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Bioactive compounds and Pharmacological Probing of Aloverae Barbadensis and Exploration of its Antimicrobial Activeness

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ABSTRACT

Innumerable variety of plants that long have been usance aimed at medicinal purposes. Various scientific investigation proved the utilization of plants as a waves to medicine and helpful to improve our health. In exploration for nature based alternative medicine for different disease conditions, plant-based compounds gained lot of interest as it possesses significant impact with efficient treatment strategies. All these types of findings were explored in India under a major streams called ethnomedicine, siddha medicine, and ayurvedic medicine. These types of medicines include not only medicinal compound, but it also includes the food habits. More number of people believe that nature-based compounds have minimal side effects, and they believe that is the sign of healthy lifestyle. Always herbal based products are showing significant advantages towards long time benefits for the mankind. Mostly, these types of nature-based therapies were predominantly used in developing and developed countries. Statistical data from WHO reports that more than eighty percent of the global population have the tendency to follow nature-based treatment strategies using raw plant source, processed plant compounds and herbal based products. We have selected and collected *Aloe vera* plant and experimentally proved its medicinal and pharmacological properties adapted in pharmaceutical sectors. Our current work aimed to elucidate the phytochemical analysis, bioactive chemical compounds, antibacterial and anticancer activity of the *Aloe vera* plants. GC- MS and was performed to know more about the secondary metabolites of Aloe vera plants and further antibacterial activity was evaluated against four pathogenic bacterial species. The outcome of results showed the impactable characteristics of the *Aloe vera* in possessing significant antimicrobial effects against the bacterial strains such as *Klebsiella pneumoniae* ATCC 700603, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, bacterial strains. Additional investigations were beleaguered going on the refinement of the new fangled compounds aimed at the irrefutable evaluation.

Keywords: *Aloe vera, bactericidal, cancer, metabolites, phytochemicals*

1. INTRODUCTION

Plants produce an enormously rich variety of specific secondary metabolites encompassing and massive number of energetic and complementary Phyto compounds. Humans hold distinct pharmacological acquaintance of the healing properties of plants. The utilization of plant extracts, based ethnomedicine comprising hundreds of biochemicals as medicinal agents. From our olden days, there is always an emerging need to find novel nature-based compounds to treat different kinds of human problems. In those type of research studies, plant and plant-based compounds play a major role with respect to treatment process. Basically, all these types of medicinal strategies help in maintaining the health status both physically and mentally. Reports suggests that all plant based compounds are very efficient in treating all kinds of human medical problems.[1] People who were known about natural medicines will not use any type of modern medicine. It have been testified that herbal drugs and its ingredients have beneficial paraphernalia on long-standing fitness and can be used competently to extravagance human illnesses or syndromes.

Yasukawa[2], reported the facts of chemo preventive nature of plant sources and supplements which are used as drugs. He also described that chemoprevention is the major requirement for all the public. The focus of this cancer chemoprevention is described to arrest the tumor growth and control the enhancement of high-grade cancers. Most of the survey related studies describes that there are majorly few important features such as food habits and other lifestyle parameters.[3] Routine intake of certain diets was known to possess essential immunity against the tumor conditions. This enlighten the significance of the outer environments including the food habits in preventing the tumor growth.[4] Further few studies focused on the identification of efficient bioactive constituents that have potential activity against cancer conditions in the plants and other herbs. These active components functions to make a longer duration and arrest the cancer development process. [5]

Fig 1. *Aloverae barbadensis* wide spreading variety in India

Aloe vera is an Angiosperms flowering plants (Fig 1) with copious, corpulent leaves. It be in the right place to the genus *Aloe* and is inherent to dry provinces in Africa and speeded and cultivated globally for countless purposes. In india the cultivate species is *Aloverae barbadensis* is a very known succulent plant. species of the *genus Aloe.* [6] It is known to have a very ancient usage as therapeutic compound. It is always known to be incredibly significant factor which is used on ancient days for nature-based medicines to treat different type of human diseases.[7] Aloe vera have been utilized in all most all countries as per significant compound for treatment options. They are known to grow in both tropical and semi tropical regions.[8] They are known to have a property to grow in less water sources and it has the property to use different metabolism, it has the property to grow even in severe dry conditions.[9]

Objectives

In this up-to-date research paper we deliver reports on the antimicrobial activity of pharmacologically important biochemical compounds with high medicinal value. The overall research findings will be exposed to prepare medicine in near future.

