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Preliminary Phytochemical, Isolation, Characterization, Antioxidant, and Antimicrobial Activity Assessments of Achyranthes aspera L

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The study evaluated the preliminary phytochemical properties, isolation, characterization, antioxidant, and antimicrobial activities of the methanol extract of *Achyranthes aspera* Linn. The plant material was collected, extracted, and subjected to thin-layer chromatography and preliminary phytochemical analysis. The antioxidant and antibacterial properties of AAME were assessed using *in-vitro* techniques, including the 2,2-diphenyl-1-picrylhydrazyl assay and the agar gel plate method. Bioactive compounds were separated and characterized using Fourier-transform infrared spectroscopy. The Rf values were obtained 0.74, 0.68, 0.19, 0.20, 0.24, 0.36, 0.44 and 0.64, these Rf values data reference sources indicated that these bioactive compounds are present in extract such as ferulic acid, caffeic acid, oleanolic acid, ursolic acid, Flavonoid-VII, Phenolic acid-VI, Flavonoid-III, and Flavonoid-I. After the Rf values confirmation were isolated a compound through the TLC method that was found to be derivatives of quercetin (Flavonoid-III). Isolated compound was confirmation using FTIR interpretation results. Additionally, the presence of the results demonstrated that AAME exhibited significant antioxidant activity, comparable to standard ascorbic acid. Moreover, its antibacterial activity against infection-causing bacterial strains was found to be comparable to that of a standard drug. These findings suggested that AAME, in its entirety, possesses potent antioxidant properties and could serve as a valuable natural source of antibacterial and antioxidant agents for topical applications.

Keywords: *Achyranthes aspera*, Antioxidant, antibacterial, flavonoid content, phenolic content.

Naturally occurring substances have undergone a great deal of research and have shown fascinating biological and pharmacological properties, which indicates that they could be used as chemotherapeutic agents or as the building blocks for contemporary medications. But in order to use these resources effectively for the sake of human health, more research is required.[1] Because plant-derived pharmaceuticals have significantly enhanced human health and

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well-being, Herbs can serve two purposes in the creation of new medications: (a) they can serve as the model for the creation of natural medications, or (b) they can be used as a phytomedicine to treat diseases. Even while modern medicine has come a long way, there are still numerous illnesses and conditions for which there is no known treatment. Herbal plants are essential to human life.[2-3] As analgesics, antioxidants, antibacterials, antimalarials, and other therapeutic remedies, people have used the bark, berries, leaves, stems, flowers, and roots of plants. Flavonoids, phenolic acids, tannins, stilbenes, lignans, lignins, and coumarins are among the bioactive compounds found in medicinal plants; these compounds have a range of biological effects, such as antibacterial and antioxidant properties.[4] Herbal remedies are frequently used in medical settings. The traditional medical system has long recognized the therapeutic potential of medicinal plants for treating a broad range of ailments. The development of safer drugs to treat inflammatory diseases, skin issues, fungal and microbial infections, diabetes, liver disorders, and gastrointestinal disorders has thus become essential. To this aim, different healthcare programs throughout nations have supplemented each other by separating active ingredients for use in contemporary medications and herbal remedies. secondary metabolites or phytochemicals that are present in medicinal plants and are in charge of their antibacterial, antifungal, and analgesic effects. Nonetheless, certain bacteria and fungi that are harmful to humans have become resistant to bioactive substances like flavonoids' antibacterial and antifungal properties.[5] The increasing prevalence of multiple drug-resistant microorganisms to conventional drugs has led to a growing interest in seeking natural sources of antimicrobial agents. The potential for plant-derived antimicrobial compounds to revolutionize modern medicine is vast and largely untapped, warranting further investigation in this field.

In Indian traditional medicine, *Achyranthes aspera* (*A. aspera*) Linn, commonly referred as chirchira in Hindi, is a source of high therapeutic properties. This native herb has long been used in Ayurvedic, Unani, and Siddha medicine, among other traditional medical systems. *A. aspera* leaves have been shown to have antibacterial qualities,[6] which makes it beneficial for treating conditions involving the skin and teeth. This document lists the traditional uses, folk cures, ethnopharmacologic properties, and ethnopharmacologic properties of this herb. *A. aspera* Linn recently reported many potential activities such as nephroprotective,[7] anticonvulsant,[8] anticancer, [9-10] wound healing activity,[11] anti-inflammatory[12-13] respectively. The annual, stiff, upright herb *A. aspera* Linn is frequently found as a weed all over India. The scientific community has recently become interested in *A. aspera* because of its possible pharmacological effects. Numerous features have been the subject of research, including its anti-inflammatory, anti-oxidative, anti-microbial, and cancer-fighting properties.[14] Studies have also looked into its possible uses in the management of diseases like diabetes, high blood pressure, and gastrointestinal issues. Therefore, we selected *A. aspera* Linn for phytochemical and pharmacological screening to explore its possibilities and therapeutic potential. [Please include previous work published on this plant.

