

ANTIMICROBIAL ACTIVITY OF ALOE VERA LEAF EXTRACT

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ABSTRACT: The antimicrobial activity of *aloe vera* extract was tested against pathogenic bacteria like *Staphylococcus aureus*, *Klebisella pneumonia* and *E.coli* and fungi like *Aspergillus niger* and *Candida* at a dose of 1:20 mg/ml and 2:40 mg/ml by using cup plate diffusion method. Various solvents such as petroleum ether, chloroform and methanol were used for extracts. The results reveal that, methanol and petroleum ether at a dose of 20 mg/ml has showed significant activity against *Klebisella pneumonia* and *E.coli* whereas in fungi, methanol extract showed significant activity against *Aspergillus niger* and *Candida*. Methanol extract has showed maximum inhibitory activity against *E.coli* and *Candida*. Petroleum ether has showed moderate inhibitory activity against *Klebisella pneumonia* and *Candida*. The zone of inhibition was measured and compared with standard Gentamycin (1 mg/ml). However, in none of the above mentioned extracts the inhibition zone was not more than that found in standard i.e., Gentamycin.

Key words: *Aloe vera*, antimicrobial activity, Gentamycin

INTRODUCTION

Traditional medicine is in practice for many centuries by a substantial proportion of the population of many centuries. It is recognized that in some developing countries, plants are the main medicinal source to treat various infectious diseases. Plant extracts represent a continuous effort to find new compound against pathogens. Approximately 20% of the plants are found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semisynthetic resources (Mothana and Linclequist, 2005). *Aloe vera* (*Aloe barbadensis* miller) is a plant, which belongs to the family of Liliaceae and is mostly succulent with a whorl of elongated, pointed leaves (Strickland et al., 2004; Beckford and Badrie, 2000). The name is derived from the Arabic word 'alloeh' which means 'bitter', referring to the taste of the liquid contained in the leaves. Aloe that is believed to have originated in the Sudan. *Aloe vera* grows in arid climates and is widely distributed in Africa, India and other arid areas. The species is frequently cited as being used in herbal medicine. *Aloe vera* is a perennial, drought resisting, succulent plant. It has stiff green, lance-shaped leaves containing clear gel in a central mucilaginous pulp. Its thick leaves contain the water supply for the plant to survive long periods of drought (Foster, 1999). The leaves have a high capacity of retaining water also in very warm dry climates and it can survive very harsh circumstances. When a leaf is cut, an orange-yellow sap drips from the open end. When the green skin of a leaf is removed a clear mucilaginous substances appears that contains fibres, water and the ingredient to retain the water in the leaf. The gel contains 99.3% of water, the remaining 0.7% is made up of solids with carbohydrates constituting for a large components (Foster, 1999). Concentrated extracts of *Aloe* leaves are used as laxative and as a haemorrhoid treatment. *Aloe* gel can help to stimulate the body's immune system (Davis, 1997). The use of plant product for pharmaceutical purpose has been gradually increased. According to World Health Organisation, medicinal plants would be the best source for obtaining a variety of drugs (Santos et al., 1995). The use of plant extracts, with known antimicrobial properties, can be of great significance in the treatment of various microbial infections. In the last decade, numerous studies have been conducted in different countries to prove such efficiency in number of medicinal plants. Most of the studies are restricted with crude extracts (Reddy et al., 2006; Erdo Urul, 2002; Atefl et al., 2003).

Many scientific studies of the use of aloe vera have been undertaken, some of them conflicting. Despite these limitations, there is some preliminary evidence that *Aloe vera* extracts may be useful in the treatment of wound and burn healing, minor skin infections, Sebaceous cyst, diabetes, and elevated blood lipids in humans. These positive effects are thought to be due to the presence of compounds such as polysaccharides, mannans, anthraquinones, and lectins.

"The use of Aloes, the common musabbar, for external application to inflamed painful parts of the body and for causing purgation [internal cleansing] are too well known in India to need any special mention."

MATERIALS AND METHODS

Collection of Plant Material:

The plant *Aloe vera* leaves were collected from in and around the city of Gulbarga, Karnataka, India.. This plant was botanically authenticated in the Department of Botany, B.V. B college of UG and PG Bidar. The leaves were shade dried and used for the extraction.

Extraction of Plant Material:

The leaves of *Aloe vera* was air dried and crushed to small piece using Mortar and Pestle and powdered in an electric grinder. The powdered plant material was subjected for successive soxhlet extraction starting from non polar to polar solvents such as petroleum ether [PE].Chloroform [CHCl_3] and methanol [MeOH] by using soxhlet extracts. The extracts were concentrated to dryness.