2. MATERIALS AND METHODS

2.1 Collection of plant material

Aloverae barbadensis samplers were poised from local cultivators of Lakhshmipuram area near Madurai, Tamilnadu. Robust and emphatic *Aloe vera* were carefully collected and identified then authenticated through comparison of the specimen preserved in herbarium, accessible in Department of Plant biotechnology, Madurai Kamaraj University, Tamilnadu, India. Tissues scrabes were taken in a disinfected plastic bag and transported to the research laboratory with an ice box for further processing.

2.2. Preparation pulp powder extract

To eliminate the sticking unwanted dirt and. substances on the surface of Aloe vera leaflets were washed using turned tap out water. After removing the spikes on the rim of the leaves the fleshy leaves were sliced. The thick green outer coat of sliced leaves were unwrapped out by a knife and the jelly part of the leaves were collected in stainless steel container. The jelly sample were dried at 60 to 70$^{\circ C}$ by using microwave up to 30 minutes. The resultant solid powder extract (Fig 2) of Aloe vera was used for the final experimental analysis.

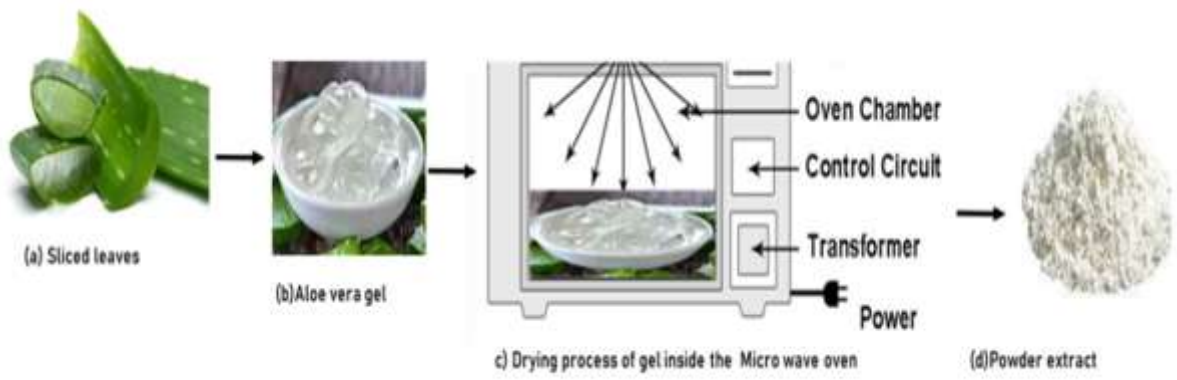

Fig 2. Conversion of Aloe vera wet jelly extract into powder extract using Microwave process

2.3. Phytochemical Qualitative Analysis

(i) Saponins Assay: The crude plant extract was added with five ml of Dis H2O in a test tube followed with vigorous mixing. The presence of saponin was confirmed with the appearance of foam.

(ii) Flavonoids: To check the presence of flavonoids, plant extract was added with two ml of 2% NaOH. The development of intense yellow colour shows the existence of flavonoids. **(iii)Terpenoids assay:** 5 ml of extract was mixed with two ml chloroform. Then the tube was kept on water path to evaporate and boiled with three ml of conc. H2SO4. The generation of grey colour shows the existence of terpenoids.

(iv) Glycosides assay: Glacial acetic acid about four ml along with one drop of 2.0% Iron (III) chloride mixture were mixed with plant extract (10 ml) and 1 ml conc H2SO4. The apperance of brown ring between layers shows the existence of glycosides.

(v)Alkaloids assay: 5 mg extract was taken in tube and one drop of dragendorff's reagent was added. A clear orange-red color precipitate shows the presence of alkaloids.

(vi) Protein assay: Few drops of biuret's reagent was added to 5 mg aloe vera extract. The resulting mixture was vigorously mixed before being allowed to warm for 1 to 5 minutes. The presence of proteins was shown by the advent of violet or red colour.

(vii) Test for Amino acids The presence of amino acids was determined by combining 5 mg of sample extract treated within 2 ml 0.2 percent Ninhydrin reagent and boiling for 2 minutes on a water bath. The emergence of violet color shows the amino acids presence.

