to be the indicator for flavonoids.

Alkaline reagent test

In this test, 2ml concentration of 2% solution of NaOH was dissolved with the extract. Appearance of yellow precipitate was noticed which on adding few drops of diluted acid turned colourless. This clearly indicated that flavonoids are present.

Tannins Test

For the detection of tannins, 0.5 g dried powdered sample was boiled with water (20 ml) and then filtered. After that, 0.1 % of Ferric Chloride was added drop wise along with 10 % Lead acetate. Consequently, white precipitates were noticed which sanctioned the presence of tannins.

Cardiac Glycosides Test

For checking the presence of glycosides, 10% Lead acetate solution was immersed in few amount of crude extract. As a result the white precipitate formed and taken as evidence for glycosides. Another tests also performed for glycosides are given below.

Liebermann's test

In this test, the crude extract was taken with 2ml chloroform and 2ml of acetic acid and then cooled in ice. After that, conc. H₂SO₄ was added carefully. This results in change of colour from violet to blue that gives information about the presence of glycone portion of glycoside.

Salkowski's test

Primarily, the extract was mixed with chloroform (2 ml). Then conc. H₂SO₄ (2ml) was added shaken very gently. Therefore, reddish brown colour was noticed and taken as indicator for cardiac glycoside.

Keller-kilani test

In this test, little amount of extract was taken with glacial acetic acid (2 ml) containing FeCl₃ soln. The mixture was transferred to another test tube which already contains conc. H₂SO₄ (2ml). As a result, brown ring appeared that endorsed presence of cardiac glycosides.

Saponin glycosides Test

5ml of distilled water was taken in a test tube and crude extract was added and energetically shaken. As a consequent, frothing takes place that indicated saponins were present.

Cyanogenic glycosides Test

Primarily, the crude extract was dissolved with distilled water and few drops of conc. H₂SO₄ were added. Sodium picrate paper was used after few minutes. If no pink colouration were noticed, then it indicated the absence of cyanogenic glycosides.

Antifungal Activity of *A.vera* Leaf Extract

The obtained extracts were also screened *in vitro* for their antifungal activity in contrast to the standard antibiotic ketoconazole (mg/ml) with the help of standard well diffusion method (Nene and Thapliyal, 1979; Delignette-Muller and Flandrois, 1994). Prior to perform this activity, all glassware were washed thoroughly with the help of detergent and rinsed with distilled water appropriately many times. The washed glassware was then air dried, wrapped with aluminium foil and sterilization was done in autoclave for 15 to 20 minutes at 121°C and 15psi of pressure.

The fungal strains tested were, cultivated in PDA medium and were incubated at a temperature of 25°C. The cultivated strains of fungus were preserved using the Castellani's method as described by Figueiredo, (1967). For the preparation of PDA medium, 9.8 grams of the Potato dextrose agar was suspended in distilled water (250 ml) in a beaker. It is then mixed well and dissolved by heating. The solution was then boiled for 1 minute and complete dissolution was ensured. It is dispensed into a suitable bottle and sterilization was done in autoclave at 121°C for 15 minutes. After sterilization the prepared medium was kept at 4-8°C.

For antifungal activity, lawn cultures were primarily set using the tested species on potato dextrose agar (PDA). The plates which have been inoculated were left aside for some time. With the help of fine cutter,

four wells were created in the plates at required distance. After that, 30 mg/L of different solvents with selected *A. Vera* leaf extract was added in to the well by means of sterilized micropipettes. The plates with fungal development were then incubated at 37°C for overnight. After 48 hours of incubation, zone of inhibition for each extract was noted with three repetitions by calculating the diameter of area which was blanked in millimetres.

As a positive control, the standardized antibiotic ketoconazole (20mg/ml) discs were employed to compare the diameter of zone of inhibition from the extracts. This was done aseptically and the zones of inhibition were measured by employing zone reader scale.

Statistical analysis

Accuracy was achieved through SPSS program. Readings were taken three times. For means, the confidence interval was 95%. Significance level was ($P < 0.05$).

