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Antibacterial and Antifungal activity of *Aloe vera* plant

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Abstract: The use of *Aloe vera* herb plant is beneficial for medicinal purposes. The main objective of study is to test antifungal properties of *Aloe vera* plant on pathogenic fungi Pucciniales (Rust) And also investigate antibacterial properties of *Aloe vera* on different types of bacteria such as *Escherichia coli*, *Proteus vulgaris*, *Acinetobacter baumanni*, *Psedomonas aerugenosa*, *Staphylococcus aureus, Bacillus Subtitis, Bacillus cereus* and *Enterococcus*. Different concentrations (10, 20, 30, 35ul) of ethanol root and shoot extract of *Aloe vera* plant was used on fungal and bacterial strains. Ethanol root and shoot extract have shown significant results in case of bacterial strains. Maximum zone of inhibition is 17mm appear in case of Escherichia coli bacterial strain which is significant result and minimum zone of inhibition observed 11.5mm in case of Acinetobacter baumanni at maximum concentration. And no zone of inhibition observed in case of fungi Pucciniales (Rust).
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Introduction

Greek researcher observed that *Aloe vera* plant present universal disease cure properties earlier 200 decades (Surjushe *et al*., 2008). *Aloe vera* plant in different kinds of illness through the adjustment of biochemical pathways they assume significant job (Salehi *et al*., 2018). Biological essential items latex and aloe gel are present in leaves of plant (Christaki & Florou-Paneri, 2010). The *Aloe vera* plant coating enhanced time span usability of papaya fruit due to its antimicrobial properties (Sharma & Gautam, 2013). The antibiotics resistant increase day by day that reason difficult to protect from pathogen as compared to natural plants are significant antimicrobial source (Abdallah *et al*., 2011). The fungi including Drechslera hawaiensis and Alternaria alternata development significantly restrict by examine *Aloe vera* gel extract. (Sitara *et al*., 2011). The *Aloe vera* gel significantly restrict the development of urinary tract infection causing bacteria during antimicrobial activity (Bukhari *et al*., 2017). *Aloe vera* potentially useful in the treatment of skin chronic disease Psoriasis (Miroddi *et al*., 2015). When used mixed extract of herb plants including *Aloe vera* then antimicrobial impact twenty five time more as compared to the single extract of *Aloe vera* (Ammayappan & Moses, 2009). Due to the presence of anti-infectant properties in *Aloe vera* plant significantly fix the scabietic disease when contrasted with benzoate careem (Oyelami *et al*., 2009). The *Aloe vera* extract with ethanol & methanol solvent tremendously restrict the development of fungi and bacteria both type when antimicrobial activity contrast with antibiotics and *Aloe vera* plant extract is better than to restrict the growth of microbial pathogen as compared to the antibiotics observed in studies (Ahmad *et al*., 2016). The used of *Aloe vera* and Sativum plant extract consolidate concentration is more reliable to restrict the growth of Salmonella Gallinarum observed in studies (Mlimbe *et al*., 2016). *Aloe vera* extract contain antimicrobial properties that reason significantly repress the growth of *Escherichia coli* bacteria (Kargaran *et al*., 2016). Due to Antifungal properties of *Aloe vera* tremendously restrict the development of a fungi involved in studies (Bajwa *et al*., 2007). Due to the presence of antibacterial effect of *Aloe vera* repress the growth of all bacteria involved in contamination of gutta percha cones (Athiban *et al*., 2012). Each plant aqueous extract including *Aloe vera* tremendously with high zone of inhibition repress the development of Trichoderma viridae fungi and no antifungal activity observed in case of penicillium chyrosegenum (Dharajiya *et al*., 2015). The *Aloe vera* plant based ointment tremendously repress the growth of examine microbes as contrast with other antimicrobial properties containing plants and fungi pathogen show more resistant as compared to bacterial pathogens against *Aloe vera* ointment (Rana *et al*., 2020; Pandey *et al*., 2010). Natural plant including *Aloe vera* has

ability to restrict the growth of fungi observed in studies (Kaur *et al*., 2015). *Aloe vera* consist restorative skin properties that reason *Aloe vera* plant have great value in cosmetic industry (Gupta *et al*., 2017). Due to the presence of germicide agent in *Aloe vera* plant have ability to restrict the growth of all pathogenic microbes also including viruses (Surjushe *et al*., 2008).