Hypothesis: A. aspera Linn has significant medicinal value due to its wide range of pharmacological properties, which can be further confirmed by pharmacological and phytochemical screening.

The goal is to identify the bioactive components of *A. aspera* Linn by doing a thorough phytochemical investigation, to assess its pharmacological characteristics, such as its antioxidant, antibacterial, and anti-inflammatory effects and to investigate its possible therapeutic uses in the management of long-term illnesses, to examine and gather data from earlier studies on A. aspera Linn in order to provide a scientific foundation for its therapeutic application. This study intends to aid in the creation of innovative herbal-based remedies for a range of illnesses by examining the bioactive components and therapeutic qualities of *A. aspera* Linn.

MATERIAL AND METHODS

Selection procurement of plant materials

A. aspera whole plant was collected from our herbal garden in Ayodhya, Uttar Pradesh, India. The collection was conducted during the flowering stage to ensure maximum phytochemical constituents. A total of 2kg samples were collected

to maintain uniformity in the study. The plant materials were authenticated by Dr. Awadesh Kumar Shukla, Department of Botany, Dr. Ram Manohar Lohia Avadh University, Ayodhya, India, and a voucher specimen was deposited in the herbarium for future reference. The whole plant was selected for preliminary phytochemical screening and antioxidant activity evaluation.

Preparation of extract

The plant materials were gathered, cleaned, dried (or freeze-dried), and powdered to produce a coarse powder for extraction. The entire A. aspera plant was roughly pulverized, sieved twenty times, and allowed to dry for two weeks in the shade. The dry powder was first treated for 24 hours at 60 to 80 °C with petroleum ether to eliminate fatty components. The plant extraction was performed using a Soxhlet apparatus with methanol as the solvent. The extracts were collected using Whatman filter paper and then dried or concentrated using a rotary vacuum evaporator. The yield percentage was determined after storing the extracts in desiccators.[15-16]

Phytochemical Evaluation Parameters

Qualitative Chemical Tests

Qualitative chemical tests were conducted on the plant extract, confirming the presence of alkaloids, glycosides, volatile oils, flavonoids, tannins, etc.[17]

Identification Test (TLC study)

Thin Layer Chromatography (TLC) was performed using conventional procedures. AAME (2 mg/ml) was dissolved in suitable solvents in small quantities. The mobile phase concentrations were adjusted to create solvent systems. The Rf values were determined using a UV-TLC chamber and compared to reference values. The TLC study is shown in Figure 3.[18-20] The Rf values interpreted and were compared to the reference values.

FTIR study

The FTIR study of AAME was performed and identified the occurrence of bioactive compounds and their active groups using Agilent FTIR Cary 630 instruments.[21-23]

Determination of total phenolic content (TPC)

0.5 ml of AAME extract and 2.5 ml of the Folin-Ciocalteu reagent were used to assess the phenolic content of AAME. Then after were added 50 ml of distilled water and 2 ml of a 7.5% sodium carbonate solution. The phenolic content of AAME samples was calculated in milligrams of gallic acid equivalents per gram of AAME using the absorbance at 765 nm. [24-25]

Total flavonoid content (TFC)

TFC was determined using quercetin as a standard. A 2% methanolic solution of aluminum chloride (1 ml) was added to serially prepared test samples of AAME. Then after UV-visible spectrophotometer was used to measure the absorbance at 420 nm following 15 minutes at room temperature. The TFC results were expressed in quercetin equivalents (µg/ml).[26-28]

***In-vitro* antioxidant activity assessment**

Antioxidant activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. A 0.1 mM DPPH solution was prepared in methanol, and the antioxidant determination was performed using the DPPH standard curve. The ascorbic acid and methanol extract test sample stock solution (10 mg/ml) was serially diluted to concentrations of 2, 4, 6, and 8 mg/ml. One milliliter of DPPH solution (0.1 mM) was added to 50 µl of each test sample, followed by the addition of methanol to achieve a final volume of 3 ml. After thorough shaking, the mixture was left to stand at room temperature for 30 minutes. The absorbance was measured at 517 nm using methanol as the reference. Ascorbic acid was used as the standard antioxidant. The percentage of DPPH free radical scavenging activity (FRSA) was calculated using the formula[29-30]:

$$\% \text{ (FRSA)} = (\text{Absorbance of control} - \text{Absorbance of test sample}) \text{ Absorbance of control} \times 100$$

Separation of chemical constituent

A 9.3 g methanolic extract was subjected to liquid chromatography on a silica gel column and eluted using petroleum ether, ethyl acetate, and methanol in increasing polarity. Bioactive compounds, including PAL-1, were obtained from methanol extracts. The structures of these compounds were determined using IR and 1H NMR spectral data. The separation and purification of each component were thoroughly explained in the experimental section. A schematic representation of the isolation procedure is provided in Figure 3.

