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Sonali D Borkar
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science & A.H., Durg, Chhattisgarh, India

Manju Roy
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science & A.H., Durg, Chhattisgarh, India

Shubham Kumar Singh
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science & A.H., Durg, Chhattisgarh, India

SG Jadhav
Department of Veterinary Parasitology, Nagpur Veterinary College, Maharashtra, India

Corresponding Author:
Sonali D Borkar
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science & A.H., Durg, Chhattisgarh, India

Phytochemical screening and *in vitro* antioxidant potential of aqueous extract of *Aloe vera* leaves

Sonali D, Borkar, Manju Roy, Shubham Kumar Singh and SG Jadhav

Abstract
Aloe vera L. is a semi-tropical plant with many medicinal applications including antibacterial, anti-inflammatory, wound healing, antioxidant and anticancer properties. The leaves are good source of volatile compounds, organic acids, phenols, enzymes, minerals etc. while the rind contains. This study evaluated the phytochemicals and reducing power and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities from aqueous extracts of *Aloe vera* leaves. The aqueous extracts of *Aloe vera* leaves were extracted and analyzed by HRLC-MS for phytoconstituents and In-*vitro* antioxidant activities (reducing power activity and DPPH free radical scavenging activity) were also evaluated using standard methods. Results obtained revealed the presence of phenols, tannins, and flavonoids having antimicrobial, anti-inflammatory and antioxidant activities. The aqueous extract of *Aloe vera* leaves produced DPPH free radical scavenging activity (IC_{50} = 700 µg/ml) compared to the control.

Keywords: Antioxidant, extract, leaves, phytochemical, therapeutic

1. Introduction
According to the World Health Organization, in developing countries, mankind relies on medicinal plants for the healthcare systems (Dar *et al*., 2017) [5]. The beneficial properties ascribed to plants have recognized and medicinal plant treatment is based on the experimental findings of several years. Most of the important drugs of the past 50 years, used in medicinal practice, have been isolated from plants.

Oxygen, being essential for life but also aggravates the damage within the cell by oxidative events. Free radicals generate during the metabolic processes in the human system leads to damage of cellular membranes, cellular organelles, and biomolecules leading to impairment of normal cellular functioning (Phaniendra *et al*., 2015) [17] hastening and development of several health disorders including Alzheimer, Parkinson, acute renal failure, cancer, diabetes, cardiovascular diseases ageing and many more (Gupta *et al*., 2015) [8].

Nature contains varieties of natural antioxidants help to combat oxidative stress by different mechanism of actions. Antioxidants function as scavengers of the free radical, thereby minimizing the deleterious effects of free radicals on organisms (Kornienko *et al*., 2019) [10]. Overproduction of oxidants causes the pathogenesis of some diseases, hence, exogenous supplementation of antioxidants is recommended to meet the adequate requirement. Antioxidant molecules donate an electron to reactive species, preventing the radical chain reaction, which prevents the formation of reactive oxidants, or behave as metal chelators, oxidative enzyme inhibitors, or enzyme cofactors (Bose *et al*., 2017) [3].

Many plants have bioactive properties due to presence of polyphenols, flavonoids, alkaloids, stilbenes, and terpenes function as natural antioxidants (Prasthakumar *et al*., 2021) [18]. Phenolic compounds are believed to confer antioxidant and anti-inflammatory activities in-vitro and in-vivo. Earlier researches reported that the presence of bioactive compounds can be affected with the solvent used in plant extraction (Mann *et al*., 2019) [13].

Aloe vera, the genus Aloe belonging to family Alliaceae is a succulent perennial herb also called wonder plant with numerous health benefits. The plant is considered as skin healer and is highly effective against allergic reactions, rheumatoid arthritis, acid indigestion, ulcers, inflammatory conditions of the digestive system and other internal organs. It acts as a natural fighter against all sorts of infection, an efficient anti-oxidant (Sharrif Moghaddasi and Res, 2011) [23]. Present study conducted for the characterization phytochemicals and investigated the usage of *A. vera* aqueous extract as a scavenger of free radicals.