Results

The morphological assessment showed that the leaves of *A. vera* plant are glabrous green while the color of dried leaves was dull green. Length of both fresh and dried leaf was measured where; fresh leaf had an average length of 43.3cm while the length of dried leaf was 38.5cm. Results indicated that dried leaf shrink in size due to dehydration. That's why; reduced length in dried leaf was noted as compared to fresh one. Leaf width was 8.2cm in fresh form and 6.1 cm in dried form. The weight of fresh leaf noted was 5.4 g whereas 5.2 g weight was noted for dried leaf as shown in table 4.

The root color of *A. vera* was grayish brown in fresh sample, while dark brown in dried sample. Length of root was 35 cm in fresh form and 34 cm in dried sample was noted. The width of fresh root noted was 5.4 whereas 5.2 cm was recorded in dried root. Weight of fresh root noted was 0.5 g while 0.4 g was note for dried root as shown in table 5. Flowers of *A. vera* were found to be pendulous type and were yellow to whitish in color as shown in table 3.

In this study, three extracts (Methanolic, Ethanolic and Aquous extracts) from *A.vera* leaves were screened to check the manifestation of significant phytochemicals. Results revealed that, in the methanolic extracts of *A. vera*, the main phytochemicals present were alkaloids, flavonoids, cardiac glycosides, reducing sugar,

phenolic compounds, steroids, terpenoids, carbohydrates, amino acid, tannins and saponin glycosides while cyanogenic glycosides were absent. In the ethanolic extracts, cardiac glycosides, reducing sugar, flavonoids, phenolic compounds, steroids, terpenoids, carbohydrates, amino acid, tannins, saponin and glycosides were present while cyanogenic glycosides and alkaloids were absent. In the aqueous extracts of *A. vera* leaves, cardiac glycosides, reducing sugar, phenolic compounds, steroids, terpenoids, carbohydrates, flavonoids, tannins and saponins were present while cyanogenic glycosides, alkaloids, amino acid were absent as shown in table 6.

Besides the screening of phytochemicals, the methanolic, ethanolic and aqueous extracts from leaves of *A. vera* L. were also assessed for their potential antifungal activity against two fungal species namely, *A. niger* and *Rhizopus*. Three different concentrations of all the extracts viz, 1 mg/ml, 5 mg/ml and 20 mg/ml were optimized to scrutinize the inhibitory influence on fungal species.

In this study, both the methanolic and ethanolic extracts of *A. vera* leaves showed better antifungal activity against tested organisms in contrast to the aqueous extracts. At the concentration of 1 mg/ml, no inhibition zones were perceived for all extracts in contradiction of the tested fungal species, while at the concentration of 5mg/ml and 20mg/ml, better inhibition zones were documented against both species.

In the mehanolic extracts, zone of inhibition verified at 5mg/ml was 7.03 mm while at 20mg/ml, zone of inhibition noted was 9.66 mm against *Rhizopus*. Zone of inhibition noted for methonolic extracts at 5mg/ml was 5.06 mm while at 20 mg/ml, zone of inhibition detailed was 19.68 mm against *A. niger* as shown in table 7. For the ethonolic extracts, zone of inhibition measured at 5mg/ml was 10.00 mm while at 20 mg/ml, uppermost zone of inhibition verified was 20.33 mm against *A. niger* as given in table 8.

The aqueous extracts of *A. vera* leaves also showed no zone of inhibition at 1 mg/ml against both fungal species. At 5mg/ml zone of inhibition documented was 5.7 mm and 10.0 mm was noted at 20mg/ml against *Rhizopus*. On the other hand, zone of inhibition against *A. niger* in aqueous extracts at a concentration of 5 mg/ml and 20 mg/ml verified were 8.23 mm and 8.0 mm as given in table 8. These consequences authorize both the methanolic and ethanolic extracts of *A. vera* leaves to be more effective against *Rhizopus* and *A.niger*.



Table - 1: Qualitatively assessed morphological characteristics of leaves of *A.vera* L.

| Plant part | Morphological characters | Observation | |
|------------|--------------------------|-------------|-------------|
| | | Fresh | Dried |
| Leaves | Colour | Green | Light Green |
| | Arrangement | Thick | Thick |
| | Margins | Serrate | Serrate |
| | Venation | Parallel | Parallel |

Table - 2: Qualitatively assessed morphological characteristics of roots of *A.vera* L.

| Plant part | Morphological characteristic | Observation | |
|------------|------------------------------|---------------|---------------|
| | | Fresh | Dried |
| Roots | Colour | Greyish Brown | Greyish Brown |
| | Type | Fibrous | Fibrous |

