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Comparative Analysis of Bioactive compounds from Leaves Sample of *Achyranthes aspera*, their Pharmacological Studies, and their Importance in the Production of Silver Nanoparticles

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ABSTRACT: *Acyranthus aspera*, commonly called as Prickly Chaff flower, is found in India and the Indian subcontinent and is also widely found in other subcontinents of Africa, America and Australia. These plants are considered to be invasive alien plants, weeds, and non-commercial plants. Scientific investigations on the medicinal potential of *Acyranthus* are limited due to a lack of knowledge on the traditional ethnobotanical importance of these plants. In this present study, bioactive chemicals from *Acyranthus aspera* have been isolated and characterized, opening the door for their possible use in a wide range of scientific fields. Additionally, studies on the potential *Acyranthus aspera* plant extracts have produced excellent results in pharmacological studies, with their constituents demonstrating strong antibacterial action against a variety of diseases. These extracts are also appealing candidates for usage in the nutraceutical and cosmeceutical sectors due to their antioxidant and anti-inflammatory effects. The extracts were rich in pharmaceutical important metabolites such as alkaloid, flavonoid, phenol, carbohydrate, amino acid, saponin, tannin, terpenoid, coumarin, etc. Higher flavonoid content (84.89±1.47 equivalent to mM of Quercetin) was found in Hydro-alcoholic leaf extract. The aqueous extracts contained higher Phenol content (121.89 ±1.39 equivalent to mM of Gallic acid) than Hydro-alcoholic leaf extract. The extracts possessed antimicrobial, antioxidant, and anti-inflammatory activity. Both aqueous and Hydro-alcohol extract had the potential for green synthesis of silver nanoparticles. The silver nano-particles thus produced have variable sizes that range from 20nm to 200nm. The current Invitro studies make them promising candidates for a safer alternative to chemical-based drugs for anti-microbial, anti-oxidant, and anti-inflammatory activities. The potential of plant extract for the greener synthesis of silver nanoparticles enables them alternatives to discover and design new integrated drug targeting and drug delivery system.

Keywords: *Acyranthus aspera*, Anti-inflammatory, Anti-oxidant, Anti-microbial, Flavonoids, Phenolics, Silver nanoparticles (AgNP).

INTRODUCTION

Secondary metabolites, in plants, refer to chemical compounds synthesized by plants. These chemical metabolites don't directly contribute to their primary metabolic functions such as growth, photosynthesis, nutrient assimilation, respiration and reproduction of the organism. These metabolites serve purpose in defense mechanisms of plants against predators and herbivores, tactical lifesaving survival entities in response to various biotic and abiotic environmental stress such as pathogens, temperature, high salt, humidity etc., aids in reproduction such as attracting pollinators, etc. (Sofowora *et al.,* 2013). The secondary metabolites can be broadly classified into three major classes: Alkaloid (containing nitrogen and nitrogen compounds), Phenolic (containing phenol group) and Terpenoid (containing non-saponifiable lipids) (Ilya *et al.,* 2002).

In recent years, *Acyranthus aspera* has gained attention and research interest due to its easy availability in various environments such as wastelands, roadsides, cultivated fields, and forest edges. There are also a wide variety of metabolite constituents present in the plants such as alkaloids, flavonoid, phenol, tannin, saponin, terpenoid, and other phyto-compounds that has a lot of pharmaceutical potential such, as antimicrobial, anti-inflammatory, antioxidant, analgesic, antidiabetic, and anticancer properties (Bhosale *et al.,* 2012; Sinan *et al.,* 2020).

Acyranthus aspera has been a part of ancient Ayurvedic medical practices since ages. The different plant parts such as leaves stem, roots, and seeds can be harnessed for their medicinal aspect. The different extracts derived from *Acyranthus aspera* commonly prepared as

decoctions, infusions, or poultices are used as a traditional medicine to address a variety and wide range of ailments such as cough, asthma, fever, dysentery, skin problems, and rheumatism. These extracts were used in both tropical application and oral consumption (He *et al.,* 2017; Sultana *et al.,* 2022).

MATERIAL AND METHODS

Sample collection: The young, disease-free, healthy specimen of the plant was obtained from Ramgarh district, Jharkhand, during September and October, when the plant's growth was active and its inflorescence was ripe (Sinan *et al*., 2020).

Sample processing: The plant sample was first washed with distilled water followed by 10% sodium chloride solution 3-4 times. Then the sample was finally washed with distilled water. The plant parts were separated and kept for drying in well-ventilated shade conditions for 5-6 days. Then the dried plants were then sorted and kept in a moisture-free zipper pouch. The plant parts were then milled and sieved to get uniform particles (Maier *et al.,* 2010).