2. Materials and Methods

The present study was carried out at College of Veterinary Science & AH, located in Durg district of Chhattisgarh, India lays between 20°54' and 21°32' north latitude & 81°10' and 81°36' east longitude, during October, 2022 –September, 2023.

2.1 Chemicals and reagents

All the chemicals of analytical grade were procured from Sigma-Aldrich (St. Louis, MO), Merck (Kenilworth, USA), Loba Chemie (Colaba, India), Qualigens Fine Chemicals (Mumbai, India) and SRL (Gurugram, India). All solutions were prepared in sterile Milli-Q water and double distilled water. To prevent photochemical reactions, all solutions were prepared in fresh with double-distilled water and stored in the dark. Before use, all glassware used in the experiments was carefully cleaned with double-distilled water and dried in a hot air oven.

2.2 Preparation of *Aloe vera* leaf extract

Fresh fully grown healthy leaves of *Aloe vera* were procured in and around the campus of College of Veterinary Science & A. H., D.S.V.C.K.V. Anjora, Durg (C.G.), India and authenticated. The leaves were shade-dried for 4 hrs at room temperature after being washed several times with distilled water to eliminate foreign materials. A colloidal solution was prepared using 10g of ground leaves in 100 ml deionized distilled water, covered with aluminium foil and left overnight at room temperature. Suspension was allowed to boil at 50 °C for 20 minutes with constant stirring. After cooling, the solution was subjected to centrifugation at 6000 rpm for 15 minutes; supernatant was filtered by using filter paper (Whatman No.1) and stored at 4 °C for further use.

2.3 Determination of Plant extract yield

The extraction yield of Aloe crude extract was calculated by the following equation: (Abbas, *et al*. 2021) [1].

$$\text{Yield (\%)} = \frac{\text{Weights of solvent free extract (gram)}}{\text{Dry weight of sample (gram)}} \text{x } 100$$

2.4. Phytochemical screening by FTIR spectroscopic analysis

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Fourier transform infrared (FT-IR) spectroscopically analyzed by Bruker-FTIR Spectrophotometer, IIT Bombay to characterize and determine the functional groups of biomolecules.

2.5 Phytochemical characterization by HRLC-MS analysis of plant fraction

HPLC–MS analysis for antioxidant and other bioactive components was performed on a TOF/Q-TOF Mass Spectrometer, (Agilent Technologies). Data acquisition and processing was accomplished using Agilent 6200 Series.

The *Aloe vera* extract was undertaken HPLC–MS characterization using the metabolite_ESI_+VE_MS/MS method to rapidly and qualitatively analyze its components. Antibacterial and antioxidant elements were analyzed via HPLC–MS on an Agilent TOF/Q-TOF Mass Spectrometer, allowing for less sample preparation and purification, reducing overall analysis time. Parameters included an injection volume of 10 µL, desorption liquid temperature at 250 °C, and specific settings for thresholds, nebulizer, and purity stringency. The analysis recorded full mass scan spectra in negative ionization mode within the range of m/z 120–1200. The method identified phytochemicals based on retention time, mass spectra, and comparison with established compound databases. The detection limit for *Aloe vera* in extracts was approximately 1 ng ml (-1) or 10 pg in SIM mode, significantly lower than existing HPLC methods in the literature (approximately 200 ng ml (-1) or 10 ng).

2.6. DPPH free radical scavenging assay

The antioxidant activity of an aqueous extract of *Aloe vera* was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay based on Brand-Williams' methodology (1985). In this assay, varying concentrations of the extract (0.2, 0.4, 0.6, 0.8, or 1 mg/mL) were mixed with a methanolic DPPH solution and incubated in the dark at 37 °C for 30 minutes. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer against a methanol-DPPH blank. The percentage of DPPH scavenging effect was calculated by the equation i.e. DPPH scavenging effect (%)/% Inhibition=A0-A1/A0×100 (Kumara and Kumar, 2018) [11]. A_0 is the absorbance of the control and A_1 is the absorbance of test. The IC_{50} value is the quantity of antioxidant required to eliminate half of all free radicals in the body.

3. Results and Discussion

3.1. Percentage yield of Aloe leaf extract using aqueous extraction method

The yield for aqueous extract of *Aloe vera* was 14.16%. The extracts were stored in tightly sealed dark containers in a freezer at −20 °C for later use. In aqueous medium good yield might be due to the fact that most of the phytochemicals bear electronegative functional groups which make the compound hydrophilic in nature (Abbas *et al*., 2021) [1].

