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PHYTOCHEMICALS AND *IN-VITRO* ANTIMICROBIAL ACTIVITY ANALYSIS OF *ALOE VERA GEL EXTRACT*

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ABSTRACT
Most widely used medicinal plants, aloe vera (A. vera), is utilized on a regular basis all over the world for the purpose of preventing or treating a variety of conditions, including cancer, metabolic issues, cardiovascular disorders, and skin troubles. Over the course of this research, we looked at the antimicrobial capabilities of A. vera gel extract, which are typically overlooked during the gel extraction process. Within the scope of this study, we evaluated the phytochemical and antimicrobial profiles of A. vera gel extracts by *prepared by cold percolation method. The required fraction of Aloe vera leave gel was soaked in (50% w/v) acetone, methanol, ethanol and distilled water*. The results of the study showed that *more or less all the fraction also exhibited remarkable activity against Grams positive and grams negative bacteria and S. cerevisiae which showed inhibitory activity against all the test extracts.*

Keywords: Aloe vera, *Phytochemicals, Antimicrobial*

INTRODUCTION
The genus ***Aloe*** belongs to order asparagales, family xanthorrhoeaceae. *Aloe vera* (*A. vera*) is a stemless or very short-stemmed succulent plant growing to 60–100 cm (24–39 in) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on the upper and lower stem surfaces. The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up to 90 cm (35 in) tall, each flower pendulous, with a yellow tubular corolla 2–3 cm (0.8–1.2 in) long. In addition to being referred to as inner leaf, inner leaf fillet, or fillet, aloe vera gel is the component that constitutes the majority of the plant. During the processing of the plant, the upper green layer, which is sometimes referred to as the A. vera skin, is typically regarded as waste or by-products [2]. The idea of a circular agro-economy has been increasingly popular in recent years as a direct result of the growing number of worries regarding the environment. It is a method that is eco-friendly and prevents trash from being produced throughout the production process. Recent research indicates that a wide variety of agricultural wastes, such as the skin of A. vera plants, have the potential to be renewable sources of bioactive compounds or biopolymers [3,4]. Since the beginning of time, A. vera has been thought to possess properties that are medicinal or healing-promoting features. It has been demonstrated through research that it possesses anti-cancer, anti-inflammatory, antiviral, anti-microbial, and antioxidant effects [5,6]. Several studies have demonstrated that aloe plants contain micronutrients and phytonutrients that have the potential to have both biological and toxicological impacts [1,6,7]. A study that was conducted not too long ago revealed that the administration of an aqueous extract of A. vera leaves could potentially greatly lessen the damage that pesticides cause to the livers of rats [8]. Additionally, a study stated that the detrimental effects of cartap were demonstrated to be greatly decreased by pre-administration of an aqueous extract of A. vera leaves, which protected the levels of oxidative markers (MDA and GSH) in Wistar rats [9]. This was shown to be the case in the study. There is a possibility that aloe vera can have a beneficial effect since it includes a large number of antioxidant molecules that are capable of combating the oxidative stress that is caused by cartap stress [10]. The usage of A. vera, on the other hand, frequently leads to interactions with other drugs, kidney difficulties, diarrhea, and an electrolyte imbalance [11].

the majority of the products made from A. vera, such as gel, are used in the cosmetics, food, and pharmaceutical spheres [12]. In A. vera processing facilities, the internal gel is routinely removed from the outer skin, which accounts for more than thirty percent of the total leaf weight. This separation results in a substantial amount of trash being produced. Such agricultural waste is often disposed of, composted, or burnt outright because it does not have any economic purpose [2]. While the inner gel of aloe vera has been the subject of much research, the

skin of the plant has been reported to be a potential source of bioactive compounds that might be used in food, food packaging, or biomedical applications. This is despite the fact that there is a very small amount of information available on the skin. Because there are several compounds with a wide range of physicochemical characteristics and solubility, the efficient extraction of these bioactive molecules from a variety of plant sources continues to be a challenging procedure. This is the case despite the fact that natural antioxidants have significant commercial and health-related benefits. The different structures and polarity of phenolic compounds in different solvents can have an effect on their solubility, extraction yield, and antioxidant activities [13]. This is due to the fact that phenolic compounds can exist in a diversity of free and conjugated forms.

