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Estimation of antibacterial action of *Aloe Vera* L. on different strains and concentrations

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ABSTRACT

Aloe vera L. belonging to family Liliaceae is a very important worldwide used plant having nutritional as well as medicinal value. It plays an important role in antimicrobial action. The current study was aimed to assess of antibacterial activity of ethanolic, methanolic and aqueous extracts of *Aloe vera* L. against six bacterial strains (*Staphylococcus aureus*; *Streptococcus pyogenes*; *Shigella sonnei*; *Escherichia coli* (1) (4C11); *Escherichia coli* (2) (ATCC 13048) and *Neisseria gonorrhoeae*). Agar well diffusion method was applied to assess the antibacterial activity of these extracts. Their antibacterial activities were associated with typical chemical like gentamicine (antibiotic) as positive control. This study revealed that *S. pyogenes, S. aureus* and *N. gonorrhoeae* had the maximum inhibition to different extracts of *A. vera* plant and selected plant extracts remained less effective against *Shigella sonnei, E. coli* (1) and *E. coli* (2). The data also revealed that methanolic extract showed relatively more inhibitory effects on bacterial growth as compared to other solvent extracts.

Keywords: *Aloe vera*, Antibacterial activity, Aqueous solution, Ethanolic extract, Methanolic extract

INTRODUCTION

Plants have been utilized as valuable source of medicine and food from the ancient times. Numerous plant extracts have constituents with anti-infectious activity. People utilized enormous quantities of plants for the medicinal purposes (Rahmatullah *et al.,* 2009). Many traditional medicines are prepared by using these plants still used in rural areas of many developing countries. These herbal medicines are relatively safe and cheaper than synthetic medicines (Mosihuzzaman *et al.,* 2008). Therefore, herbal medicines are valuable source of treating disease for people living in rural community. *Aloe vera* L. is a perennial fleshy xerophyte, which stores water reserve in the leave tissue to survive in dehydrated regions of little and unpredictable rainfall. Internal chunk of the leaf is moist, soft, glassy, pulpy and lubricious which contains numerous thin-walled parenchyma tissues where water is available in the shape of gummy mucilage (Araújo *et al.,* 2013). Its cell wall comprises of cellulose and hemicelluloses as well as accumulated carbohydrates such as acetylated mannan. Many people use gel of Aloe as a therapy against skin infections such as sunburn, heat burns, psoriasis, cold sore and frostbite (Mushtaq *et al.,* 2012). It is additionally used to treat bowel diseases, osteoarthritis, fever, body inflammation and itching. It is also used for stomach ulcers**,** diabetes and asthma intended for soothing side-effects of radio therapy. It is helpful in natural treatment **of** constipation and depression (Haq, 2004). *A. vera* has many medicinal uses and from the ancient time has been used to treat different diseases like, genital herpes, epilepsy and dandruff (Qadir, 2009). When used as a mouth rinse, pure juice of Aloe is very effective at reducing dental plaque build-up as regular mouthwash and also aid in the recovery of mouth ulcers (Eamlamnam *et al.,* 2006). It is also used as natural healing agent for many diseases of skin. It was observed that there were special properties of *A. vera* extract with respect to different organisms specially human

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being (Channa *et al.,* 2014). However, due to curative and therapeutic properties Aloe has been used for many decades containing numerous active ingredients in its gel, out of them over 75 have been recognized (Rice-Evans *et al.,* 2004; Hamman, 2008). Polysaccharides are present in the inner parenchyma tissues of pulp of Aloe leaf are used as medicines (Purohit *et al.,* 2012).