3.2. Phytochemical screening by FTIR spectroscopic analysis of Aloe leaf extract

The major active biocompounds of the ALE were investigated and evaluated using Fourier transform-infrared spectroscopy (Fig 1).

The spectra have prominently indicated towards the presence of hydroxyl (O-H stretching, 3437.27 cm-1). Furthermore, a central peak is attributed to the amino groups present in alcohol, phenol, and amines in the *Aloe vera* (C=C stretching, 1619.30 cm-1).

Peaks at aromatic (C-O/C-H bending, 1420.61 cm-1), geminal methyl groups (C-H bending, 1319.08 cm-1), esters and ether (C-O stretch, 1049.15 cm-1) are representative of phytocompounds in Aloe leaves extract (Rasli *et al*., 2020; Fardsadegh and Jafarizadeh 2019) [7].

These phytochemicals include polysaccharides, flavonoids, carbohydrates, coumarins, tannins, chromones, alkaloids, anthraquinones, organic compounds, pyrones, phytosterols, anthrones, sterols, vitamins, proteins, and mineral constituents (Salehi *et al*., 2018) [20].

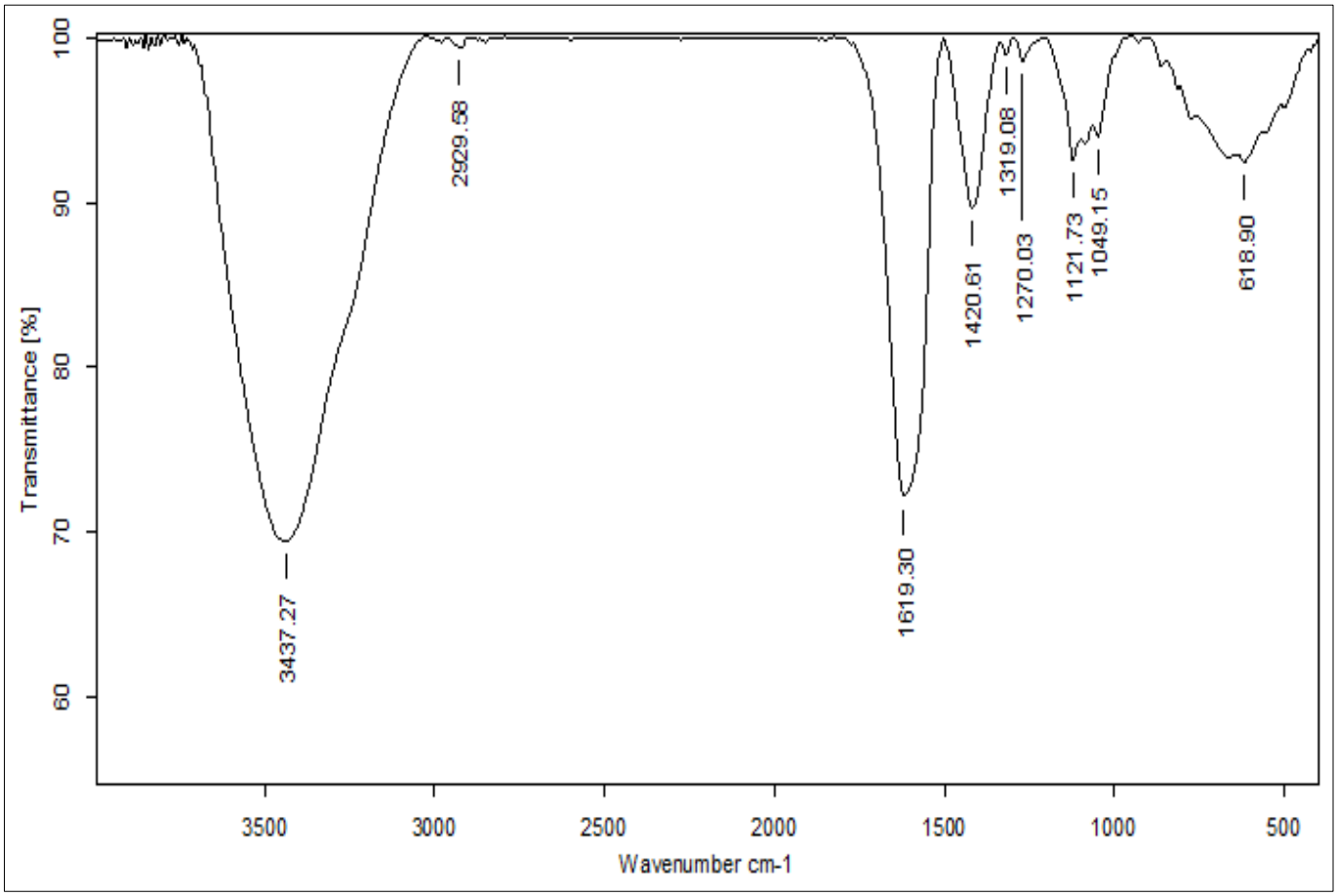


Fig 1: FTIR spectra for aqueous extract of *Aloe vera* leaves.

These species have been reported to have pharmacological activities including anti-inflammatory, immunomodulatory, antibacterial, antifungal, antiviral, anti-proliferative, anti-diabetic, laxative, wound healing, moisturizing, anti-aging, and skin protection (Banik and Sharangi, 2018) [2].

3.3 DPPH (1,1-diphenyl- 2-picryl hydrazyl) free radical scavenging assay

The results of the HPLC-MS studies confirm the presence of a wide array of phytoconstituents with antioxidant properties.