MATERIAL AND METHOD:

Qualitative analysis of phytochemicals

i. **Test for Saponins:** 5 ml of distilled water was mixed with aqueous plant extract in a test tube and shaken well. Then observed 1 cm layer of foam indicated the presence of Saponins.

ii. **Test for Flavonoids:** 1 ml of crude extract was taken in a test tube and added few drop of dilute NaOH solution. An intense yellow color was appeared in test tube. It became colorless when on addition of a few drop of dilute acid that indicated the presence of Flavonoids.

iii. **Test for Alkaloids:** 1 gm powder sample were taken in conical flask and added 3 ml ammonia solution. It was allowed to stand for few minutes to evaluated free alkaloids. 10 ml chloroform was added to the conical flask and shaken by hand and then filtered. The chloroform was evaporated from the crude extract by water bath and added 3 ml Mayer's reagents. A cream color precipitation was obtained immediately that showed the presence of Alkaloids.

iv. **Test for Steroids:** 1 mg of extract mixed with 10 ml of chloroform and concentrated H2SO4 to the test tube by sides and adding a few drops of anhydride. A green color layer indicated the presence of Steroids.

v. **Test for Tannins:** 1 ml of crude extract was taken in a test tube and added 1 ml of FeCl3. A brownish green or a blue-black coloration showed positive test.

vi. **Test for Terpenoids:** 1 ml of crude extract added 1 ml of concentrated H2SO4. Then it was heated for 2 minutes. A reddish brown color indicated the presence of Terpenoids.

Collection of Plant Sample and Processing

The collection of *Aloe vera* plant sample was made from different part of Raipur *viz.* Botanical garden of I.G.K.V., local garden and parks of Raipur, shops etc. Systematic surveys were undertaken during 2021. Plant sample was identified by Dr. K. K. Shukla, Professor, SoS in Biotechnology. Pt. R.S. University, Raipur, (C.G.). The leaves of collected plant material were then processed and extracts were prepared by cold percolation method. The required fraction of *Aloe vera* leave gel was soaked in (50% w/v) acetone, methanol, ethanol and distilled water for 48 h. The prepared mixtures were stirred using a sterile glass rod at 24 h interval. The extracts were filtered by Whatmann filter paper no 1 [14]. The filtrates were then concentrated in water bath.

Determination of Antimicrobial

The antimicrobial profile of each extract was evaluated against a set of Gram positive and negative pathogenic bacteria using Kirby – Bauer method determined by NCCLS standards (National Committee for Clinical Laboratory Standards, 2002). Well diffusion method was utilized to assess antibacterial action of *A. vera* leave gel extract as portrayed by [15]. In well diffusion method sterile petri plates were set up with 20 ml of Muller Hinton Agar. The standard inoculums of bacterial suspension acclimated to 0.5 McFarland turbidity standard which is comparable to $1x10^8$ CFU/ml of microorganisms were cleaned on the seeded media and permitted to dry for 15 min. Then a well or a cup was formed in the seeded agar with the help of a borer having inner diameter of 6 mm. 40µl of aliquot of different concentration of essential oil ranging from 100 mg/ml and then positioned onto the inside of the hardened agar medium. Petri plates were then brooded for 24 h at 35 ± 1^0C. At last the zone of

restraint shaped by *A. vera* leave gel was recorded after 24 h hatching at 35±1^{0}C. The impacts were contrasted and that of standard, streptomycin (positive control). Concentrate was tried in triplicate alongside streptomycin (1mg/circle). The plates were kept at 4°C for 1h for dispersion of concentrate, from that point were hatched at 37±2°C for 24 h. Zone of hindrance (IZ) or discouraged development of microorganisms was estimated and the 'Activity Index' (AI) for each concentrate was determined.

$$\text{Activity index (AI)} = \frac{\text{Inhibition Zone of the}}{\text{Inhibition Zone of the standard}}$$

Fig.1. Preparation of crude extract of test plant (*A. vera*)

Test Organisms used for the Analysis

MTCC bacterial isolates viz., *Aeromonas hydrophilla* (MTCC 966), *B. cereus* (MTCC 633), *B. brevis**, *Enterobacter aerogenes**, *E. facalis**, *Escherichia coli* (MTCC 1591), *Klabsiella pneumoniae* (MTCC 2405), *Salmonella typhi* (MTCC 531), *Vibrio cholerae* (MTCC 1168), *S. dysentery **, *Candida albicans* (MTCC 1022), *Saccharomyces cerevisiae (*MTCC1732).

RESULT AND DISCUSSION

Photochemical Screening:

The phytochemical screening of aqueous extract of the plant (*A. vera)* material showed the presence of four secondary metabolites as saponins, steroids, tannins, and terpenoids.

Table.1: Result of plant extract screening assay.