A group of compounds has been reported in *A. vera* L. leaf that perform biological activities especially in cancer (Habeeb et al., 2007; Masaldan *et al.,* 2011). It has more than thirty active components used in cosmetics as well as in medicines production. Aloe contains about 98% water. Water removed from those active constituents by applying reverse osmosis system and save those active constituents from thermal damage of active compounds present in the juice (Abdullah *et al.,* 2014). It has been reported that some essential heavy metals like Nickel (Ni), Chromium (Cr), Cobalt (Co), Iron (Fe), Copper (Cu) and Zinc (Zn) are present in *A. vera*. Besides them some of the non-essential and toxic metals like, Cadmium (Cd) and Lead (Pb) were also found in Aloe leaves (Iqbal *et al.,* 2013). Its juice comprises of pulp containing natural ingredients and fibres. As herbal medication for the skin it also has high role in internal healing, cleansing and refurbishing. People prefer plant based medicines than synthetic medicines and Himalaya Agri herbal Aloe products are in high demanding in the international markets such as Asia, USA, Australia and whole Europe (Cristiano *et al.,* 2016). Aloe species provide commercial platform and extraction of its pulp has been done in industries at vast scale around the globe. Therefore, the current study was planned to evaluate antibacterial activity of *A. vera* L.

MATERIALS AND METHODS

To determine the antibacterial activity of *Aloe vera* L. experiments were carried out in Ethnobotany Laboratory of the Department of Botany, University of Gujrat (UOG), Punjab, Pakistan.

Identification and collection herb

Aloe vera L. plants were purchased from local nursery of Gujrat, Punjab, Pakistan, and identified with the aid of Flora of Pakistan (Freitag, 2001)

The plant leaves were washed with distilled water to remove dirt and after that these were air-dried for 2 weeks at 37°C. Then, finely crushed to form decent uniform paste by means of crusher and kept in dark in air tight vessels (Sandasi *et al.,* 2010).

Extraction of plant materials

Based on polarity, gel-like Aloe leaves samples were thoroughly mixed to prepare ethanolic, methanolic and aqueous extracts. Plant samples (10 g) were mixed with 100 ml solvents and placed these solutions for 24 hours at 190-220 rpm on incubator shaker. Thereafter, the solvents were filtered with Whatman filter paper and poured in to the falcon tubes. Methanolic and ethanol extract were placed in a rotatory evaporator at 60 and 40 º C at 250 rpm to evaporate 60% of the solvent to get crude extract. After that, the remaining 40% solvents were vaporized in an incubator. The extracted material was kept in a refrigerator at 18 ºC for further use. The crude extract was further mixed in 1% of Dimethyl Sulfoxide (DMSO) solution to formulate various concentrations as described by Alipour *et al.* (2011).

Culturing of bacterial strains

Six bacterial strains were obtained from the Biochemistry Department Lab. University of Gujrat. These bacterial strains were identified using biochemical techniques and further sub-cultured on Luira Broth (LB) Medium for 18-24 hours at 35-37 °C (Shameem *et al.,* 2017).

Table 1: Pre-Cultured Bacterial strains used for antibacterial assay

	Bacterial Strains	Accession No.
1.	*Streptococcus pyogenes*	ATCC 15224
2.	*Staphylococcus aureus*	ATCC 25922
3.	*Shigella sonnei*	ATCC 35318
4.	*Escherichia coli* 1	4C11
5.	*Escherichia coli* 2	ATCC 13048
6.	*Neisseria gonorrhoeae*	TC-11-2

Agar well diffusion assay

To calculate the antibacterial activity of Aloe extracts, agar well diffusion method was used with some modifications (Rana *et al.,* 2011). The bacterial strains were incubated on LB medium and after 12-18 hrs, spread with sterilized spreader on agar containing petri plates. With the help of Glass borer about 6mm wells were developed in the agar in the Petri-plates. Crude extracts of Aloe prepared in 1 % DMSO were poured into wells to evaluate antibacterial activity. All the three extracts of *Aloe vera* in Methanol, water and Ethanol were used for the said purpose. Six clinical strains of bacteria (Table 1) *S. pyogenes, S. aureus, S. sonnei, E. coli* 1, *E. coli* 2 and *N. gonorrhoeae* were used to investigate the activity of Aloe extracts. Three concentrations (25, 50 and 75 mg/ml) of plant extract residues in DMSO were used to investigate of the lowermost effective concentration of Aloe.