Material and method

Preparation of *Aloe vera* Extract

Aloe vera is therapeutic herb plant. The non-woody stem of *Aloe vera* made of skewered shape leaves. The remdiel and restorative properties contain gel present in leaves of *Aloe vera* (Hamman, 2008). Several bioactive compounds such as flavonoids, lectin, terpenoids, pectins, fatty acids, hemicelluloses, minerals, salicylic acid, lignin, saponins, vitamin A (beta-carotene) are present in the *Aloe vera* (Surjushe *et al*., 2008). The height of *Aloe vera* plant 23 to 39 inches. The plant cleaned through the ordinary water to clear the tainting. At that point with the utilizing of air-dryer dried *Aloe vera* plant. Then through the electronic balance samples were weighed the entire *Aloe vera* plant. By utilizing of disinfected sharp edge separate the plant leaves and roots. At that point right off the bat weight the root and besides shoot of *Aloe vera* plant. Now by the utilizing of strilized cutting edge squashed the root of plant convert into pieces and then on plant shoots applying the equivalent method. Now take two container cleaned well with ordinary water. And then spot in the oven for sanitization. Then two products root and shoot are placing into the two strilized jar. Now both container become full through the 99.5 ethanol and jar immovably fix with the cap. Then putting the jar for 72 hours at room temperature and shake the jars through hands time to time. And then strained the both sample. After these both plant samples extracs with ethanol solvents separately are passing through the column Chromatography technique. After this rotary evaporator technique (present in the pharmacy lab of University) apply on the both plant extracts samples to getting end product ethanol *Aloe vera* plant extract of root and shoot separately. After this the sample of ethanol root extract and ethanol leaves extract of *Aloe vera* plant are prepared. And then these extracts samples test look at on microbes. Then made the plant ethanol extract accompanied by the various concentration 10,20,30,35 ug/ml sample of ethanol root extract. Then plant shoot extract same method apply them. Then plant root and shoot ethanol extract sample test on microbes including fungi and bacterial strains through the Agar disc diffusion method Kirby-Bauer (Biemer, 1973).

Inoculum preparation

Arranged stock culture of microbes in the Lab. To set up culture of microbes includes such as pathogenic fungi Pucciniales (Rust) and gram negative bacteria *Escherichia coli, Proteus vulgaris, Acinetobacter baumanni, Psedomonas aerugenosa* and gram positive bacteria *Staphylococcus aureus, Bacillus Subtitis, Bacillus cereus, Enterococcus faecalis*. Culture of these microbes using nutrient agar medium and at temperature of 4°C conserve it. By using nutrient broth medium active culture formed at the time of performed experimental work (through the shifting of culture cell into test tube by the using of strilized loop. Then temperature of 37°C for 24 hours place in incubator. And in case of fungi at temperature 37°C for 72 hours putting in incubator (Sood *et al*., 2011).