Preparation of the Extract:

The plant material were shade dried, powdered and subjected to Soxhlet extraction (1kg) with solvents ranging from non-polar that is Petroleum ether (60-80⁰), Chloroform (70-80⁰), Methanol (60-90⁰) respectively. The extracts were concentrated to dryness in a flask evaporator under reduced pressure and controlled temperature. The petroleum ether extract (20gm), chloroform (20gm), and ethanol extract (40gm). All the extracts were prepared in Tween-80 (1%) suspended in distilled water. The extracts preparations were done as previously described by Alade and Irobi [6]. The plant extracts were prepared by using soxhlet apparatus collected and stored in a vial for further studies.

Disc Preparation: The 6mm (diameter) discs were prepared from whatmann No. 1 filter Paper the discs were sterilized by autoclave at 12°C. After the sterilization the moisture discs were dried on hot air oven at 50°C. Then various solvent extract discs and control discs were prepared.

Antibacterial and Antifungal Activity Of *Aloe Vera*:

The antibacterial and antifungal activity studies were carried out by disc diffusion technique [12]. The sterile nutrient agar plates and potato dextrose agar plates were prepared. The bacterial test organisms like *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli* were spread over the nutrient agar plates by using separate sterile cotton buds. Then the fungal test organism like *Aspergillus niger* and *Candida* were spread over the potato dextrose agar plates After the microbial lawn preparation three different extracts of plant disc were placed on the organism inoculated plates with equal significant difference between extract used and also distance control discs were also prepared. All bacterial plates were incubated at 27°C for 24 hrs and fungal plates at 24°C for 72hrs. The diameter of the minimum zone of inhibition was measured in mm. For each test, three replicates were performed.

Statistical Analysis: Data were expressed as mean±standard deviation. The data obtained were subjected to ANOVA test to determine whether there was significant difference between extract used and also between the lengths of incubation.

RESULTS

The present study carried out on the *Aloe vera* revealed to evaluate antimicrobial activities of various extracts of *Aloe vera*. The successive leaf extracts using petroleum ether, chloroform and methanol of *Alovera* were tested for their antimicrobial efficiency against pathogenic bacteria and fungi (*Staphylococcus aureus*, *Klebsiella pneumonia*, *E.coli*,) and fungi like (*Aspergillus Niger*, *Candida*) at a dose 1: 20mg/ml and 2:40mg/ml. The standard drugs used for comparison were Streptomycin and Fluconazole against bacteria and fungi. Among the extracted tested for their antibacterial activity, the leaf extracts showed moderate to high activity against both gram positive and gram negative bacteria. The extracts using petroleum ether, chloroform, and methanol of *Aloe vera* showed active antimicrobial activity against *Staphylococcus aureus*, *Klebsiella pneumonia*, *E.coli* and, and antifungal activity against *Candida* and *Aspergillus niger*.

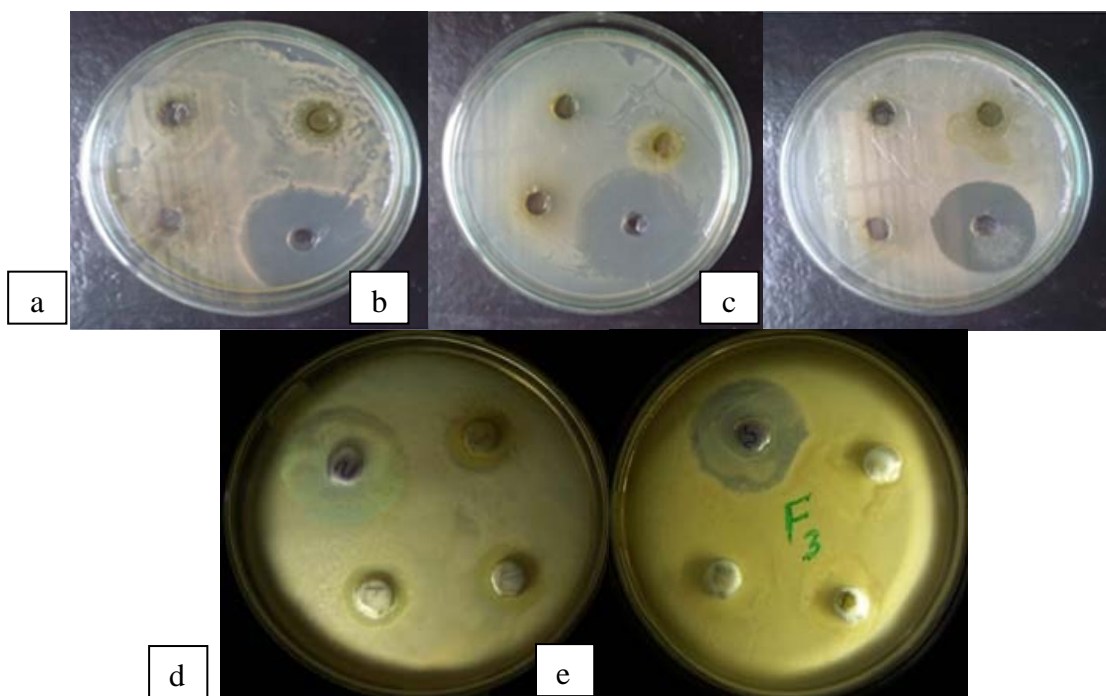
The chloroform and methanolic extract showed highest inhibition zone at higher concentration (i.e. 40mg/ml). Overall the methanolic extracts showed greater inhibition of all pathogenic microorganisms used when compared to chloroform and petroleum ether extracts.