Plant Extract preparation. One part of the powdered plant sample was taken in an amber colour bottle, and to the powdered plant sample ten parts of the organic solvent of different polarities were added separately. The above plant sample-solvent mixture was then kept for 36-48 hours with occasional agitation for extraction of metabolites from plant parts into the solvents. Then the extraction mixture was filtered using Whatman #1 filter paper. The filtrate was collected in a clean and dry amber-coloured collection bottle and stored in a cool and dry place until further analysis (Ćujić *et al.,* 2016; Liu *et al.,* 2022).

Extractive value: The extractive value indicates the efficiency of the method by which the crude medicine was extracted from the plant sample (Chandel *et al.,* 2011). The study was carried out simultaneously on both organic solvent extracts (Ajazuddin, 2010).

Qualitative screening for plant secondary metabolite production: Qualitative screening plays a crucial role in the detection of various plant secondary metabolites within plant extracts. These secondary metabolites, such as alkaloid, flavonoid, terpenoid, and phenolic compounds, are organic compounds synthesized by plants that are not directly involved in their growth and development but often possess important pharmacological properties (Stochmal *et al.,* 2022; Sahoo *et al.,* 2015).

For qualitative detection of Alkaloids, Dragendorff's and Mayer's reagent test can be carried out that produced characteristic precipitates to confirm the presence of alkaloids. Carbohydrates can be identified using tests like Benedict's or Barfoed's test, where a colour change indicated the presence of reducing sugars. For glycosides, hydrolysis tests were employed to break down the glycosidic bond and release aglycones, which can then be identified using specific chemical tests. Flavonoid and phenolic were often detected through colour changes using the Alkaline reagent test and ferric chloride test respectively. Amino acids and protein were detected using the Ninhydrin or Biuret tests. Saponins were identified by their characteristic frothing properties. Tannins produce a colour change or precipitate when treated with ferric chloride or lead acetate. Terpenoids are identified using tests like the Liebermann-Burchard test, which yields distinctive bluish-green colour changes. Resins can be detected by their solubility in organic solvents with the formation of turbidity. Coumarins can be detected by the formation of distinctive yellow colour after the addition of an alkaline reagent (Boutaoui *et al.,* 2018; Sinan *et al.,* 1973).

Quantitative Estimation of Flavonoids and Phenolic: Quantitative estimation of flavonoids was estimated by the Aluminium chloride-based Spectrophotometric method. Flavonoids react with Aluminium chloride ($AlCl_3$) to form a complex that exhibits a characteristic colour change. The intensity of the colour is directly proportional to the concentration of flavonoids present in the sample. The absorbance values obtained for the standard solutions (Quercetin) are plotted against their respective concentrations to construct a standard calibration curve (Quercetin standard curve). The concentration of flavonoids in the plant extract can be determined by measuring its absorbance and using the calibration curve to interpolate the corresponding concentration (Pękal and Pyrzynska, 2014; Sahoo *et al.,* 2015).

The quantitative estimation of Phenol content in plant samples is estimated by Folin-Ciocalteu (F/C) method. This is based on the reaction of phenol with Folin-Ciocalteu reagent to form a blue-coloured complex in alkaline conditions. The F/C reagent consists of phosphomolybdic and phosphotungstic acids that are reduced by phenol and other phenolic compounds in alkaline conditions to form blue-coloured compounds that can be analyzed by UV Visible spectrophotometer at λmax of 760nm. The intensity of the colour formed is directly proportional to the concentration of phenol present in the sample. By preparing a calibration curve using known concentrations of phenol, the concentration of phenol in an unknown sample can be determined accurately using the absorbance values obtained from the spectrophotometer (Sahoo *et al.,* 2015).

In vitro assay for plant-derived extracts: The plant extracts were then tested in-vitro for various pharmacological actions, such as antibacterial, antioxidant activity and anti-inflammatory activities (Tlili *et al.,* 2019).

Anti-microbial activity: The anti-microbial activity studies of plant extract (Balouiri *et al.,* 2016) against different pathogenic bacteria such as *E. coli, Staphylococcus* sp., *Pseudomonas sp., Salmonella* sp, *and Bacillus* sp. were done by Agar well diffusion method where the presence of clear zone around the wells (no growth of microorganism) indicate that the test microorganism is susceptible to the plant extract whereas the absence of clear zone indicates that the test microorganism is resistance to the plant extract.