Table - 3: Qualitative assessed morphological characteristics of flower of *A. vera* L.

| Plant part | Morphological characteristic | Observation | |
|------------|------------------------------|-------------|-----------|
| | | Fresh | Dried |
| Flower | Colour | Yellow | Yellow |
| | Type | Pendulous | Pendulous |

Table - 4: Quantitatively assessed morphological characteristics of leaves of *A. vera* L.

| Plant parts | Length (cm) | Width (cm) | Weight (cm) |
|-------------|-------------|------------|-------------|
| Fresh leaf | 40.3±1 | 8.2±0.13 | 5.4±0.2 |
| Dried leaf | 38.5±0.64 | 6.1±0.12 | 4.5±0.3 |

Values are mean of three readings

Table - 5: Quantitatively assessed morphological characteristics of roots of *A.vera* L.

| Plant parts | Length(cm) | Width(cm) | Weight(cm) |
|-------------|------------|-----------|------------|
| Fresh Root | 35±1 | 5.4±0.2 | 0.5±0.1 |
| Dried Root | 34±1 | 5.2±0.1 | 0.4±0.2 |

Values are mean of three readings



Table – 6: Phytochemicals analysis of three *A. vera* L. leaf extracts

| Phytochemicals | Ethanollic extract | Methanollic extract | Aqueous extract |
|--------------------------|--------------------|---------------------|------------------|
| | Presence/absence | Presence/Absence | Presence/Absence |
| Alkaloids | - | - | - |
| Cardiac Glycosides | + | + | + |
| Reducing sugar | + | + | + |
| Phenolic compounds | + | + | + |
| Steroids and Terpenoids, | + | + | + |
| Carbohydrates | + | + | + |
| Amino acid | + | + | + |
| Flavonoids | + | + | + |
| Tannins | + | + | + |
| Saponin glycosides | + | + | + |
| Cyanogenic glycosides | - | - | - |

Table – 7: Antifungal activity of methanollic extract of *A. vera* L. leaves against *A. niger* and *Rhizopus*.

| Sr/No | Microorganisms | Zone of inhibition after 48 hours | | | |
|-------|--------------------------|-----------------------------------|-----------|------------|--------------|
| | | Methanollic extract | | | Ketoconazole |
| | | 1mg/ml | 5mg/ml | 20mg/ml | 20mg/ml |
| 1 | <i>Rhizopus</i> | 0.00±0.00 | 7.03±0.12 | 9.66±0.76 | 15.50±0.12 |
| 2 | <i>Aspergillus niger</i> | 0.00±0.00 | 5.06±0.11 | 19.68±0.70 | 24.40±0.00 |

Values are mean of three readings

Table – 8: Antifungal activity of ethanollic extract of *A. vera* L. leaves against *A. niger* and *Rhizopus*.

| S/No | Microorganisms | Zone of inhibition after 48 hours | | | |
|------|--------------------------|-----------------------------------|-----------|------------|--------------|
| | | Ethanollic extracts | | | Ketoconazole |
| | | 1mg/ml | 5mg/ml | 20mg/ml | 20mg/ml |
| 1 | <i>Rhizopus</i> | 0.00±0.00 | 7.03±0.12 | 9.66±0.76 | 16.00±0.00 |
| 2 | <i>Aspergillus niger</i> | 0.00±0.00 | 10.0±0.20 | 20.33±0.57 | 24.40±0.60 |

Values are mean of three readings

Table – 9: Antifungal activity of aqueous extract of *A. vera* L. leaves against *A. niger* and *Rhizopus*.

| S/No | Microorganisms | Zone of inhibition after 48 hours | | | |
|------|--------------------------|-----------------------------------|-----------|-----------|--------------|
| | | Aqueous extract | | | Ketoconazole |
| | | 1mg/ml | 5mg/ml | 20mg/ml | 20mg/ml |
| 1 | <i>Rhizopus</i> | 0.00±0.10 | 5.07±0.6 | 10.0±0.20 | 15.70±0.00 |
| 2 | <i>Aspergillus niger</i> | 0.00±0.10 | 8.23±0.25 | 8.0±0.00 | 23.90±0.00 |