(viii)(Test for carbohydrates 5 mg extract was mixed with 2 to 4 drops of Benedict's chemical reagent and permissible to hot water boil. A reddish or brownish precipitate formation shows the presence of carbohydrates.

(ix)Test for phenols The aloe vera powder was boiled and the filtrate 2 ml was treated 4 drops of 0.1% ferric chloride solution the colour vicissitudes to green or blue shows the presence of phenols.

2.4. GC- MS Analysis:

For GC-MS assay sample, the Aloe vera powder sample was grinded with GC graded methanol and the resulting methanolic extract was centrifuged 6000rpm and the collected supernatant was used to trial injected into GC-MS system. The supernatant containing chemical derivatives were analysed using a 5HT capillary mode column (30m- 0.32mm- 0.25m) within EI (electron impact) ionization applying GC-MS System, QP-ultra-2010- Shimadzu, Japan. At 1.57 mL min-1, helium gas cast-off as a transporter (carrier) gas. The temperature of injection in GC was reserved at 250ºC and ion cause temperature considered to be 230ºC. The boundary and MS ion source were upheld at 230ºC and 300ºC, respectively. The analysed Mass spectra can reserved at 70 eV; one scan intermission reserved as 0.2 seconds. The bioactive compounds identification were based on contrast of the standard mass spectra documented in NIST Libraries with the Software implemented to NIST 2014 (2.2.0.0) within AMDIS v.2.72 type.

2.5. Antimicrobial Activity

All the test microorganism were purchased from MTCC, Chandigarh and the strains were maintained on Mueller Hinton Agar (MHA). Antimicrobial outbreak were achieved by means of agar well diffusion technique in Muller Hindan Agar plates. The testified micro organisms all are inoculated in nutrient broth and well-cultured overnight at 37°C. A uniform well of 3mm were created in culture plates. To each well the Aloe vera extract filled in the range of 50mg, 100mg, 150 mg and

200mg /ml and streptomycin 25 mcg as the positive control and DMSO used as negative control respectively. The plates were allowable to diffuse at chamber temperature for about 30 minutes earlier actuality nurtured at 37°C for 24 hours. The zone of inhibition (ZOI) was experiential and measured in mm.

3. RESULTS AND DISCUSSION

3.1 Qualitative Phytochemical Analysis

Phytochemical analysis shows that the aqueous extract of *Aloe vera* contained important medicinal phytochemicals such as Alkaloids, Carbohydrates, Glycosides, Saponin, Protein, Amino acids, Phenolic compounds and Terpenoids (Table 1). Results showed that Carbohydrates, Saponin, Alkaloids and phenolic compounds and Terpenoids were present in Aloe vera extracts.

Table 1: Qualitative Analysis of Phytochemical assays

S.No	Phytochemical Tests *A. vera* extract	Observation	Results
1	Alkaloids	Orange/red colour	P
2	Carbohydrates	Brownish precipitate	P
3	Glycosides	Brown ring	P
4	Saponin	Foam formation	P
5	Protein	Advent violet colour	p
6	Amino acid	Light colour	p
7	Phenolic Compounds	Green colour	P
8	Terpenoids	Grey colour	P
9	Flavonoids	Yellow colour	P

AB – Absent, P - Present

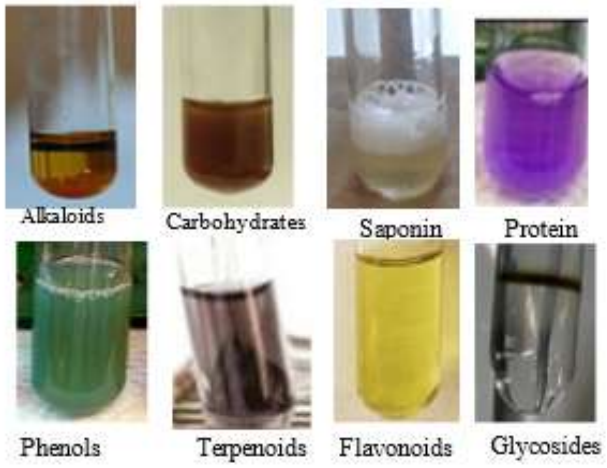

Fig 3 .Phytochemical Determination

3.2. GC- MS analysis:

GC MS analysis was performed for *Aloe vera* extracts to analyze the presence of bioactive compounds presents in them. GC MS spectrum analysis Fig 4 and Table2, represents the complete details with bioactive compound list identified in the GC MS analysis. It possesses many important secondary metabolite components which are known to have significant bioactivities in wide range of applications. Most of the components were found to be essential fatty acids and its metabolites and they were well known to play major role in restructuring, firming, regenerating and as well moisturizing the skin. It is also showed to enhance the smoothness of the human skin.[10]. These components were known to possess antimicrobial property and hence it has been applied in treating many conditions including atopic eczema, acne, wounds and other