The extracts of petroleum ether at the dose level of 20mg/ml showed the inhibition zone of *Staphylococcus aureus* (15mm), *Klebsiella* (19mm), *E.coli* (14mm), (Fig. 1) whereas the extracts of petroleum ether at the dose level of 40mg/ml showed the inhibition zone of *Staphylococcus aureus* (20mm), *Klebsiella* (20mm), *E-coli* (10mm) (Plate-I, & Fig. 2). The extracts of chloroform at dose level of 20mg/ml showed the inhibition zone of *staphylococcus aureus* (15mm), *Klebsiella* (11mm), *Escherichia coli* (14mm) where as extracts of chloroform at the dose level of 40mg/ml showed the diameter by zone of inhibition of *Staphylococcus Aureus* (14 mm), *Klebsiella*(10mm), *E-coil* (12mm) (Table-1). The extract of methanol at the dose level of 20mg/ml showed the diameter of zone of inhibition of *Staphylococcus aureus* (13mm), *Klebsiella* (13mm), *E.coli* (22mm) where as extracts of methanol at the dose level of 40mg/ml showed the diameter of zone of inhibition of *Staphylococcus aureus* (15mm), *Klebsiella* (15mm), *E.coli* (16mm).

Table 1: Antimicrobial Activity Of Leaf Extract of *Aloe vera*

Leaf extracts	Zone Of Inhibition (mm)*				
	<i>Staphylococcus aureus</i>	<i>Klebsiella sp.</i>	<i>E. coil</i>	<i>A. niger</i>	<i>Candida</i>
Chloroform (20mg)	15mm	11mm	14mm	10mm	15mm
Chloroform (40mg)	4mm	10mm	12mm	12mm	20mm
Petroleum ether (20mg)	15mm	19mm	14mm	12mm	14mm
Petroleum ether (40mg)	20mm	20mm	10mm	14mm	19mm
Methanol (20mg)	13mm	13mm	22mm	15mm	16mm
Methanol (40mg)	15mm	15mm	16mm	17mm	15mm
Standard (40mg)	12mm	20mm	30mm	15mm	28mm
Standard (20mg)	15mm	20mm	28mm	18mm	25mm

Data represents average of three replicates., mm* = Mean of three replicates.
Dose – 20 mg/ml and 40 mg/ml.



a) *Staphylococcus aureus* b) *Klebsiella pneumoniae* c) *E. coli* d) *Candida* e) *Aspergillus niger*.

Fig: I Plates Showing Zone of Inhibition

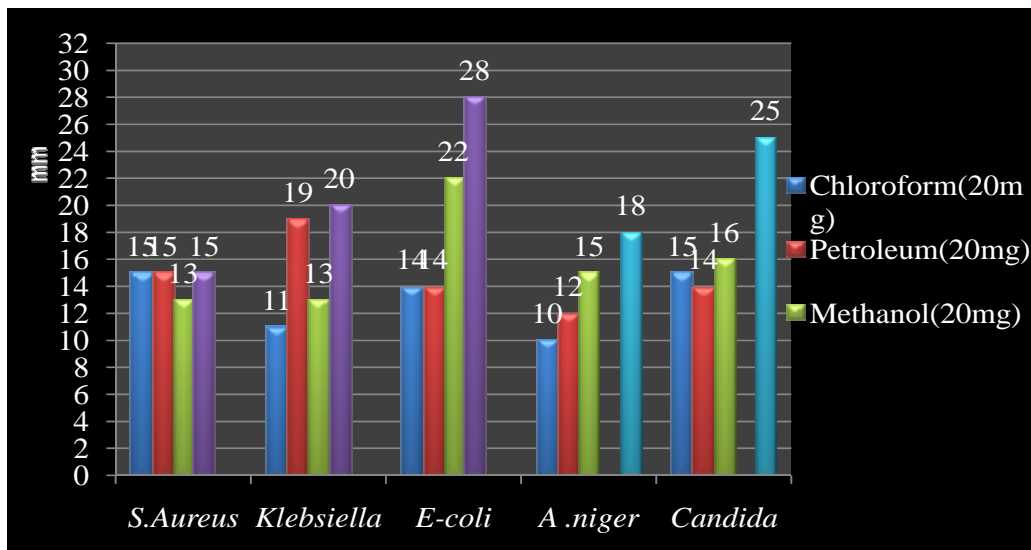


Figure 1: Antimicrobial Activity of Leaf Extract of *Aloe vera* in 20mg Concentration