Most of flavonoid biocompounds are dissolved is ethyl acetate solvent. Therefore

mostly select this solvent. It is semi polar organic solvent due to this reason, easily to evaporate and TLC separation fast. Bioactive compound PAL-2 20mg successfully was isolated (80ethylacetate:10 methanol) and fraction-3 PAL-3 respectively. These isolated bioactive compounds stored properly for the further investigation.[28-30]

Assessment of *In-vitro* antimicrobial activity of AAME

The antimicrobial activity of AAME was evaluated using the agar well diffusion method against Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) bacteria.

Using agar-agar and 8% nutritious broth in double-distilled water, nutrient broth medium (NBM) was created. It was autoclaved for 25 to 30 minutes to sterilize it at 15 Ibs pressure. After growing the bacterial strains in an agar gel petri plate and aseptically adding 15 milliliters of NBM, the plates were left to stabilize at room temperature. Bacterial cell cultures were maintained in peptone saline solution by routine sub-culturing. The cultures were then kept at 37°C for a whole day. [31-34]

Agar plate preparation

Bacterial strains were streaked across the sterile surface of agar plates to spread the inoculum evenly. After curing at room temperature, wells of 9 mm diameter were drilled into the agar plates. AAME at concentrations of 50, 100, 150, and 200 mg/ml was prepared using dimethyl sulfoxide (DMSO) as the solvent. Ofloxacin (10 mg/ml) was used as a standard antibiotic. The plates were incubated at 37°C for 24 hours. The zone of inhibition was measured using a calibrated digital Vernier caliper. Each test was performed in triplicate.[33-34]

RESULTS

It was found that the extract from the AAME stem segment had a 9.31% yield. The methanolic extract of the entire AAME component was semisolid, and all of the extracts had a dark brown color. In phytochemical screening tests, the methanolic extract of AAME was shown to include proteins, amino acids, carbohydrates, glycosides, tannins, saponins, alkaloids, phenolic compounds, and flavonoids.[35–38] Semi-qualitative information on the extract's active elements is provided by the bioactive compounds. A phytochemical analysis revealed a variety of phytoconstituents in the methanolic extract of AAME. Consequently, more in-vivo studies were conducted on AAME.

The TLC study of AAME was studied, calculated Rf vale and interrelated the Rf value. The Rf value represented and found to be Ferulic acid, Caffic acid, Oleanolic acid, Ursolic acid, Flavonoid-VII, Phenolic acid-VI, Flavonoid-III, and Flavonoid-I were present. The TLC values showed that AAME test sample having potential sources of phenol and flavonoid components which revealed that AAME having potential activities.[19-20] The computed Rf values for the various sites were then compared to the reference values from studies. Results are shown in table 1.

The quercetin equivalent of flavonoids was used to calculate the TFC of the AAME. For the concentration of TFC, there are strong linear correlations between the standard and sample extracts. It was found that the TFC was 35.9±0.13µg/ml quercetin equivalent using the aluminum chloride colorimetric method.

The gallic acid equivalent of phenols was used to calculate the TPC of AAME. For the concentration of total phenol, there are strong linear correlations between the standard and sample extracts. It was calculated using the Folin-Ciocalteu method and was found to be 9.9±70.15 µg/ml of equivalent to gallic acid, the results demonstrated that AAME had the greatest potential.

FTIR study of AAME

Figure 4 reports the FTIR spectrum of AAME leaves. The OH group of phenolic compounds is represented by the absorption band at 3438.03 cm–1. It attests to the plant's phenolic component content. The results of AAME are listed in Table 2.

Antioxidant evaluation of AAME Extract

AAME composition was selected based on its distinct in-vitro efficacy, or % free radical scavenging activity. It was shown that AAME whole component extracts have potent antioxidant qualities.[23-24] Table 3 show the findings of the DPPH method's examination of the AAME capacity to scavenge free radicals. When compared to the conventional ascorbic acid, the test samples' comparative antioxidant activity amply demonstrated the impact of AAME.

Separation of chemical constituent

A silica gel column (100-200 mesh) was used to isolate bioactive compounds, were 9.3 gm AAME loaded in column chromatography. It was then eluted using a step-by-step gradient using petroleum ether: ethyl acetate, followed by methanol (90:10, 80:10, 70:20, 60:30, and 50:50 by volume) solvents, as indicated in Table 4. After collecting 25 fractions, the identical fractions check by Thin Layer Chromatography (TLC), and were identical fractions were combined. For the detection of flavonoid bioactive compounds spots were identified by applying a 10% methanol: sulfuric acid spray reagent.[25-27]

Fraction-1

Using the gradient elution technique, the residue (450 mg) from these fractions was collected on a silica gel (100–200 mesh) column using methanol: ethyl acetate (70:30). Methanol, glacial acetic acid, toluene, and ethyl acetate make up the residue on TLC. A single, noticeable pinkish red patch with an Rf value of 0.44 was visible in 3.5:1:0.1:0.1. The Rf value, FTIR interpretation data and reference sources revealed that isolated compounds was derivative of quercetin (Flavonoid-III). Addition ferulic acid, Caffic acid, Oleanolic acid, Ursolic acid, Flavonoid-VII, Phenolic acid-