dermatological conditions. Because of its extensive healing activities, oleic acid was mostly adapted in many cosmetic applications.[11] The GC MS analysis revealed the presence of different type of phytosterols, phenolic compounds, tocopherols, carotenoids and other polyphenolic compounds which eventually signifies its therapeutic importance of our AV extract. In addition, phenolic compounds, tocopherols, and other phytochemicals also act as natural antioxidants..[12]

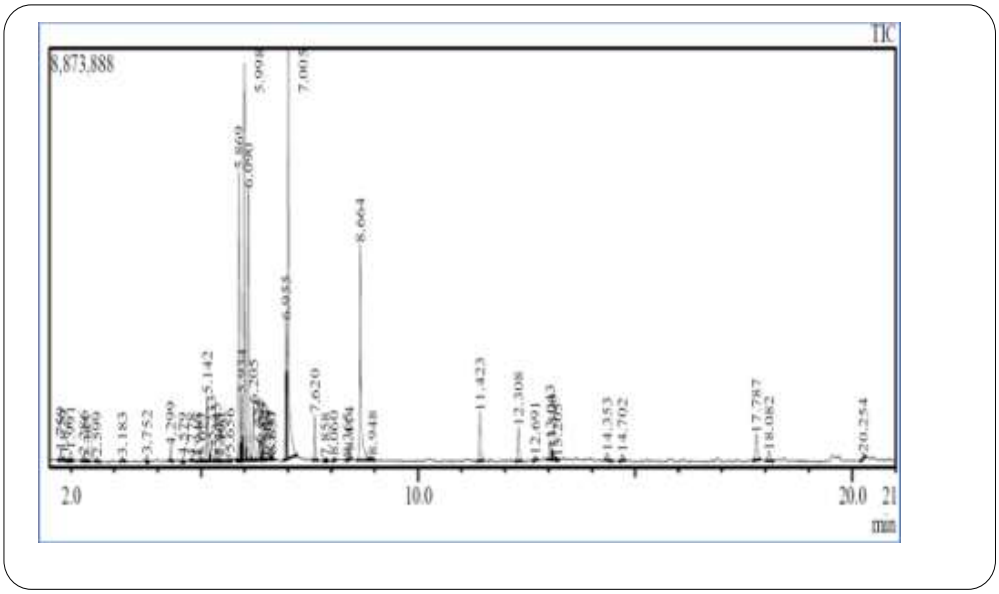

Figure 4. GC- MS spectral analysis of *Aloe vera* extracts

Table 2 : GC MS analysis of *Aloe vera* extracts

Peak#	R.Time	Area%	Name
1	1.759	0.4	BUTANEDIOIC ACID, [(TRIMETHYLSILYL)OXY]-, BIS(TRIMETHYLSILYL) ESTER
2	1.829	0.23	TRIETHYLENE GLYCOL, DI-TMS
3	1.991	0.51	BUTANAL, 2,3,4-TRIS[(TRIMETHYLSILYL)OXY]-, (R*,R*)-
4	2.286	0.23	1-Deoxypentitol, 4TMS derivative
5	2.385	0.15	(R*,S*)-2,3-Dihydroxybutanoic acid, tris(trimethylsilyl) deriv.
6	2.599	0.17	L-Threonic acid, tris(trimethylsilyl) ether, trimethylsilyl ester
7	3.183	0.19	DODECANOIC ACID, TRIMETHYLSILYL ESTER
8	3.752	0.23	D-Arabinose, tetrakis(trimethylsilyl) ether, ethyloxime (isomer 2)
9	4.299	0.55	XYLITOL, 1,2,3,4,5-PENTAKIS-O-(TRIMETHYLSILYL)-
10	4.579	0.17	2-Deoxy-pentos-3-ulose, bis(methoxime),O,O'-bis(trimethylsilyl)-
11	4.778	0.22	Ribonic acid, 2,3,4,5-tetrakis-O-(trimethylsilyl)-, trimethylsilyl ester
12	4.94	0.1	Ribonic acid, 2,3,4,5-tetrakis-O-(trimethylsilyl)-, trimethylsilyl ester
13	5.027	0.17	Ribo-hexos-3-ulose, 2,4,5,6-tetrakis-O-(trimethylsilyl)-, bis(O-methyloxime)
14	5.142	3.1	D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer 2)
15	5.233	0.96	D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer 1)
16	5.345	0.56	ISO-CITRIC ACID-TETRATMS
17	5.405	0.15	D-(-)-Fructose, pentakis(trimethylsilyl) ether, methyloxime (anti)
18	5.49	0.1	2,2,9,9-TETRAMETHYL-5,6-BIS[(TRIMETHYLSILYL)OXY]-3,8-DIOXA-2,9-DISILADECA
19	5.656	0.44	.beta.-D-Galactofuranose, 1,2,3,5,6-pentakis-O-(trimethylsilyl)-
20	5.869	11.58	D-FRUCTOSE, 1,3,4,5,6-PENTAKIS-O-(TRIMETHYLSILYL)-, O-METHYLOXIME
21	5.934	2.49	.BETA.-D-GLUCOPYRANOSE, 1,2,3,4,6-PENTAKIS-O-(TRIMETHYLSILYL)-
22	5.998	16.04	D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime
23	6.09	10.18	D-(+)-Talose, pentakis(trimethylsilyl) ether, methyloxime (syn)