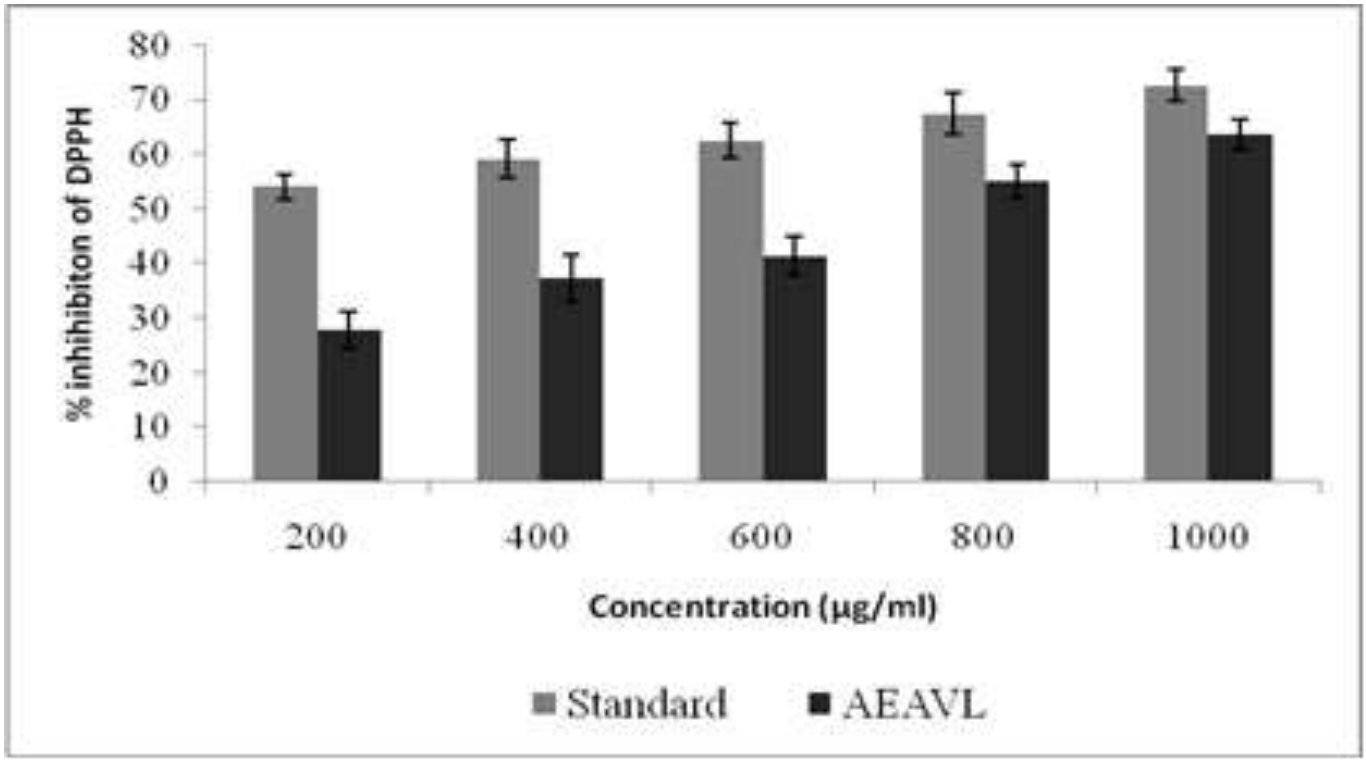


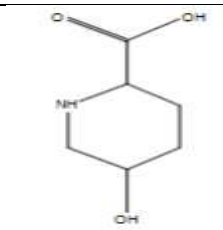
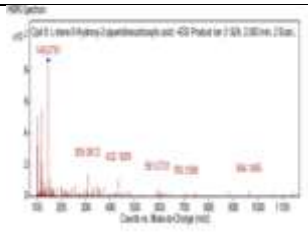
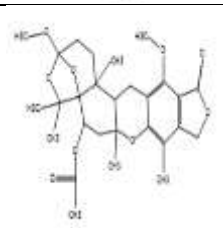
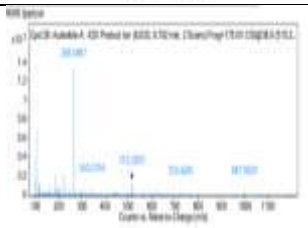
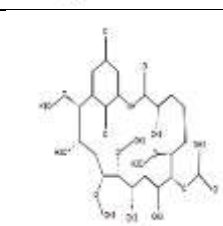
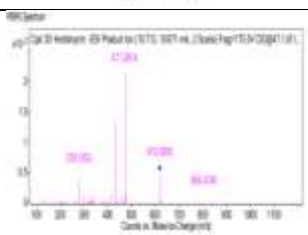
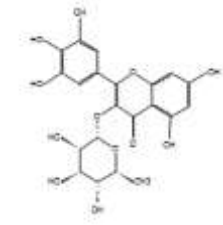
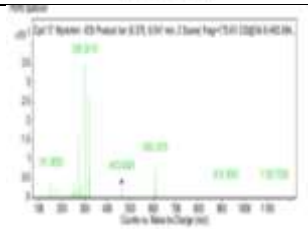
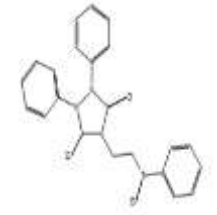
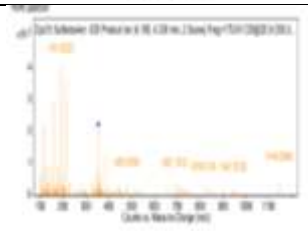
Fig 2: Percentage inhibition of DPPH free radical by Standard (Ascorbic acid) and Aqueous extract of *Aloe vera* leaves (AEAVL)