Method for disc diffusion

Nutrient Agar media used for anti-bacterial activity. Then calculate the needed amount of nutrient agar (13g/ml) takes into the colonial flask and add distilled water. And flask closed by using the cotton and covered by the using of aluminum foil. Then autoclave the media. After making the nutrient agar media poured on the strilized petri dish plates wait until the solidifying nutrient agar media. After than bacterial culture swab on petri dish plate by the using of strilized cotton bud stick. After this through the forceps different concentration discs put on the solidifying nutrient agar media. After this petri dish plates kept it in incubator for overnight at 37°C temperature. After this check the zone of Inhibition and measured. Muller-Hinton Ager media used for antifungal activity. Then require muller-hinton ager put into strilized colonial flask and add distilled water and closing the flask mouth by the using of cotton wool and covered by the using of aluminum foil, autoclave the media. After the making media poured onto the strilized petri dish plate and wait until the solidifying it. Then fungus culture appropriately swab on the solid medium. After this through the different concentration of discs put in the solid medium. After this petri dish plates kept it in incubator for 72 hours at the temperature of 37°C. After 72 hours checking the zone of Inhibition and measured it (Agarry, *et al*., 2005).

Results

Antibacterial activity of Ethanol Root Extract on Gram- Bacteria

The different concentration of Ethanol root extract of *Aloe vera* plant antibacterial efficacy tested against the gram negative bacteria including *Escherichia coli, Proteus vulgaris, Acinetobacter baumanni, Psedomonas aerugenosa*. The zone of

inhibition of *Psedomonas aerugenosa* is 15mm illustrated in Table 1 and Figure 1. The maximum zone of inhibition is 16mm of *Escherichia coli*. The *Acinetobacter baumanni* is 12.5mm is least zone of inhibition as compared to the other examine gram negative bacteria illustrated in Table 1 and Figure 1.

Table 1. Ethanol Root Extract Concentration And Zone of Inhibition Size

Microbes	10ug/ml	20ug/ml	30ug/ml	35ug/ml	Control
Escherichia coli	7	10	13.5	16	_
Proteus vulgaris	8	9.5	11	14	_
Acinetobacter baumanni	7.5	9	10.5	12.5	_
Psedomonas aerugenosa	7	11	12.5	15	_

Antibacterial activity of ethanol root extract on Gram+ Bacteria

The ethanol root extract antibacterial efficacy examine on the gram positive bacteria and results illustrated in Table 2 and Figure 2. The *staphylococcus aureus* display 16mm zone of inhibition of as contrast with other gram positive strains. And *Enterococcus faecalis* bacteria display 12mm is least zone of inhibition illustrated in the Table 2 and Figure 2.

Table 2. Ethanol Root Extract Concentration And Zone of Inhibition Size

Microbes	10ug/ml	20ug/ml	30ug/ml	35ug/ml	Control
Staphylococcus aureus	8	9	14	16	_
Bacillus Subtitis	8	11	12	15	_
Bacillus cereus	9	10.5	13	14.5	_
Enterococcus faecalis	7	9	11	12	_

Antibacterial Activity of *Aloe vera* Shoot Extract against Gram- Bacteria

The observation of ethanol shoots extract against gram negative bacteria illustrated in the Table 3 and Figure 3. At the concentration on 35ug/ml maximum zone of inhibition is 17mm observed in case of *Escherichia coli* bacteria illustrated in table 3 and Figure 3. And the least zone of inhibition 11.5mm noticed in case of *Acinetobacter baumanni* bacteria illustrated in the table.3 and figure.3.

Table 3. Ethanol Shoot Extract Concentration And Zone of Inhibition Size

Microbes	10ug/ml	20ug/ml	30ug/ml	35ug/ml	Control
Escherichia coli	11	14.5	16	17	_
Proteus vulgaris	8	10	11.5	13	_
Acinetobacter baumanni	7	8.5	10	11.5	_
Psedomonas aerugenosa	8	9	11	13	_

Antibacterial Activity of *Aloe vera* Shoot Extract against Gram+ Bacteria

The *Aloe vera* plant shoot extract tested on gram positive bacteria by the zone of inhibition method and the observation are illustrated in Table.4 and figure.4. The maximum zone of Inhibition 15mm measured in case of *staphylococcus aureus* and the least zone of Inhibition 12mm in case of bacillus cereus at concentration of 35ug/ml. And the highest zone of inhibition is 10mm observed in case of *bacillus subtitis* at concentration of 10ug/ml.