24	6.205	2.15	D-(+)-Talose, pentakis(trimethylsilyl) ether, methyloxime (syn)
25	6.325	0.63	D-Sorbitol, 6TMS derivative
26	6.393	0.54	D-Glucitol, 6TMS derivative
27	6.425	0.4	L-(+)-Rhamnopyranose, 4TMS derivative
28	6.509	0.14	Levoglucosan, 3TMS derivative
29	6.59	0.1	D-(-)-Lyxofuranose, tetrakis(trimethylsilyl) ether
30	6.637	0.2	D-Gluconic acid, 6TMS derivative
31	6.955	5.54	Talose, 5TMS derivative
32	7.005	18.96	Palmitic Acid, TMS derivative
33	7.62	1.74	Myo-Inositol, 6TMS derivative
34	7.858	0.14	Heptadecanoic acid, TMS derivative
35	8.06	0.14	MANNOSE, 6-DEOXY-2,3,4,5-TETRAKIS-O-(TRIMETHYLSILYL)-, L-
36	8.365	0.09	9,12-OCTADECADIENOIC ACID (Z,Z)-, TRIMETHYLSILYL ESTER
37	8.414	0.34	OLEIC ACID, TRIMETHYLSILYL ESTER
38	8.664	10.64	Stearic acid, TMS derivative
39	8.948	0.11	D-(+)-Cellobiose, (isomer 1), 8TMS derivative
40	11.423	2.09	BIS-O-TRIMETHYLSILYL-PALMITINIC ACID-GLYCERIN-(1)-MONOESTER
41	12.308	1.59	Sucrose, 8TMS derivative
42	12.691	0.18	D-(-)-Ribofuranose, tetrakis(trimethylsilyl) ether (isomer 2)
43	13.043	1.12	Glycerol monostearate, 2TMS derivative
44	13.1	0.51	D-(+)-Trehalose, octakis(trimethylsilyl) ether
45	13.205	0.14	D-(+)-Cellobiose, octakis(trimethylsilyl) ether, methyloxime (isomer 1)
46	14.353	0.56	D-(+)-Cellobiose, octakis(trimethylsilyl) ether, methyloxime (isomer 1)
47	14.702	0.43	.beta.-Tocopherol, TMS derivative
48	17.787	1.67	Stigmast-5-ene, 3.beta.-(trimethylsiloxy)-, (24S)-
49	18.082	0.58	Lupeol trimethylsilyl ether
50	20.254	0.35	Lup-20(29)-en-28-al, 3-(trimethylsilyl)oxy, (3.beta.)-
		100	

3.3.Antimicrobial activity

Antimicrobial activity elucidation of the of the plant extract was analyzed by the agar well dissemination method in contrast to five diverse microorganisms together with *Klebsiella pneumoniae* , *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* . The selected organisms were normally encountered by diverse infectious diseases. The current work showing that Aloe vera leaf extracts in variable degree of antimicrobial action against all verified microorganism. Streptomycin was used as positive control; plant aqueous extracts were used to analyses the antimicrobial activity. The details about the antimicrobial activity were represented in Table 3. Fig 5.