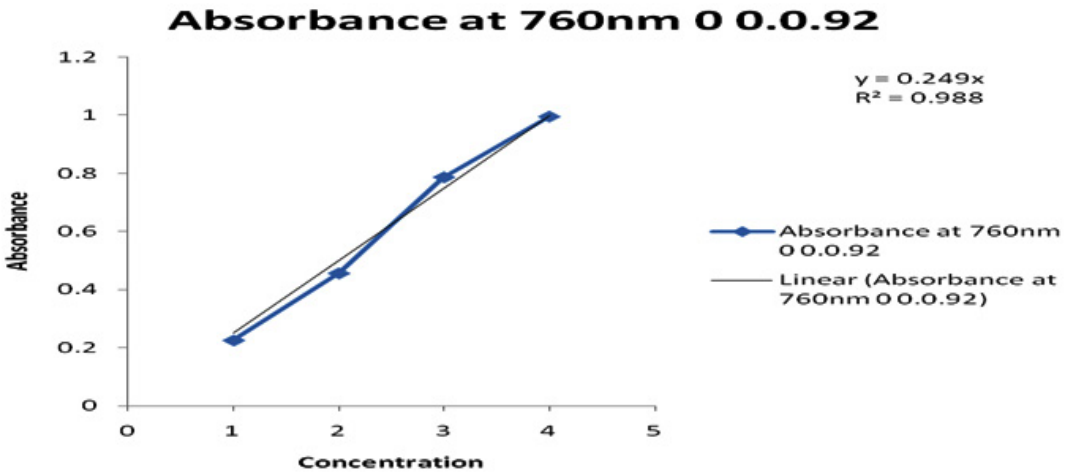

Fig. 1. The standard curve of Gallic Acid

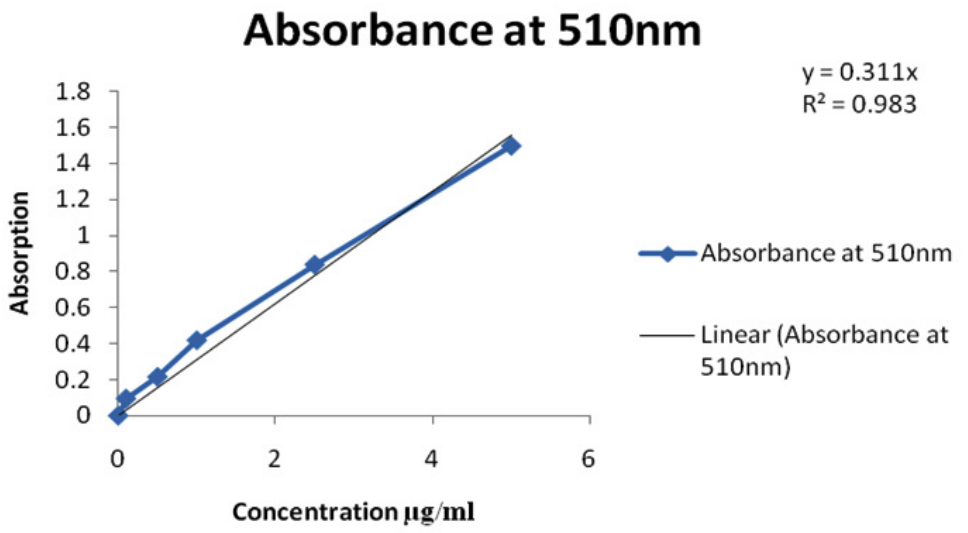

Fig. 2. Quercetin's standard curve

VI, Flavonoid-III, and Flavonoid-I were present in AAME. These results also indicated that AAME whole part having potential activities.[28-30]

Antimicrobial test

The agar well diffusion technique was used to determine the zone of inhibition for the entire AAME. The antimicrobial spectrum demonstrated that AAME has concentration-dependent efficacy against both gram-positive and gram-negative bacteria. When tested against *S. aureus, S. bacillus, E. coli*, and *P. aureginosa* at 50 to 200 mg/ml, the AAME sample was found to be effective (Maximum ZI among all test samples). Comparing AAME to the ofloxacin standard, the results demonstrated its antibacterial activity (Fig. 5). The antibacterial effectiveness of AAME against bacterial infections is shown in Table 8.