Leaf Extract and tests	Secondary Metabolites		Test Observation
	Present	Absent	
Saponins	++		Layer of foam Present
Flavonoids	+	-	No Change
Alkaloids	-	+	No Change
Steroids	+++		Green color shown
Tannins	++		Brownish green color shown
Terpenoids	++		Reddish brown color appeared

The results of the phytochemical analysis of leaves of *A. vera* can be found from above table. For medicinal values of this plant lies in some chemical substances that have a definite physiological action on human body. The

most important of these bioactive constituents of the plant are alkaloids, terpinoids, tanins, steriods, flavonoids, saponins. The results have showed that saponins, terpinoids, steroids and tanins are present in aqueous extract from leaves. The saponins in the pharmaceutical industry are used as adjuvants to enhance absorption of other drugs to high the solubility. In aqueous solution form foam, this property comes from the aglycone, lipophilic part and sapogenin, hydrophilic moiety. Among other mechanisms of action of some saponins, it is the cell lysis ability, exhibit action on membranes, being capable of causing the disruption of the membranes of blood cells, conferring hemolytic action. It can complex with steroids and, therefore, giving it the antifungal and hypocholesterolemic action.

Tannins which have bactericidal and fungicidal due to its complexation of proteins, in which they explain their influence on the control of insects, fungi and bacteria. Recent studies have shown that Tanin is capable of capturing free radicals, which interested the active oxygen, to form stable radicals. They affect the locking of lipid peroxidation in leaves mitochondria, blocking leukocyte lipooxygenase and suppression the formation of superoxide of anion radicals. Thus, tanins have a role in preventing and treating diseases caused by lipid peroxidation.

Terpinoids show the analgesic properties. Terpinoids are substances in volatile oils and having medicinal properties such as anti-inflammatory, bacterial, fungicidal, antiviral, cardiovascular and antitumor. Steroid reduces redness and swelling. This can help with inflammatory conditions such as asthma and eczema. Steroids also reduce the activity of the immune system, which is the body's natural defense against illness and infection.

Antibacterial Activity using well Diffusion Method

Filter paper disc diffusion bioassay obtained from various fractions of *A. vera* leaves gel (Table 2 & 3) revels that the effect was varied significantly against various organism. Ethanolic fraction of *A. vera* leaves gel was most effective against the tested organisms than methanolic fraction. Maximum inhibition was recorded against *B. cereus* which was followed by *B. brevis*, *Enterobacter aerogenes* and *E. coli* when ethanolic fraction was applied. Minimum antibacterial activity of both ethanolic and methanolic fraction was observed against *S. typhi*. Ethyl acetate and acetone fraction was most effective against *B. brevis* and least effective against *S. typhi*. Aqueous extract of *A. vera* gel exhibited its maximum inhibition activity against *E. coli* followed by *B. brevis* and *B. cereus* while minimum activity was recorded against *Aeromonas hydrophilla*.

Methanolic fraction also showed maximum activity against *S. cerevisiae*. Ethyl acetate, ethanolic and acetone fraction exhibited some moderate activity against *S. cerevisiae*. Aqueous extract was least effective against *S. cerevisiae*.

Methanolic fraction obtained from the extract of *A. vera gel* was found to be most effective against a wide range of Gram positive and Gram negative bacteria in case of disc diffusion method. Data presented in Table 5 clearly indicates that methanolic fraction displayed maximum activity against *B. cereus* which was followed by *Enterobacter aerogenes* and *B. brevis*. Acetone fraction of *A. vera* gel also showed good activity against tested bacterial strains which was maximum in *B. cereus* followed by *Enterobacter aerogenes* and *B. brevis*. Minimum activity of both the fraction recorded against *S. typhi*. Both ethyl acetate and ethanolic fraction were highly effective against *B. cereus* and *B. brevis* respectively while least activity of these fractions was recorded against *Vibrio cholerae*. Aqueous fraction of *A. vera*flower display best activity against B. cereus while it was least against *E. facalis*.

More or less all the fraction also exhibited remarkable activity against *S. cerevisiae* which showed inhibitory activity against all the test extracts. Activity of methanolic fraction was maximum against *S. cerevisiae*. Poor activity was shown by acetone extract only against S. cerevisiae (11.00mm).

Table 2: Antimicrobial activity of extract of *A. vera* leaves gel on some bacteria and yeast through WDM

S.N.	Test micro organism	Acetone	MeOH	EtOAc	EtOH	D/W	Ref.
1	*Aeromonas hydrophilla* (MTCC 966)	11.00±0.05	12.00±0.00	11.00±0.02	15.0±0.21	9.80±0.02	38 ± 0.04
2	*B. cereus* (MTCC 633)	24.00±0.02	19.50±0.83	26.00±0.30	28.50±0.00	13.60±0.00	40 ± 0.07
3	*B. brevis* *	23.20±0.10	25.83±0.02	26.00±0.30	28.00±0.00	20.0±0.05	32 ± 0.04
4	*Enterobacter aerogenes* *	20.00±0.00	23.50±0.00	17.00±0.08	25.50±0.30	14.16±0.04	34.8 ± 0.07
5	*E. facalis* *	18.50±0.15	18.20±0.08	ND	18.50±0.30	10.16±0.07	35 ± 0.07
6	*Escherichia coli* (MTCC 1591)	17.16±0.09	20.00±0.07	16.10±0.04	21.00±0.09	15.16±0.33	36 ± 0.02
7	*Klabsiella pneumoniae* (MTCC 2405)	-	-	-	16.00±0.02	ND	30.01±0.02
8	*Salmonella typhi* (MTCC 531)	-	10.16±0.03	16.33±0.02	10.50±0.15	ND	28.15±0.00
9	*Vibrio cholerae* (MTCC 1168)	13.67±10.00	10.0±0.40	10.00±0.09	14.50±0.00	ND	26.18±0.00
10	*Salmonella. dysentery* *	-	-	-	-	-	24.58±0.33
11	*Candida albicans* MTCC 1022	-	-	-	-	-	NT
12	*Saccharomyces cerevisiae* MTCC1732	12.00±0.08	15.00±0.22	10.00±0.21	11.00±0.26	10.00±0.07	NT