Values are mean of three readings



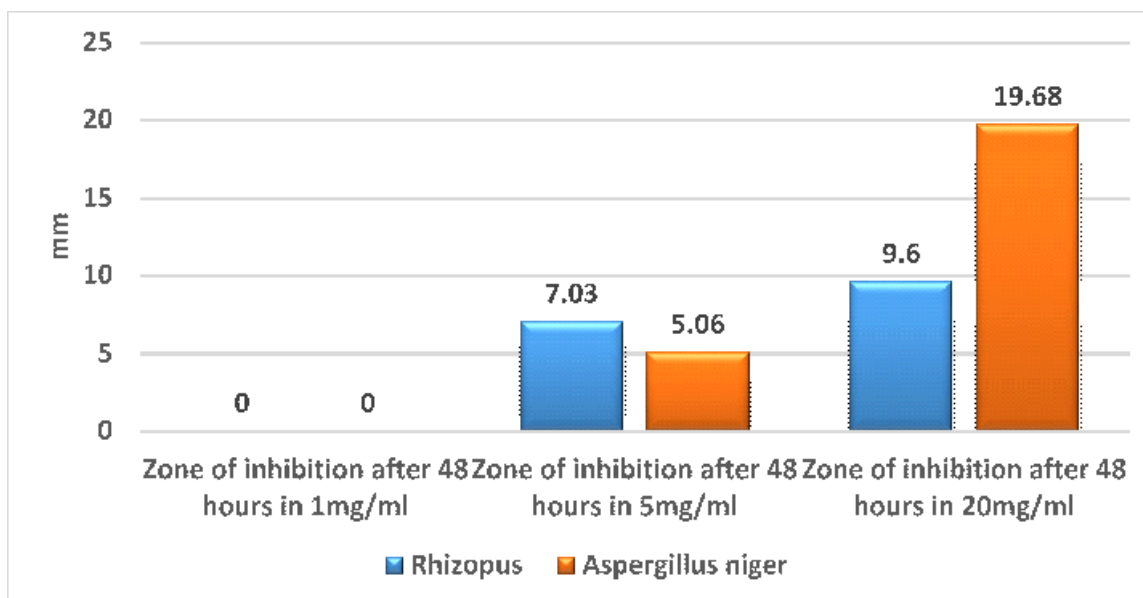


Figure 1. Antifungal activity of methanolic extract of *Aloe vera* L. Leaves against *Aspergillus niger* and *Rhizopus*