Table 4. Ethanol Shoot Extract Concentration And Zone of Inhibition Size

Microbes	10ug/ml	20ug/ml	30ug/ml	35ug/ml	Control
Staphylococcus aureus	8	11	13	15	_
Bacillus Subtitis	9	10.5	12	13.5	_
Bacillus cereus	7	9	10.5	12	_
Enterococcus faecalis	9	11	12.5	14	_

Antifungal Activity of Ethanol Root Extract in case of *Pucciniales* (rust fungi)

To determine the antifungal efficacy of ethanol root extract of *Aloe vera* test on pathogenic fungi *Pucciniales* (common name rust). The disc diffusion method is used in experiment. The different concentration of ethanol root extract tested on Inhibit the growth of rust fungi but there is no zone of Inhibition observed illustrated in Table 5 and Figure 5.

Table 5. Ethanol Root Extract Concentration And Zone of Inhibition Size

Microbes	10ug/ml	20ug/ml	30ug/ml	35ug/ml	Control
Pucciniales (Rust)	0	0	0	0	_

Antifungal Activity of Ethanol shoot Extract in case of Pucciniales (rust fungi)

The ethanol leaves extract of *Aloe vera* plant also examine on the *Pucciniales* (rust) through the disc diffusion method. The different concentration of *Aloe vera* leaves extract applies on the *Pucciniales* (rust). But no clearly zone of Inhibition observed illustrated in Table 6 and Figure 6.

Table 6. Ethanol Leaves Extract Concentration And Zone of Inhibition Size

Microbes	10ug/ml	20ug/ml	30ug/ml	35ug/ml	Control
Pucciniales (Rust)	0	0	0	0	_

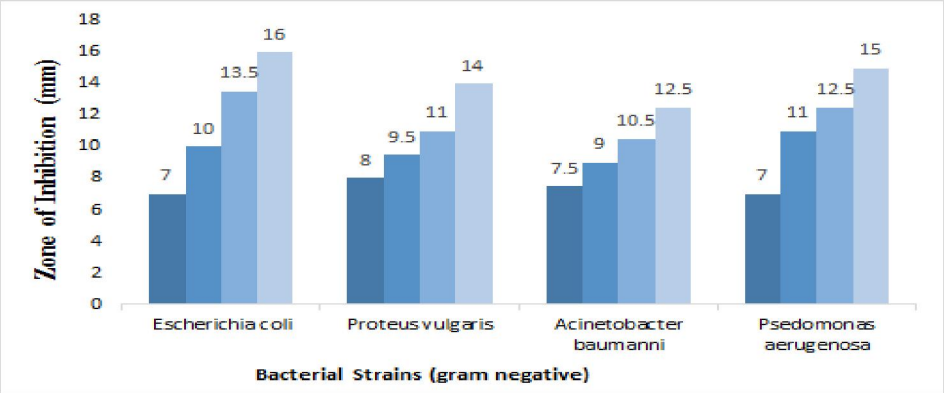

Figure 1. Ethanol Root Extract Concentration and Zone of Inhibition Size.