DMSO was used as a negative control in this experiment. At the same time, standard antibiotic (control) was added as positive control in adjacent well and inhibition zones (IZ) diameter was calculated for each extract well. The experiments were set up with three replicates. The readings were noted, their means were determined for determining the antibacterial activity of plant extracts (Saleem *et al.,* 2010). The corresponding inhibition zone diameter was measured after 24 hours and 48 hours, respectively.

RESULTS

Antibacterial action of aqueous extract of *Aloe vera* after 24 and 48 hours

Aqueous extract of Aloe at conc. (25 mg/100, 50 mg/100 and 75 mg/100 ml) in DMSO solvent showed generally decline in bacterial growth after 24 and 48 hours of incubation. *Escherichia coli* (1) (4C11) and *Escherichia coli* (2) (ATCC 13048) were reported to be sluggish at 25 mg/ml treatment of aqueous Aloe extract but, they showed some inhibition in growth at 50 mg and 75 mg of plant extract. However, all bacterial strains showed variable inhibition zones at different conc. of aqueous extract. *Streptococcus pyogenes* indicated significantly high antibacterial activity while, *Escherichia coli* (1) showed least antibacterial activity in aqueous extract after 24 hours (Fig. 1a) and 48 hours (Fig. 1b) of incubation. The antibacterial activity of Aloe was comparable with standard antibacterial agent i.e., Gentamicin of Pfizer company.

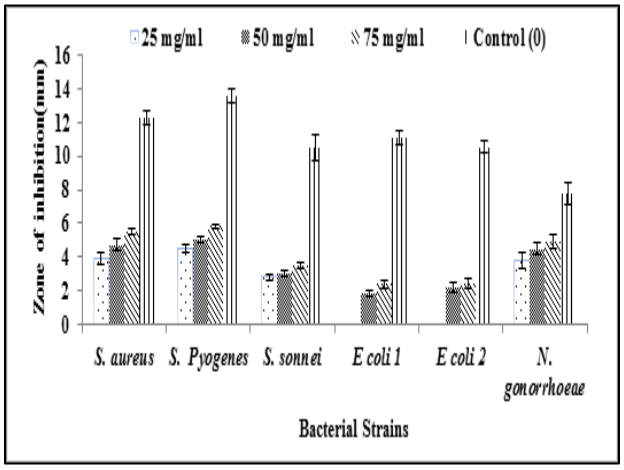

Fig. 1a: Antibacterial activity of aqueous extract of *Aloe vera* after 24 hours of incubation

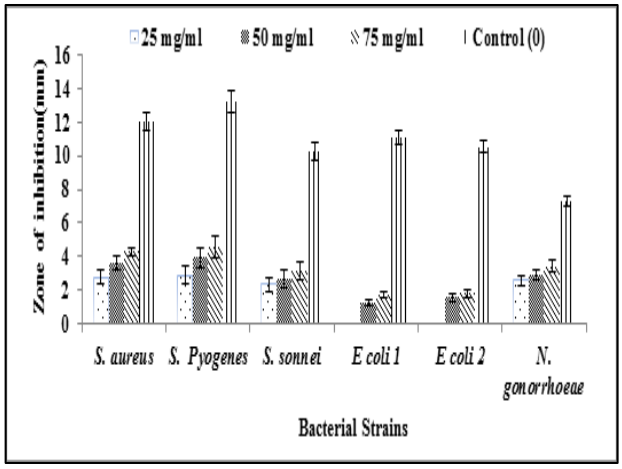

Fig. 1b: Antibacterial *activity of aqueous extract* of *Aloe vera* after 48 hours of incubation

Antibacterial action of methanolic extracts of *Aloe vera* after 24 and 48 hours

Methanolic extracts of Aloe in DMSO indicated the effective inhibition bacteria strains after 24 and 48 hours incubation at conc. (25 mg/ml, 50 mg/ml and 75 mg/ml). All bacterial strains showed maximum IZ at 75 mg/ml conc. methanolic extract. However, *S. aureus* and *S. pyogenes* exhibited the maximum IZ as compared to *E. coli* 1 and *E. coli* 2 after 24 hours (Fig. 2a) and 48 hours (Fig. 2b) of incubation.