DISCUSSION

The amount of alkaloids, tannins, polyphenols, and flavonoids in the AAME was

Table 1. Solvents, spots and Rf values of AAME

Solvents	Spots	Standard Rf value	Rf	Results
Butanol: Glacial Acetic Acid: Water (8:2:10)	2	0.73	0.74	Ferulic acid
		0.69	0.68	Caffic acid
Toluene : EA : Formic Acid (6.7:0.75:0.15)	6	Oleanolic acid 0.19	0.19	Oleanolic acid
		Ursolic acid 0.20	0.20	Ursolic acid
		Derivative of quercetin	0.24	Flavonoid-VII
		Chlorogenic acid	0.36	Phenolic acid-VI
		Derivative of quercetin	0.44	Flavonoid-III
		Kampferol	0.64	Flavonoid-I

Table 2. Analysis of AAME's FTIR spectrum

S.. No	Frequency cm^{-1}	Indication
1	3462	-OH Stretching of bioactive phenol
2	2984	=C-H stretching of aromatic Ring
3	1734	C=O Stretching of secondaryAmide group
4	1447	C=C of aromatic Ring
5	1373	N-H bending of secondry amine
6	1236	C-O Carbonyl group

Table 3. Antioxidant effects of AAME and Ascorbic acid

Sr. No.	Sample	IC_{50}
1	Ascorbic acid	11.10 µg/ml
2	AAME plant methanolic, extract	135.802 µg/ml

"(Mean ± SD, n = 3)"

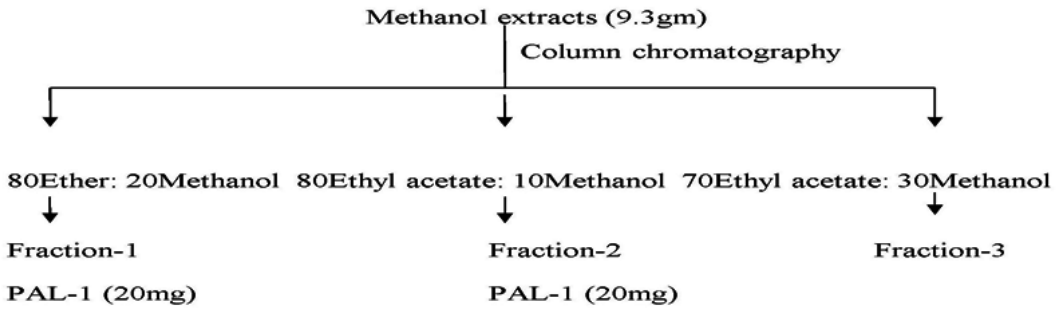

Fig. 3. Chromatographic separation of Ethyl acetate extract of the *Achyranthas aspera plant*

determined using Thin Layer Chromatography (TLC) and validated against reference standards. These bioactive molecules, particularly flavonoids, play a crucial role in shielding biological systems from oxidative stress and damage caused by free radicals. Oxidative stress is a key factor in the

Table 4. Antimicrobial efficacy assessment of AAME

Strains	Extract	Zone of inhibition (in mm), mean ± SEM				Ofloxacin (10µg/ml)
		50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	
S. aureus	AAME	12.50±0.411	20.50±0.414	24.10±0.12	26.00±0.381	31.0±0.661
S. bacillus	AAME	11.29±0.654	21.69±0.420	23.75±0.613	25.00±0.512	33.4±0.750
E. coli	AAME	15.10±0.488	18.20±0.530	22.00±0.450	20.55±0.237	29.50±0.63
P. aureginosa	AAME	16.30±0.537	19.15±0.401	21.50±0.380	23.50±0.510	31.8±0.503

All results are shown in form of mean ± SEM, n = 3. For every bacterial strain, data were evaluated using a two-way ANOVA, Dunnett's multiple comparisons test, and ****p<0.0001.