Table 3: Antimicrobial activity with zone of inhibition

S. No.	Organisms Used	Zone of inhibition (mm)			
		Aloe vera extract			
		A	**100**	**125**	**150**
1	*Klebsiella pneumoniae* ATCC 700603,	10	2	4	6
2	*Staphylococcus aureus* ATCC 25923,	11	2	3	4
3	*Pseudomonas aeruginosa* ATCC 27853,	11	2	4	5
4	*Escherichia coli* ATCC 25922,	11	2	4	6

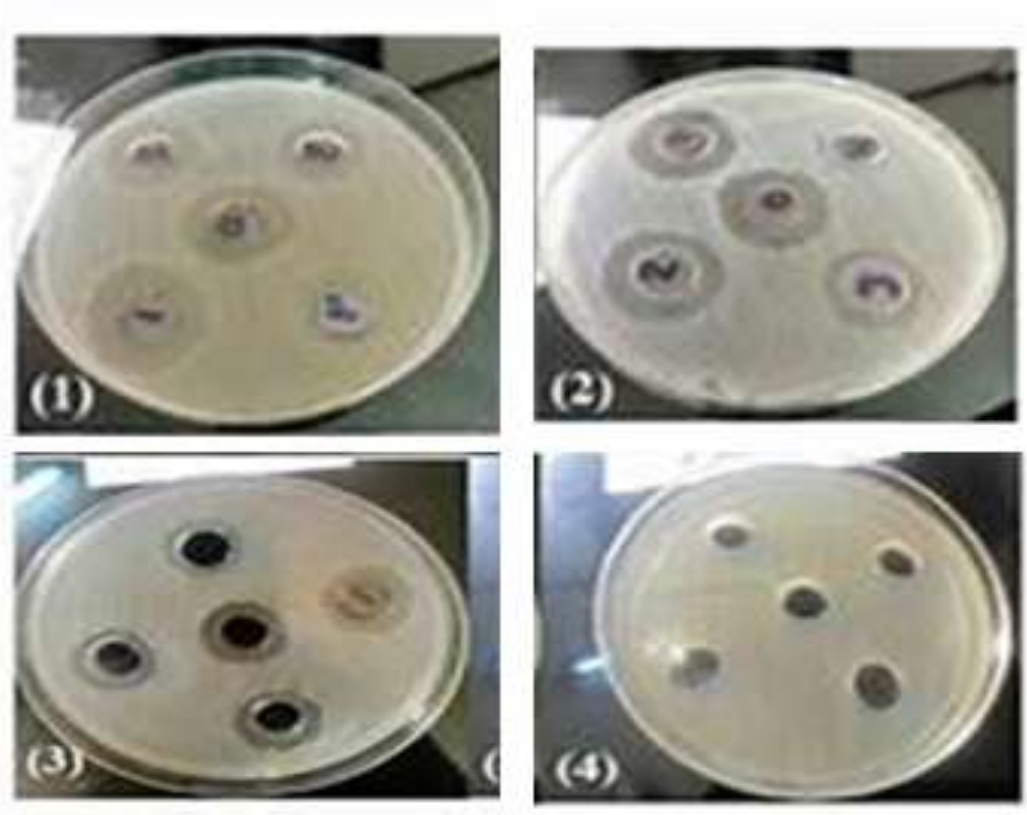

Fig 5. Antimicrobial effect of Aloe vera extract against 1. *Klebsiella pneumoniae* , 2. *Staphylococcus aureus* 3.*P. aeruginosa* 4. *Escherichia coli*

The gel of Aloe vera was identified for its remedial and medicinal chattels from very older periods and it was studied that they have more than 80 bioactive compounds. Presence of polysaccharide compounds are the important for their medicinal properties[13]. The functional property of the Aloe vera gel were due to their multiple combined effect of the chemical compounds than a single compound reaction. One of the important secondary derivative was Anthraquinones and it was the

main component involved in different kind of bio functions as following as hemo-static, astringent, antidiabetic, antibacterial, antiulcer, anti-oxidant, anticancer properties, and anti-inflammatory, in addition for gastro-intestinal disorder treatment (e.g., diarrhoea, dysentery and constipation), burns, radiation injury, and wound.