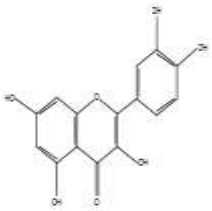
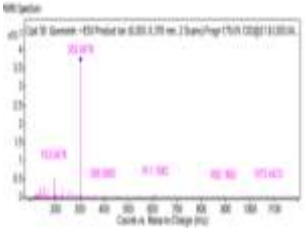
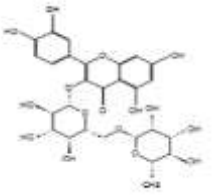
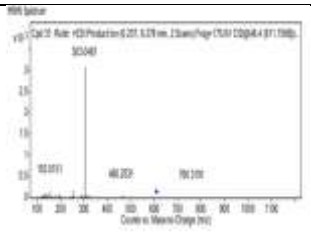
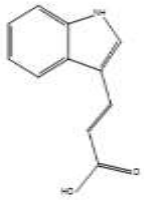
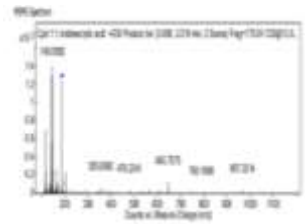
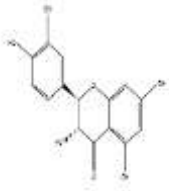
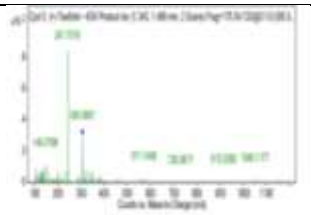
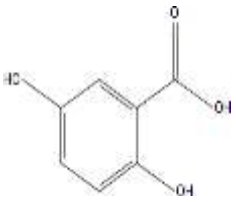
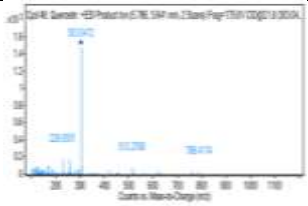
The reactivity of the aqueous extract was analyzed with 2, 2-Diphenyl-1-Picryl hydrazyl, a stable free radical which is got reduced by accepting hydrogen or electron from the donor molecule. Free radical scavenging activity of extract and standard ascorbic acid was expressed as percentage inhibition. The percentage DPPH inhibition activities of aqueous extract of *A. vera* was 55.05% at 800 µg/ml as compared to 54.1% at 200 µg/ml with standard (Ascorbic acid) (Fig. 2). As DPPH takes up one electron in the presence of a free radical scavenger, the absorption reduces and the resulting discoloration is stechiometrically related to the number of electrons gained (Thorat, 2013) [24]. Plant polyphenolic chemicals have been found to have potent antioxidant capabilities that help to protect cells from the oxidative stress caused by free radicals. Ascorbic acid, used as positive control which showed high percentage inhibition of free radicals about 72.68% at 1000 µg/ ml as compared to 63.73% with aqueous extract of *Aloe vera*. Hes *et al*., (2019) [9] reported that Aloe gel inhibited the generation of DPPH radical and its IC_{50} value was found to be 572.14 µg/mL, which is the concentration of substrate that causes 50% loss of the DPPH activity (colour).

3.4 HPLC- MS profiling of aqueous extract of *Aloe vera* leaf

In Table 1, the phytochemical screening of the *Aloe vera* revealed the presence of phenolics and tannins in aqueous extract. The LC-MS-based untargeted analysis showed the phytochemical profile of the leaves of *Aloe vera* in which phytochemicals from different chemical classes were annotated, including organic acids, phenolic acids, flavonoids, and other compounds and ESI-MS fingerprint spectra of above discussed phytochemicals from the leaves of *Aloe vera* with wide significance were postulated.

Table 1: The phytochemical content was determined by using HPLC- MS analysis revealed several peaks in aqueous extract of *Aloe vera*.

Name of compound	Structure	Bioactivity with reference	MSMS Zoomed Spectrum	m/z	RT value	Mol.wt.
L-trans-5-Hydroxy-2-piperidinecarboxylic acid		Serve a role as defense or signalling molecules (Yannai 2004) [28].		146.0801	2.004	145.0728
Austalide A		Antimicrobial and anti-inflammatory potential (Chen *et al*. 2015) [4]		515.2208	8.711	16.2281
Herbimycin		Displays antitumour effects, antimicrobial agent and an apoptosis inducer (NCBI 2023) [15].		619.2852	10.792	574.287
Myricitrin		a flavonoid found in various plants, exhibits antioxidant, anti-inflammatory and antifibrotic activity (Domitrović *et al*. 2015) [6].		463.0947	6.458	464.1019
Sulfadoxine and Sulfadimethoxine		These are both antimicrobial agents used in the treatment of malaria and bacterial infections (Oliveira *et al*. 2022) [16].		355.0724	4.263	310.0745