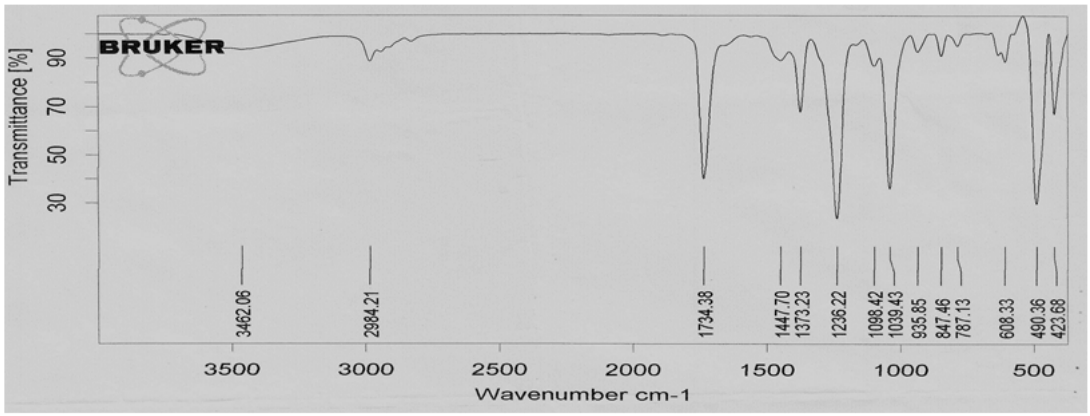

Fig. 4. FTIR study of AAME

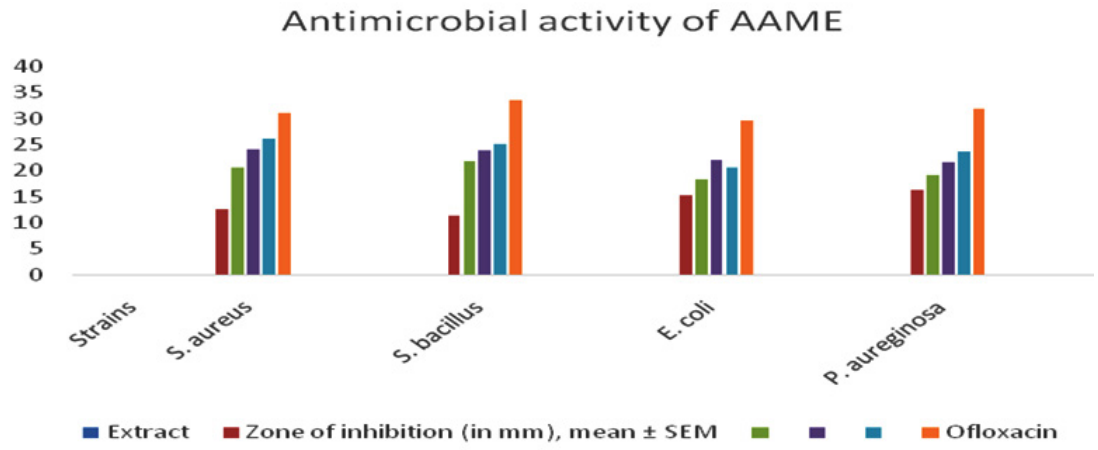

Fig. 5. Antimicrobial assessment of AAME
All results are shown in form of mean ± SEM, n = 3. For every bacterial strain, data were evaluated using a two-way ANOVA, Dunnett's multiple comparisons test, and ****p<0.0001

pathogenesis of numerous diseases, including cardiovascular disorders, neurodegenerative conditions, and various cancers. The significant presence of bioactive compounds in AAME suggests its potential role in mitigating oxidative stress and associated disease progression.

The phytochemical screening of AAME confirmed the presence of alkaloids, polyphenols, flavonoids, glycosides, triterpenoids, tannins, and other bioactive constituents, which exhibit strong antioxidant potential. Flavonoids and polyphenols, in particular, are well-recognized for their potent antioxidant properties and their ability to enhance the body's natural defense mechanisms. Studies have shown that rutin and its metabolites, which contain vicinyl dihydroxyl groups, contribute to the destruction of harmful microorganisms and offer protective effects against a variety of infectious bacterial and viral diseases. Additionally, rutin has been linked to the prevention of glucose-induced radical formation, thereby playing a role in diabetes management.[28-30]

Furthermore, gallic acid (GA), a well-known polyphenol (3,4,5-trihydroxybenzoic acid), has been identified as possessing both antioxidant and pro-oxidant activities.[31-33] This dual role of GA can be beneficial in modulating cellular responses to oxidative stress and promoting immune responses. The polyphenolic components in A. aspera extract have demonstrated immunomodulatory and anti-cancer properties, emphasizing its therapeutic potential. Moreover, the PCA extract contains known phenolic acids and flavonoid components such as kaempferol, quercetin, chrysin, quinic acid, and chlorogenic acid, all of which contribute to its bioactivity.[34-36]

Despite these promising findings, further in-depth studies are required to validate the specific therapeutic activities of these bioactive compounds. Advanced analytical techniques, including High-Performance Liquid Chromatography (HPLC), Mass Spectrometry (MS), and Nuclear Magnetic Resonance (NMR) spectroscopy, should be employed to identify and quantify these compounds with greater accuracy.[37-38] Additionally, in vivo and clinical studies are essential to substantiate the pharmacological relevance of AAME and its bioactive constituents in disease prevention and management.

CONCLUSION

Natural antioxidants derived from food supplements and traditional medicines offer significant potential in preventing oxidative damage, in contrast to synthetic therapies, which often come with undesirable side effects. The findings of this study provide valuable insights into the potential application of natural antioxidants, either as standalone treatments or in combination with innovative chemical formulations, to counteract oxidative stress-related diseases. The strong antibacterial activity observed in the AAME whole plant extract can be attributed to its diverse array of physiologically active compounds. These bioactive constituents contribute to the extract's broad-spectrum antimicrobial efficacy, making it a promising candidate for further research in the development of alternative therapeutic agents. However, to fully establish the therapeutic potential of AAME, additional studies are needed to validate the bioefficacy, safety, and mechanisms of action of its active compounds through rigorous preclinical and clinical evaluations.

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Data Availability Statement

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Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials**.**

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Not Applicable.