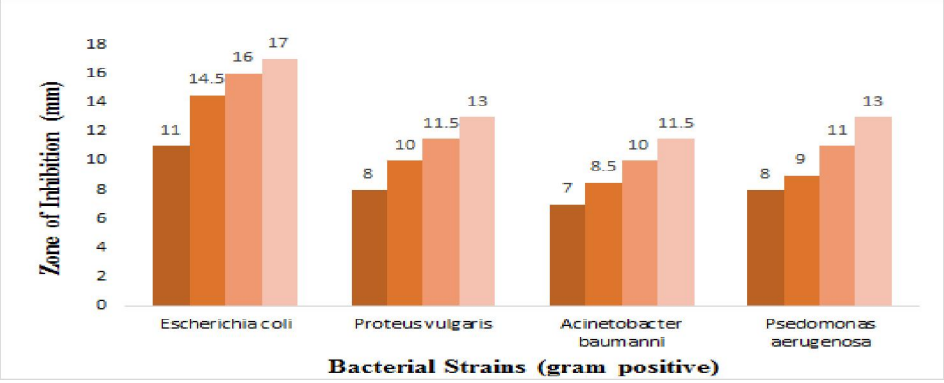

Figure 2. Antibacterial activity of ethanol root extract on Gram+ Bacteria

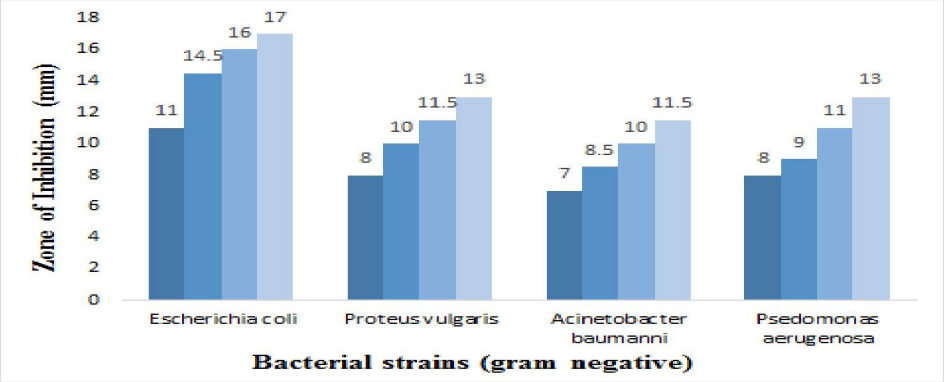

Figure 3. Antibacterial Activity of *Aloe vera* Shoot Extract against Gram- Bacteria

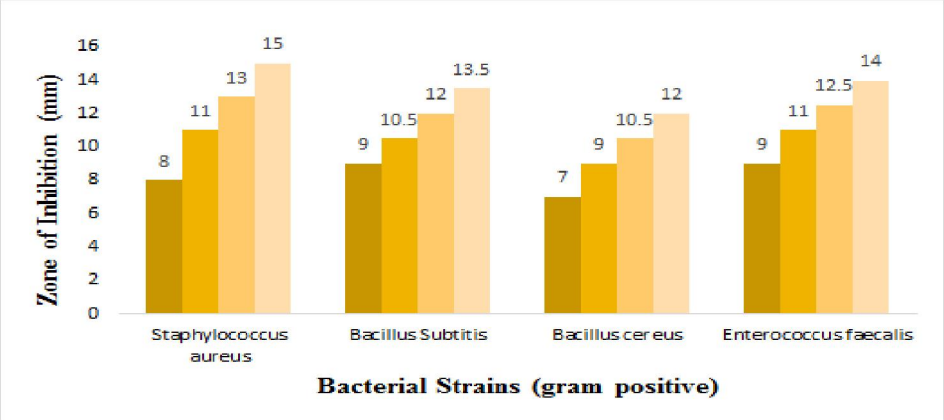

Figure 4. Antibactrial Activity of *Aloe vera* Shoot Extract against Gram+ Bacteria

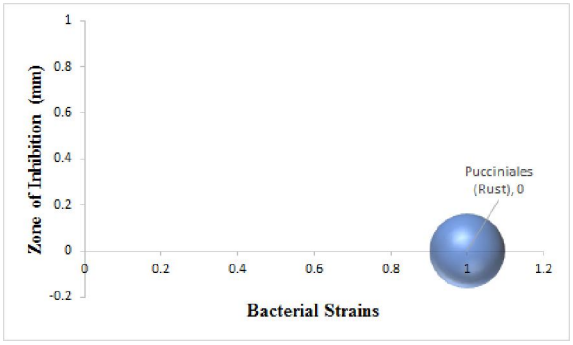

Figure 5. Antifungal Activity of Ethanol Root Extract in case of Pucciniales (rust fungi)