Quercetin		a flavonoid found in many fruits, vegetables, leaves, and grains. It's being researched for its potential antioxidant and anti-inflammatory effects (Li *et al*. 2016) [12].		303.0476	5.864	302.0404
Rutin		anti-tumor activities, reduction of inflammatory cytokines and antimicrobial activities (Shanmugasundaram and Roza, 2022) [22]		611.1568	6.293	610.1496
Indole-acrylic acid		promotes intestinal epithelial barrier function, mitigates inflammatory responses and immune system modulating properties (Wlodarska *et al*. 2017) [27]		188.0696	3.143	187.0623
(+)-Taxifolin		A flavonoid antioxidant (Topal *et al*. 2016) [16].		305.0616	1.709	304.0538
Gentisic acid		anti-inflammatory, antirheumatic and antioxidant (Mardani-Ghahfarokhi and Farhoosh, 2020) [14]		153.0209	2.014	154.0281

The plant has demonstrated numerous health benefits, including skin problems (burns, wounds, and anti-inflammatory processes).

Moreover, *Aloe vera* has shown other therapeutic properties including anticancer, antioxidant, antidiabetic, and antihyperlipidemic (Sanchez *et al*., 2020) [21]. Therefore, in this study, the phytochemical constituents by high-performance liquid chromatography analysis and antioxidant property of aqueous extracts of *Aloe vera* leaf were investigated with both positive and negative ion mode to assess the potential protective benefits of this plant against degenerative reactions induced by free radicals. The phytocompounds identified were of pharmacological importance. The saponins, flavonoids and alkaloids were only present in the aqueous extract, while steroids were only present in the ethanol extract (Zhang *et. al*., 2018) [29].

5. Conclusion

It is evident from the present study that the *A. vera* aqueous extract could be utilized as a good source of antioxidants in pharmaceutical industry and a primary platform for further pharmacological studies to discover therapeutic targets.

6. References

1. Abbas A, Naqvi SAR, Rasool MH, Noureen A, Mubarik MS, Tareen RB. Phytochemical analysis, antioxidant and antimicrobial screening of *Seriphidium oliverianum* plant extracts. Dose-response: a publication of the International Hormesis Society. 2021;19(1):15593258211004739.
2. Banik S, Sharangi AB. Phytochemistry, health benefits, and toxicological profile of Aloe. J Pharmacogn. Phytochem. 2019;8(3):4499-4506.
3. Bose B, Choudhury H, Tandon P, Kumaria S. Studies on secondary metabolite profiling, anti-inflammatory potential, *in vitro* photoprotective, and skin-aging-related enzyme inhibitory activities of *Malaxis acuminata*, a threatened orchid of nutraceutical importance. J Photochem. Photobiol. B: Biol. 2017;173:686-695.
4. Chen CJ, Zhou YQ, Liu XX, Zhang WJ, Hu SS, Lin LP, *et al*. Antimicrobial and anti-inflammatory compounds from a marine fungus *Pleosporales* sp. Tetrahedron Lett. 2015;56(45):6183-6189. https://doi.org/10.1016/j.tetlet.2015.09.079
5. Dar RA, Shahnawaz M, Qazi PH. Natural product medicines: A literature update. J Phytopharmacol. 2017;6(6):349-351.
6. Domitrović R, Rashed K, Cvijanović O, Vladimir-Knežević S, Škoda M, Višnić A. Myricitrin exhibits antioxidant, anti-inflammatory, and antifibrotic activity in carbon tetrachloride-intoxicated mice. Chem. Biol.