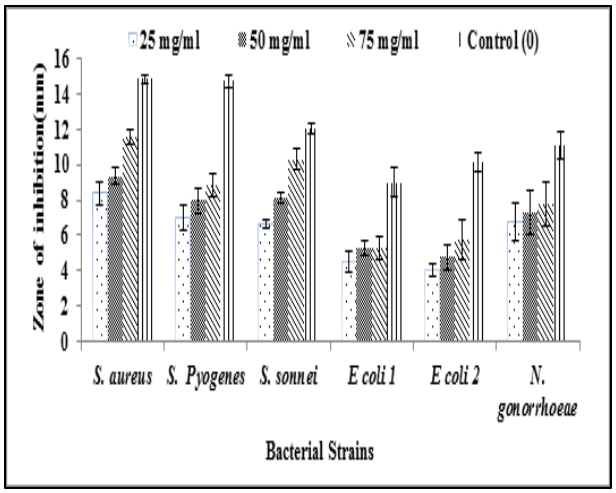

Fig. 2a: Antibacterial action of methanolic extract of A. vera after 24 hours of incubation

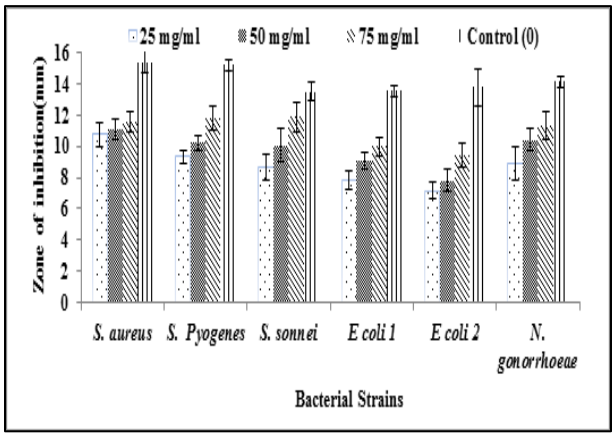

Fig. 2b: Antibacterial action of methanolic extract of A. vera after 28 hours of incubation

Antibacterial action of ethanolic extract of *Aloe vera* after 24 and 48 hours

The ethanolic extract of *A. vera* at various conc. In DMSO exhibited the remarkable reduction in bacterial strains growth after 24 hrs and 48 hrs incubation at conc. (25, 50 and 75 mg/ml). However, *S. aureus* showed maximum inhibition as compared to *E. coli* 2. The ethanolic extract of Aloe displayed maximum antimicrobial activity against *S. aureus* and minimum against *E. coli* 2 after 24 hrs (Fig. 3a) and 48 hrs (Fig. 3b) of incubation.

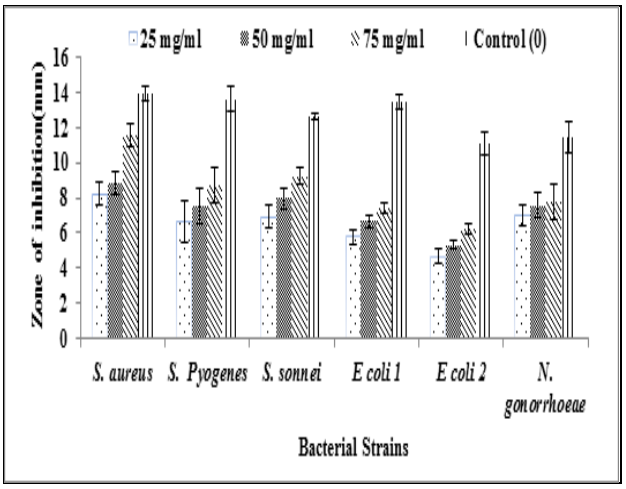

Fig. 3a: Antibacterial action of *Aloe vera* extract in ethanol after 24 hours of incubation**.**