Authors Contribution

Ajay Kumar Shukla: Conceptualization, Research work, Writing Original Draft; Aarti Tiwari: Checking article Draft; Vimal Kumar Yadav: Data Collection, Analysis; Vishnu Prasad Yadav and Kunal Agam Kanaujia: Writing – Review & Editing; Manoj Kumar Mishra: Visualization, Supervision, Project Administration; Maya Sharma and Garima Verma: Funding Acquisition, Resources, Supervision.

REFERENCES

1. Abubakar MG, Yerima MB, Zahriya AG et al.,. Acute toxicity and antifungal studies of ethanolic leaves, stem, and pulp extract of *Tamarindus indica*. *Res J Pharm Biol Chem Sci*. 2010;1(4):104-11.
2. Iwu MM, Duncan AR, Okunji CO. New antimicrobials of plant origin. In: Janick J, editor. Perspectives on New Crops and New Uses. Alexandria, VA: *ASHS Press*; 1999. p. 457-62.
3. Yadav MK, Sharma K, Shukla AK. Dermoprotective efficacy of ursolic acid: An overview. *Int J Green Pharm*. 2024;18(3):114-23. doi:10.22377/ijgp.v18i03.
4. Abera A, Mekonnen A, Tebeka T. Studies on antioxidant and antimicrobial activities of *Plumbago zeylanica* Linn. traditionally used for the treatment of intestinal worms and skin diseases in Ethiopia. *Res J Med Plant*. 2015;9(6):252-63.
5. Oladeji O. The characteristics and roles of medicinal plants: some important medicinal plants in Nigeria. *Nat Prod* - An Indian J. 2016;12(3):1-8.
6. Jain NK, Anand S, Keshri P, et al. A comprehensive review of ethnomedicinal, phytochemical and pharmacological activity profile of *Achyranthes aspera*. *Pharmacog Res*. 2024;16(3):472-82.
7. Lin SY, Chang CL, Liou KT, Kao YK, Wang YH, Chang CC, et al. The protective role of *Achyranthes aspera* extract against cisplatin-induced nephrotoxicity by alleviating oxidative stress, inflammation, and PANoptosis. *J Ethnopharmacol*. 2024 Jan 30;319(Pt 1):117097. doi: 10.1016/j.jep.2023.117097.
8. Gawande DY, Druzhilovsky D, Gupta RC, et al.,. Anticonvulsant activity and acute neurotoxic profile of *Achyranthes aspera* Linn. *J Ethnopharmacol*. 2017 Apr 18;202:97-102. doi: 10.1016/j.jep.2017.03.018. PMID: 28315457.
9. Singh RK, Verma PK, Kumar A, et al.,. *Achyranthes aspera* L. leaf extract induced anticancer effects on Dalton's lymphoma via regulation of PKCá signaling pathway and mitochondrial apoptosis. *J Ethnopharmacol*. 2021 Jun 28; 274:114060. doi: 10.1016/j.jep.2021.114060. PMID: 33771640.
10. Subbarayan PR, Sarkar M, Nagaraja Rao S, et al. *Achyranthes aspera* (Apamarg) leaf extract inhibits human pancreatic tumor growth in athymic mice by apoptosis. *J Ethnopharmacol*. 2012 Jul 13;142(2):523-30. doi: 10.1016/j.jep.2012.05.030.
11. Fikru A, Makonnen E, Eguale T, D et al.,. Evaluation of in vivo wound healing activity of methanol extract of *Achyranthes aspera* L. *J Ethnopharmacol*. 2012 Sep 28;143(2):469-74. doi: 10.1016/j.jep.2012.06.049.
12. Lee JO, Yang WS, Park JG, et al. contribute to the anti-inflammatory activities of *Achyranthes aspera* ethanolic extract. *J Ethnopharmacol*. 2017 Jul 12;206:1-7. doi: 10.1016/j.jep.2017.05.013.
13. Nigussie D, Makonnen E, Tufa TB, et al.. Systematic review of Ethiopian medicinal plants used for their anti-inflammatory and wound healing activities. *J Ethnopharmacol*. 2021 Aug 10;276:114179. doi: 10.1016/j.jep.2021.114179.
14. Singh A, Singh SK. Phytochemical investigation and evaluation of anti-inflammatory activity of *Achyranthes aspera* Linn. in rats. *Indian J Exp Biol*. 2013;51(9):741-5. PMID: 24151751.
15. Charde M, Shukla A, Bukhariya V, et al.,. Herbal remedies as antioxidants: an overview. *Int J Pharm Res*. 2012;1(2):25-34.
16. Gupta M, Lodhi S, Shukla A. Preliminary phytochemical analysis and in vitro anti-