Interact. 2015;230:21-29.
7. Fardsadegh B, Jafarizadeh H. *Aloe vera* leaf extract mediated green synthesis of selenium nanoparticles and assessment of their *in vitro* antimicrobial activity against spoilage fungi and pathogenic bacteria strains. Green Process. Synth. 2019; 8:399-407.
8. Gupta P, Authimoolam SP, Hilt JZ, Dziubla TD. Quercetin conjugated poly (β-amino esters) nanogels for the treatment of cellular oxidative stress. ActaBiomate. 2015;27:194-204.
9. Hes M, Dziedzic K, Górecka D, Jędrusek-Golińska A, Gujska E. " *Aloe vera* (L.) Webb.: Natural Sources of Antioxidants – A Review. Plant Foods for Human Nutrition. 2019;74:255-265.
10. Kornienko JS, Smirnova IS, Pugovkina NA, Ivanova JS, Shilina MA, Grinchuk TM, *et al*. High doses of synthetic antioxidants induce premature senescence in cultivated mesenchymal stem cells. Sci. Rep. 2019;9(1):1296.
11. Kumara PK, Kumar BA. Determination of DPPH Free Radical Scavenging Activity by RP-HPLC: Rapid Sensitive Method for the Screening of Berry Fruit Juice Freeze Dried Extract. Nat. Prod. Chem. Res. 2018;6(5):1-7.
12. Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, *et al*. Quercetin, Inflammation and Immunity. Nutrients. 2016;8(3):167. https://doi.org/10.3390/nu8030167.
13. Mann AA, Nazir A, Khan MKI, Ahmed T, Zia, V, Murid, M, *et al*. The therapeutic properties and applications of Aoe vera: a review. J Herb. Med. 2019;12:1-10.
14. Mardani-Ghahfarokhi A, Farhoosh R. Antioxidant activity and mechanism of inhibitory action of gentisic and α-resorcylic acids. Sci. Rep. 2020;10(1):19487.
15. National Center for Biotechnology Information. PubChem Compound Summary for CID 5311102, Herbimycin A. Retrieved October 30, 2023, from https://pubchem.ncbi.nlm.nih.gov/compound/Herbimycin-a.
16. Oliveira IS, Manzano CM, Nakahata DH, Santiago MB, Silva NBS, Martins CHG, *et al*. Antibacterial and antifungal activities *in vitro* of a novel silver(I) complex with sulfadoxine-salicylaldehyde Schiff base. Polyhedron. 2022;225:116073.
17. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin. Biochem. 2015;30(1):11-26.
18. Prasathkumar M, Anisha S, Dhrisya D, Beck R, Sadhasivam S. Therapeutic and pharmacological efficacy of selective Indian medicinal plants – A review. Phytomed. Plus. 2021;1(2):100029.
19. Rasli W, Basri H, Harun Z. Zinc oxide from *Aloe vera* extract: Two-level factorial screening of biosynthesis parameters. Heliyon. 2020;6:e03156.
20. Salehi B, Albayrak S, Antolak H, Kręgiel D, Pawlikowska E, Sharifi-Rad M, *et al*. Aloe genus plants: from farm to food applications and phyto pharmacotherapy. Int. J Mol. Sci. 2018;19(9):2843.
21. Sanchez M, Gonzalez-Burgos E, Iglesias I, Gomez-Serranillos MP. Pharmacological Update Properties of *Aloe vera* and its Major Active Constituents. Molecules (Basel, Switzerland). 2020;25(6):1324-1332.
22. Shanmugasundaram D, Roza JM. Assessment of anti-inflammatory and antioxidant activity of quercetin-rutin blend (SophorOx™) - an *in vitro* cell-based assay. J Complement. Integr. Med. 2022;19(3):637-644.
23. Sharrif Moghaddasi M, Res M. *Aloe vera* their chemicals composition and applications: A review. Int J Biol Med Res. 2011;2(1):466-471.
24. Thorat I. Antioxidants, their properties, uses in food products and their legal implications. Int J Food Stud. 2013;2:81-104.
26. Topal F, Nar M, Gocer H, Kalin P, Kocyigit UM, Gülçin İ, *et al*. Antioxidant activity of taxifolin: an activity-structure relationship. J Enzyme Inhib. Med. Chem. 2016;31(4):674-683. https://doi.org/10.3109/14756366.2015.1057723
27. Wlodarska M, Luo C, Kolde R, d'Hennezel E, Annand JW, Heim CE, Krastel P, *et al*. Indoleacrylic Acid Produced by Commensal Peptostreptococcus Species Suppresses Inflammation. Cell Host Microbe. 2017;22:25–37.
28. Yannai S. Dictionary of Food Compounds with CD-ROM: Additives, Flavors, and Ingredients. Boca Raton: Chapman & Hall/CRC; c2004.
29. Zhang Y, Bao Z, Ye X, Xie Z, He K, Mergens B, *et al*. Chemical investigation of major constituents in *Aloe vera* leaves and several commercial aloe juice powders. J AOAC Int. 2018;101:1741-1751.