- Data are multiple of three replicates
- Values ± standard error (SE)
- WDM: Well diffusion method
- Ref.: Reference antibiotic
- ND: Not detectable, -: No activity, NT: Not tested

Table 3: Antimicrobial activity of extracts of *A. vera* gel on some bacteria and yeast through DDM

Test micro organism	Acetone	MeOH	EtOAc	EtOH	D/W	Ref.
Aeromonas hydrophilla (MTCC 966)	10.50±0.08	13.83±0.00	ND	ND	9.00±0.28	38 ± 0.04
B. cereus (MTCC 633)	25.5 ± 0.99	30.12±0.02	20.0±0.14	20.16±0.02	18.00±0.01	40 ± 0.07
B. brevis *	18.50±0.02	21.00±0.10	17.16±0.15	17.63 ± 0.11	16.00±0.05	32 ± 0.04
Enterobacter aerogenes *	22.83±0.10	23.00±0.07	20.83±0.84	15.83 ± 0.20	11.50±0.30	34.8 ± 0.07
E. facalis *	11.83±0.25	15.50±0.01	13.6±0.33	10.5±0.00	11.83±0.07	35 ± 0.07
Escherichia coli (MTCC 1591)	19.00±0.00	22.63±0.22	18.00±0.14	19.0±0.12	16.16±0.01	36 ± 0.02
Klabsiella pneumoniae (MTCC	-	-	-	-	-	30.01±0.02

2405)						
Salmonella typhi (MTCC 531)	10.0±0.04	11.83±0.00	-	-	-	28.15±0.00
Vibrio cholerae (MTCC 1168)	ND	12.50±0.10	ND	8.50±0.04	-	26.18±0.00
S. dysentery *	-	ND	-	-	-	24.58±0.33
Candida albicans (MTCC 1022)	-	13.20±0.20	-	11.60±0.20	-	NT
Saccharomyces cerevisiae (MTCC1732)	11.50±0.15	17.67±0.15	17.0±0.36	18.00±0.11	14.0±0.01	NT

- Data are multiple of three replicates
- Values ± standared error (SEM)
- DDM: Disc diffusion method
- Ref.: Reference antibiotic
- ND: Not detectable, -: No activity, NT: Not tested

It was determined from the findings of the phytochemical study that the by-products of A. vera included a variety of phytochemicals. According to the findings, the phytochemical content of the gel extract of *A. vera* demonstrated that the phytochemical content is strongly dependent on the extraction techniques [16, 17]. Tannin content, saponins, phenolics, flavonoids, and terpenoids were found to be considerably present in our research, as evidenced by the findings of our investigation.

CONCLUSION

Results obtained through antibiotic bioassay reveals that ethanolic fraction of *A. vera* exhibited maximum inhibition against *E. coli* followed by *B. cereus*. Similar trend was also observed against yeast cells and was maximum against *S. cerevisiae*. Methanolic extract of *A. vera* leaves was found to be effective against a wide range of bacterial strains which was most pronounced against *B. cereus*. Activity of this extract was also good against *S. cerevisiae*. It was observed that well diffusion method was more sensitive method than others for evaluating antimicrobial activities because there is a better contact and diffusion of the extracts into the media and organisms but filter paper disc may act as barrier between the extract and the organisms. There may not be proper diffusion and total release of active compounds absorbed by the disc into the media.

In this present study an attempt is made to investigate the phytochemical analysis in aqueous solvent extract of *A. vera*. Qualitative analysis of *A. vera* plant confirms the presence of various phytochemicals like terpenoids, saponins, tannins, steroids etc. in aqueous extracts of its leaves gel. The phytochemical ingredients in the screened plant seemed to have the potential to act as a source of useful drugs. Also these ingredients help to improve the health status of the people due to the presence of various compounds therein that are essential for good health. The plant used in our research showed that the *Aloe vera* species possess a good quality of phytochemicals which have ornamental purposes to help directly or indirectly in the health maintenance of living beings.

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