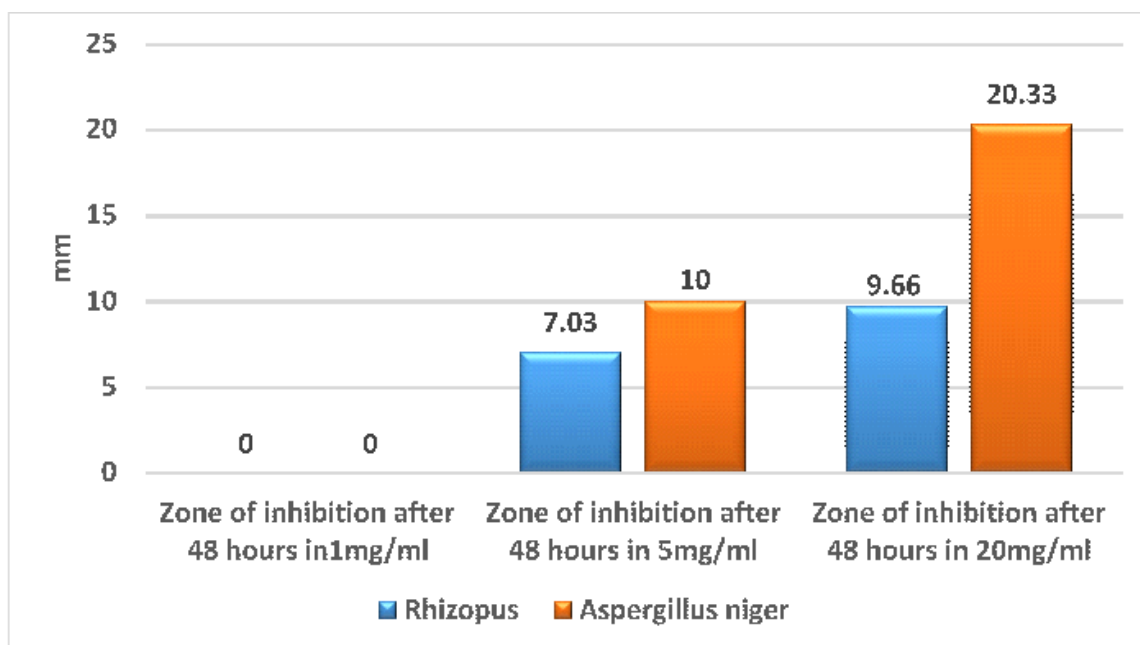


Fig 2. Antifungal activity of ethanolic extract of *Aloe vera* L. Leaves against *Aspergillus niger* and *Rhizopus*.

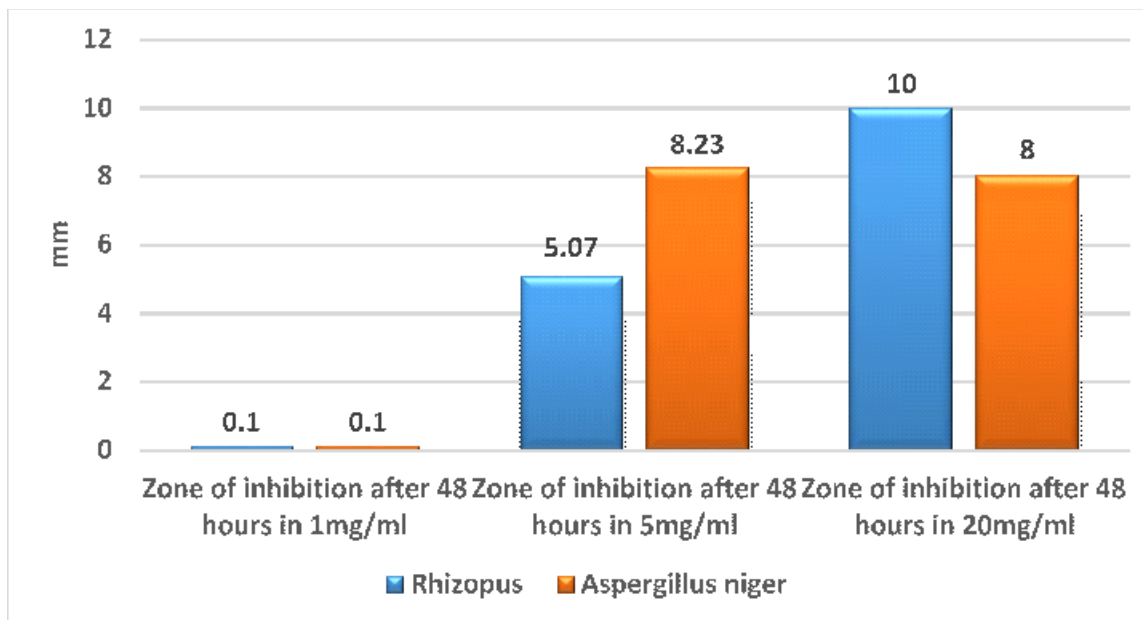


Fig 3. Antifungal activity of aqueous extract of *Aloe vera* L. leaves against *Aspergillus niger* and *Rhizopus*

Discussion

The present study examined the morphological attributes, presence of some important phytochemicals and the antifungal activity of leaf extracts of *A. vera* against *A. niger* and *Rhizopus* fungal strains. This study confirmed the presence of important phytochemicals in different extracts of *A. vera* leaves. In one study, Yebpella et al., (2011) also confirmed the presence of important phytochemicals in *A. vera* and found maximum quantity of alkaloids and glycosides in *A. vera* leaves gel when compared to the lyophilized gel with green rind. They found saponins in larger amount in the green rind. On the other hand, they also noticed steroids and cardiac glycosides with negative results in all the extracts.

Among the phytochemicals present in plant extracts, Alkaloids are the most efficient and are therapeutically significant ones. These constituents are purely quarantined and their synthetic derivatives are employed as the predominant medicinal agents. This is because of their antispasmodic, analgesic and antibacterial properties (Stray, 1998). One study corroborates that, due to the presence of these alkaloids, *A. vera* plant holds effective anti-malarial tendency since alkaloids comprises quinine, which have better anti-malarial activity (Robinson, 1985). Other crucial constituents of this plant are glycosides,

these compounds have the ability to upsurge heart-beat power without maximizing oxygen quantity desirable for muscles of the heart. Thus the effectiveness of heart can be improved without damaging to the organ (David, 1983).