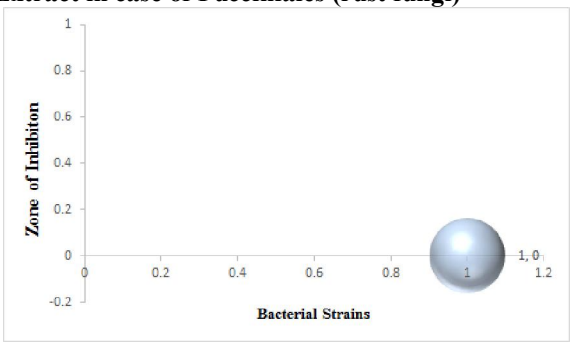

Figure 6. Antifungal Activity of Ethanol Shoot Extract in case of Pucciniales (rust fungi)

Discussion

The main objective of study was that to examine the Antifungal and antibacterial activity of *Aloe vera* on plant pathogenic fungi rust and gram positive and gram negative bacteria. Zone of inhibition method used to check the Antibacterial and Antifungal activity in experimental study. The concentrations (10ug, 20ug, 30ug, 35ug) of ethanol root and leaves extract to repress the growth of bacteria and plant pathogenic fungi rust. The ethanol root and leaves extract effectively restrict the growth of gram negative as well as gram positive bacteria. The zone of inhibition maximize when the concentration level of ethanol and root extract enhance. The ethanol root and leaves extract illustrated maximum Inhibitory effects as contrast with plant pathogenic fungi *Pucciniales* (rust). The *Aloe vera* root and leaves extract clearly no zone of inhibition in case *Pucciniales* (rust). Another studies observed *Aloe vera* leaves extract tremendously restrict the growth of all tested microbes as contrast with *Aloe vera* gel (Agarry, *et al*., 2005). Another studies revealed that due the presence of bioactive ethanol and methanol extracts tremendously restrict the development of fungi microbes including in studies. (Khaing, 2011). Another studies observed that aqeous extracts and ethanol have ability & reliable to dissemination the development of pathogenic

microbes (Stanley *et al*., 2014). In this study maximum zone of inhibition 17mm in case of Escherichia coli Bacteria (cause urinary tract infection) by utilization of *Aloe vera* extracts that reason *Aloe vera* have ability to cure the urinary tract infection observed in another studies (Ahmad *et al*., 2020; Bukhari *et al*., 2017). In this study *Aloe vera* extract also restrict the development of *Acinetobacter baumanni* (causes wound infection) that reason *Aloe vera* used reliable in burning wound infection as contrast with silver sulfadiazene observed in another studies (Hosseinimehr *et al*., 2010). In these studies *Aloe vera* extract illustrated good zone of in case of *staphylococcus aureus* bacterial strain (cause skin infection). Due to this reason *Aloe vera* tremendously help cure skin infection caused by microbes including *staphylococcus aureus* observed another studies (Selamoglu, 2018). The study revealed that ethanol extract of *Aloe vera* repress the growth investigated microbes excluding the *Pucciniales* (rust) fungi (Masood *et al*., 2020; Khalil *et al*., 2020ab; Ali *et al*., 2020; Iqra *et al*., 2020; Nazir *et al*., 2020; Asif *et al*., 2020; Danish *et al*., 2020). It illustrated good observation to Inhibit the development of microbes at low concentration of extract. Its need to more research to utilize the *Aloe vera* plant exttac effective way.

Conclusion

This study observed that *Aloe vera* plant significantly restrict the growth Bacteria except tested fungi and fungi strain less sensitive in contrast to bacterial strain against *Aloe vera* ethanol extract. *Aloe vera* plant is reliable and has potential to cure bacterial infection in contrast to conventional antibiotics.

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