Hence it is been applied as alternative treatment strategies for many disease condition, used as additional supplements in daily nutrition and even adapted in many different of cosmetic products. Reports described that Aloe vera plant possess seventy five different biologically important derivatives[14]. Other reports suggested that aloe gel additionally possess sugars (17%), polysaccharides (55%), proteins (7%), lipids (4%), minerals (16%), and also phenolic compounds (1%). Among them more healing property was described for the polysaccharide compound[15]. Reports states that corytophanid and aloe-emodin corytophanid are main obviously stirring anthraquinone composites[16]. A study described compound present in the leaf extracts of aloe possess enormous antioxidant potential activity [17]. In addition, aloe gel had the ability to reduce the inflammation response by activating the generation of prostaglandin along with increasing leucocyte infiltration (Reynolds & Dweck 1999). An interesting study found that polysaccharide from aloe reduces the cerebral ischemia when treated in haemorrhagic rats[18] and in addition it was noted that it increases the properties of phagocytosis [19].

With respect to severe infectious diseases, it always remains a bigger threat to mankind, though many advancements happened in pharma sector. The main issue is with gaining the multi drug resistance[20].Researchers started exploring the plant based antimicrobial derivatives which possess significant efficacy in treating the diseases with minimal side paraphernalia[21,22]. Reports had already described the efficiency of aloe gel activity compared to both Gram-negative and Gram-positive bacteria[23].

Among the secondary metabolites, it was studied that Anthraquinones, dihydroxy anthraquinones and saponins possess effective anti-microbial activities (Nejatzadeh-Barandozi 2013). Reports suggested that emodin found to possess activity against E.coli and the process was initiated by inhibiting the membrane solute transport[24}.

Saponins were reported to act on synthesis of protein in bacteria by acting on the A site of ribosome. Further this eventually lead to growth of bacterial arrest in the media with aloe extract. Moreover alkaloids, flavonoids and phenols act on the stimulation of phagocytosis and kill the bacteria[25]. It is found that the crude extract of *Aloe vera* showed efficient activity against all the microorganism. The extract from *Aloe vera* showed inhibition zone of 6 mm against *Escherichia coli*, and also Methylene resistant *S. aureus* and *Candida albicans*. Several studies concentrated on understanding and proving the anti-bacterial properties of Aloe vera. A study aimed at identifying and quantifying the importance of A.vera leaves, hence they tried with various solvents to isolate the bioactive compounds and look for their nature of function specifically for antibacterial role against different type of bacteria. It is observed that acetone-based extract showed significant antibacterial role [26].

But in contrast, earlier reports stated that comparing different extraction methods by different type of solvents and observed that methanol and ethanol extracts found to be showing increased activity than acetone extract which showed decreased activity for pathogenic organism. [27] In addition, this study found that different factors regarding immune response & antioxidant response were found to be showing higher efficiency when given to animal with oral ulceration. *Aloe vera* hold powerful antifungal, antiviral and antibacterial, properties.[28] The antimicrobic properties of *Aloe vera* has been accredited to the natural plants derived phytochemicals, they confirmed *in vitro* hang-up of various pathogens of microbial origin.[29,30] Aloe extract have been initiate antimicrobial effects in contrast to *Salmonella paratyphi*, *Streptococcus pyogenes*, and *Staphylococcus aureus* it is due to the presence various medicinal compounds

4. CONCLUSION

Our study has reported that the powder extract of Aloe vera leaf gel has envisioned consequence of antibacterial achievement against selected bacterial species. This analysis further pledges that the Aloe vera leaf extracts might be used and aimed at the dealing of microbial contagions. Henceforth, our present-day findings resolve to be suggested that Aloe vera could be cast-off as an antibacterial for inhibition of around microbial infections. It can be believed about the current study may well to be used to recognize novel and superfluous effective antimicrobial medicine originated from natural resources. Most earlier reports suggest that crude extracts of Aloe vera always showed better efficiency against microorganism. This could be due to the presence of different secondary metabolites which possess significant antibacterial activity.

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