helminthic activity of *Martynia annua* Linn. and Permotrema reticulatum. *Asian J Biomater Res*. 2015;1(2):72-74.
17. Gupta R, Sharma P, Garg A, et al.,. Investigation of in vitro anthelmintic activity of *Ficus elastica* leaves. *J Drug Discov Ther*. 2013;1(5):1-3.
18. Sharma OP, Bhat TK, Singh B. Thin-layer chromatography of gallic acid, methyl gallate, pyrogallol, phloroglucinol, catechol, resorcinol, hydroquinone, catechin, epicatechin, cinnamic acid, p-coumaric acid, ferulic acid, and tannic acid. *J Chromatogr A*. 1998;822(1):167-71.
19. Mediæ-Šariæ M, Jasprica I, Smolèiæ-Bubalo A, et al.,. Optimization of chromatographic conditions in thin layer chromatography of flavonoids and phenolic acids. *Croat Chem Acta*. 2004;77(1):361-6.
20. Svendsen AB. Chromatography of alkaloids, Part A: Thin-Layer Chromatography. *J Chromatogr Lib*. 1983;23:1-9.
21. Sasidharan S, Chen Y, Saravanan D, et al.,. Extraction, isolation and characterization of bioactive compounds from plant extracts. *Afr J Tradit Complement Altern Med*. 2011;8(1):1-10.
22. Shukla AK, Yadav A, Vishwakarma RK, et al.,. Applications, isolation and characterization of fenugreek seed gum as pharmaceutical excipient. *J Med Pharm Allied Sci*. 2020;9(2):2484-91.
23. Shukla AK, Kumar M, Bishnoi RS, Jain CP. Isolation and characterization of natural and modified seed gum. *Asian J Pharm Pharmacol*. 2019;5(2):409-18.
24. Bishnoi RS, Kumar M, Shukla AK, et al.,. Comparative fingerprint and extraction yield of Prosopis cineraria (L.) Druce leaves with phenolic compounds (gallic acid) and flavonoids (rutin). *J Drug Deliv Tech*. 2019;9(3-s):560-8.
25. Bishnoi RS, Kumar M, Shukla AK, et al.,. Development and validation of novel HPLC method for the estimation of rutin in crude hydromethanolic leaf extract of Prosopis cineraria. *J Drug Deliv Tech*. 2018;8(6):68-73.
26. Garg S, Garg A, Shukla A, et al.,. Review on antioxidant evaluation methods: In-vitro and in-vivo models. *Asian J Pharm Pharmacol*. 2018;4(2):147-54.
27. Tiwari R, Jain R, Agrawal OP, et al.,. Evaluation of total phenolic and flavonoids content and their relation with antioxidant properties of *Tagetes patula* flower using in vitro assay method. *Bull Environ Pharmacol Life Sci*. 2023;12(5):204-8.
28. Yadav MK, Sharma K, et al.,. Extraction, characterization, screening, and assessment of the anti-inflammatory properties of bioactive compound. *J Nat Rem*. 2024;24(2):293-9. doi:10.18311/jnr/2024/33540.
29. Otohinoyi DA, Ekpo O, et al.,. Effect of ambient temperature storage on 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical for the evaluation of antioxidant activity. *Int J Biol Chem Sci*. 2014;8(3):1262-8.
30. Mourya P, Shukla A, et al.,. Hypoglycemic and hypolipidemic effects of ethanolic and aqueous extracts from *Ziziphus oenoplia* (L.) Mill on alloxan-induced diabetic rats. *Beni-Suef Univ J Basic Appl Sci*. 2017;6:1-9.
31. Jahan F, Lawrence R, et al.,. Evaluation of antimicrobial activity of plant extracts on antibiotic susceptible and resistant *Staphylococcus aureus* strains. *J Chem Pharm Res*. 2013;3(4):777-89.
32. Tiwari J, Shukla A. Investigations on *Calliandra haematocephala* flowers extract for in vitro anthelmintic activity. *Adv Pharm J*. 2016;1(1):17-20.
33. Sharma RA, Sharma PA, et al.,. Antimicrobial screening of sequential extracts of Datura stramonium L. *Int J Pharm Pharm Sci*. 2013;5(2):401-4.
34. Mahto BK, Patel R, et al. Development and standardization of poly-herbal formulation. *Sci Temper*. 2022;13(2):118-25.
35. Sharma P, Pandey P, et al.,. Development of quality control parameter of henna powder. *Int J Pharm Sci Rev Res*. 2013;21(1):293-5.
36. Shukla A, Gupta RC, Sharma P. Comparative study of microwave-assisted with conventional extraction of calcium sennosides from senna leaf. *Res J Pharm Biol Chem Sci*. 2013;4(3):103-9.
37. Dubey RS, Verma NK, et al.,. Hepatoprotective activity of poly-herbal formulation against paracetamol-intoxicated Wistar-albino rats. *Res J Pharm Technol*. 2024;17(7):1-7.
38. Tiwari R, Jain R, et al.,. of anti-inflammatory effect of *Premna reticulata, Curcuma caesia*, and *Tagetes patula* using in vitro study. *Bull Environ Pharmacol Life Sci*. 2023;12(5):209-14.