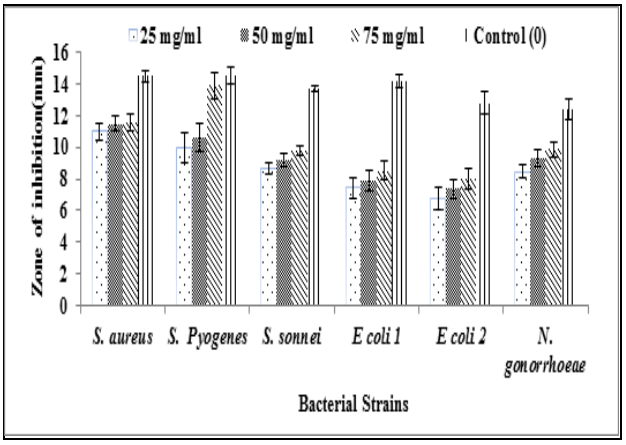

Fig. 3b: Antibacterial action *Aloe vera* extract in ethanol after 48 hours of incubation.

DISCUSSIONS

The data showed that *Aloe vera* had a great potential of controlling growth different bacterial strains. Methanolic, ethanolic and aqueous extracts of Aloe caused inhibition in bacterial growth. Our results are coherent with the findings that *Psidium guajava* extracts exhibit antimicrobial effects against both gram positive and negative bacteria. The antibacterial activity of Aloe might be owing to the prevalence of the bioactive polyphenolic elements (Amoo *et al.,* 2009; Biswas *et al.,* 2013). Antibacterial bioactive compounds are prevalently either polar or non-polar when extracted in organic medium. Organic crude extracts of the plants showed enhanced antimicrobial activity than of aqueous extracts (Jeyachandran *et al.,* 1995). Therefore, methanolic and ethanolic extracts of Aloe considerably reduced the growth of the

targeted bacterial strains. Similar results were reported by (Al-inke, (2011) and Egamberdieva *et al.,* (2017).

The accumulative evidences have been reported that there was reduction in growth of negative gram bacteria (*S. aureus, E. coli* and *K. pneumonia*) and gram positive bacteria (*M. luteus, E. aerogenes* and *B. subtilis*). Aloe extracts were found to be ineffective against *E. coli* 1 and 2 when applied as different conc. while, *Staphylococcus aureus* and *Streptococcus pyogenes* showed their susceptibility towards all the treatments (Elisha *et al.,* 2017). The highest IZ 13.9mm ± 1.3 was measured at 75 mg/ml of ethanolic extract of *A. vera* after 48 hours of incubation against *S. pyogenes*. However, the minimum inhibition zone was recorded at 25 mg/ml of aqueous extract of Aloe after 24 hours incubation against *E. coli* 1 and *E. coli* 2 that might be due to less solubility of organic constituents in water. It has been recorded that methanolic and ethanolic extracts of *A. vera* were enriched with ingredients like flavonoids, steroids, saponins and glycosidic compounds (Kumar *et al.,* 2017). Due to these ingredients in Aloe inhibitory activity against bacteria has been found (Palombo *et al.,* 2001; Sriram *et al.,* 2010; Mostafa *et al.,* 2018).

CONCLUSION

Based on data, it is concluded that Aloe extract in Methanol solvent caused greatest inhibition of *S. pyogenes* in comparison with other bacterial stains.

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