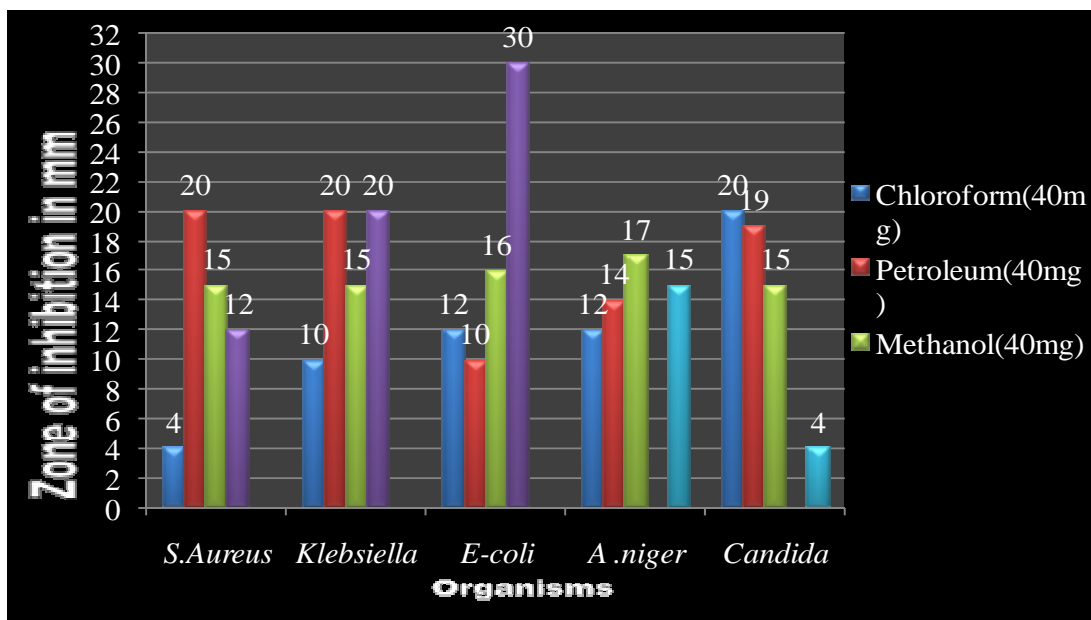


Figure 2: Antimicrobial Activity of Leaf Extract of *Aloe vera* in 40mg Concentration

DISCUSSION

In this present study the petroleum ether extract, has shown high zone of inhibition in *Escherichia coli*, *Klebseilla pnemoniae*, the fungi *Aspergillus niger* and moderate zone for *Staphylococcus aureus*, and *Candida*. Chloroform extract has shown a high zone of inhibition in *Klebsiella pneumonia*, *Escherichia coli* and *Aspergillus Niger* but moderate zone of inhibition in *Staphylococcus aureus*, and *Candida*. Methanol extract as shown high zone of inhibition in *Staphylococcus aureus*, *Klebseilla pnemoniae* and *Candida* but moderate zone in *Escherichia coli* and *Aspergillus Niger*. When compared the zone of inhibition with the standard drugs like streptomycin and flucanazole. The plant extracts have shown almost equal to the standard drug. The above parameter supports the strong scientific basis for the use of these plants in traditional treatment of microbial diseases.

The antimicrobial activity of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter. Only alcoholic extract was found to be a better solvent for extraction of antimicrobially active substances compared to water and hexane (Ahmad *et al.*, 1998). Agarry *et al.*, (2005) compared the antimicrobial activities of the gel and leaf of *Aloe vera* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Trichophyton mentagrophytes*, *T. schoeleinii*, *Microsporium canis* and *Candida albicans*. The antimicrobial analysis it was confirmed that this plant leaf extracts showed positive results against bacterial species such as *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* and fungi *Aspergillus niger* and *candida*. Hence, it can be concluded that the leaf extracts of *Aloe Vera* can effectively act as an antimicrobial agent which have ability to replace most of medium medicines of this era.

CONCLUSION

The present study has revealed the importance of natural products to control antibiotic resistant bacteria, which have been a threat to human health. It is, therefore highly essential that medicinal plants whose properties have not been fully characterized should form a top agenda of top management in developing nations whose citizens are sometimes unable to afford expensive orthodox medicine. This study has revealed the presence of many secondary metabolites in the leaves of *Aloe vera*. It has the further confirmed that the plant extracts could be used for the treatment of various infections including skin transmitted infections. The results lend credence to the folkloric use, if this plant in treating microbial infection and shows that *Aloe vera* could be exploited for new potent antimicrobial agents.

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