These kinds of extracts from plants including ethanol extracts, its fractions, essential oils and some other resins were documented with efficient antifungal potentials and show their capability to control pathogenic fungal strains (Gahukar, 2012).

Plants have the tendency to depend upon certain mechanisms to protect themselves from toxic effects of pathogens. That's why in the case of fungal infection, plants prepare their bioactive organic compounds (Morrisey and Osbourn, 1999), peptides (Broekaert et al., 1997) and antifungal proteins (Selitrennikoff, 2001).

In this study, we tried to optimize the concentrations of crude extracts of *A. vera* leaves to achieve better antifungal activity against *Rhizopus* and *A. niger*. Banso et al, (1999) suggest that the antifungal substances present in plant crude extracts become fungistatic at lower concentrations, while at greater concentrations, they may become fungicidal. On the other hand, one study suggests that the dilution of extracts with each other may increase or decrease the inhibiting effect of one extract on the other to get better antimicrobial activity (Dellavalle et al., 2011).

Contrariwise, in our study, when aqueous extracts of *A. vera* leaves were tested, where the inhibition of fungal growth was lower in all concentrations, however, the methanolic and ethanolic extracts of *A. vera* leaves showed better results. This may be due to the presence of numerous compounds in the extracts inquisitive with the actions of one another in the methanolic and ethanolic extracts of *A. vera* leaves.

Studies suggests that the secondary metabolites obtained from plants have been previously documented as having inhibitory act in contradiction to certain pathogenic microbes (Xiaotian and Weishuo, 2006; Freeman & Beattle, 2008). However, other revelations have also been done in order to delineate the potential antimicrobial action of different elements like anthraquinones (Wu et al., 2006; Garcia-Sosa et al., 2006; Dabai et al., 2007) and saponins (Reynolds and Dweck, 1999).

Our findings corroborating the antifungal activity of *A. vera* leaves extracts are in agreement with the studies of Yebpella et al., (2011). They found that the *A. vera* gel suppressed development of both Gram negative and Gram positive bacteria with diminutive consequences on fungal strains. The reason behind this may be due to the manifestation of some active compounds present in this plant.

Our findings regarding the antifungal activity of *A. vera* plant are also in complete agreement with the study of Kedernath et al., (2012). They scrutinized chloroform, ethanol and petroleum extracts of *A. vera* and got superlative antifungal happenings in petroleum extract as compared to other extracts. They achieved supreme growth suppression by *A. vera* extracts in *Neurospora crassa* than *Aspergillus fumigates* and *Aspergillus niger*. Our results also found that the ethanolic extracts of *A. vera* leaves are effective against *Aspergillus niger*

Studies of Ferro et al., (2003) have corroborated the antimicrobial potential of *A. vera* whole gel. Extracts from this plant possess inhibitory influences on growth of fungi that cause dental diseases. Nonetheless, indication for proper control underneath human skin still has to be assessed. Along with the fungal growth inhibition, the inner leaf gel from *A. vera* plant also inhibits growth of some bacterial species like *Klebsiella* and *Streptococcus* in vitro.

This inhibition by plant extracts might not be associated with the action of only one active compound, but it is the consequence of collective efforts of different compounds that are present in trifling amount in the plant (Davicino et al., 2007).

From this study, it could be authenticated that the crude extracts from leaves of *A. vera* possess noteworthy fungicidal possessions and this plant could be traditionally used as better antiseptics.

Conclusion

This study revealed the presence of important phytochemicals in the leaf extracts of *A. vera* plant. This plant is very crucial from medicinal point of view and could be used as alternative to chemicals that will help to reduce their toxicity in medications. It is recommended that both ethanol and methanol extractions are most preferable methods than other methods for better antifungal examination. It is also confirmed that *A. vera* could be employed as a potent antimicrobial agents. More work should also be carried out on *A. vera* stem and root extracts to reveal potent antimicrobial agents for their better use in pharmaceutical industries.

Conflict of interest statement

The authors declare no conflict of interest in this research.

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