Anti-oxidant activity: The potential for plant extracts demonstrating antioxidant activity was evaluated by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity (Blois, 1958; Brand-Williams *et al.,* 1995, Jain *et al.,* 2023; Sahoo *et al.,* 2015) where

Ascorbic acid was used as a reference standard. The DPPH molecule is extremely stable, with a maximum absorbance of 517nm. The DPPH molecule can be reduced by the anti-oxidant molecule which leads to a decrease in absorbance. The scavenging activity percentage is computed as follows:

Percentage of scavenging activity=((Absorbance control-Absorbance test))/(Absorbance control)*100

Anti-inflammatory activity: The standard albumin denaturation assay with indomethacin was used to investigate the plant extracts' anti-inflammatory effect (Dharmadeva *et al.,* 2019). The percentage of denaturation activity is estimated by spectrophotometric method by recording the absorbance of the test sample and the blank sample at 680nm and using the following equation: Percentage of denaturation=((Absorbance control-Absorbance test))/(Absorbance control)*100

Screening for plant extracts capable of the production of Silver nanoparticles (AgNP's) from silver nitrate (AgNO3): Considering their unique characteristics as well as potential applications, silver nanoparticles (AgNPs) have become increasingly popular as a result of recent advances in nanotechnology. One intriguing strategy is to use plant extracts as eco-friendly and cost-effective nanoparticle production agents (Hano and Abbasi, 2021). A positive reaction for screening of silver nanoparticles was indicated by the change in coloration of the silver nitrate solution from transparent/light yellow coloration into dark brown/reddish brown coloration.

Characterization of production of Silver nanoparticles by Spectral analysis: UV-Vis spectrophotometry offers a simple and efficient means of determining the optical properties and plasmonic behavior of AgNPs. During the synthesis process, the reduction of silver ions to nanoparticles leads to the formation of characteristic surface plasmon resonance (SPR) peaks, typically observed in the UV-Vis spectra (Dubeya *et al.,* 2010) and further morphological studies by Scanning Electron microscope (Begum *et al.,* 2009).

RESULTS

Acyranthus aspera plant leaves that appeared young, tender, and disease-free were selected for study (Fig. 1). In the month of October, the leaves were collected. The leaves were dried in an aseptic environment with minimal contamination, pulverized, and extracts from the plants (Aqueous and Hydro-alcohol) (Fig. 2) were obtained by a maceration procedure (cold extraction technique).

Fig. 1. Leaves sample of *Acyranthus aspera.*

Fig. 2. Dried Powdered leaf of *Acyranthus aspera.*

For the preparation of aqueous plant extract, 100% water was used whereas for the preparation of Hydro-alcoholic extract, a mixture of water and ethyl alcohol was used in the ratio of 55 parts and 45 parts respectively.

The plant material and solvent were mixed in a ratio of 1gm: 20ml of solvent. The mixture was kept in an amber-coloured bottle with mild stirring and stored at room temperature for 36-48 hours. After extraction, the mixture was filtered and the filtrate was stored in an amber-coloured bottle for further analysis. The aqueous extract was Reddish brown in colour whereas the hydro-alcoholic extract was greenish in colour (Fig. 3, 4).

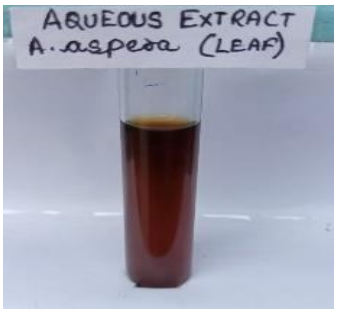

Fig. 3. Aqueous Leaf Extract of *Acyranthus aspera*.

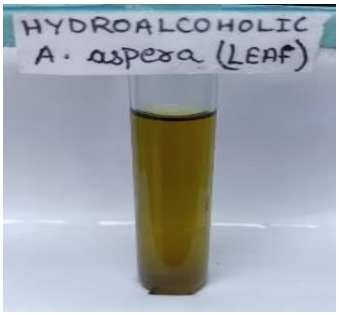

Fig. 4. Hydro-alcoholic Leaf Extract of *Acyranthus aspera*.

Extractive value: The extractive value of a crude drug for aqueous extract and hydro-alcoholic extract was found to be 3.25% and 2.75% respectively.

Qualitative screening for bioactive compounds: After extraction of plant bioactive compounds, the extracts were considered for Qualitative screening for the presence of different bioactive compounds. The aqueous extract has shown positive for alkaloids, carbohydrates, glycosides, phenol, amino acid, saponin, steroid, tannin, terpenoids, and coumarins. The hydro-alcoholic extract contained phytochemicals such as carbohydrates, flavonoids, phenol, quinone, and coumarin.

Quantitative estimation of Flavonoid and Phenolic: The Total flavonoid and phenolic compounds were quantified for aqueous and hydro-alcoholic extracts for leaf samples of *Acyranthus aspera.* For quantitative estimation, the Quercetin standard curve (Fig. 5) and Gallic acid standard curve (Fig. 6) were established for Flavonoid content and Phenol content, respectively.

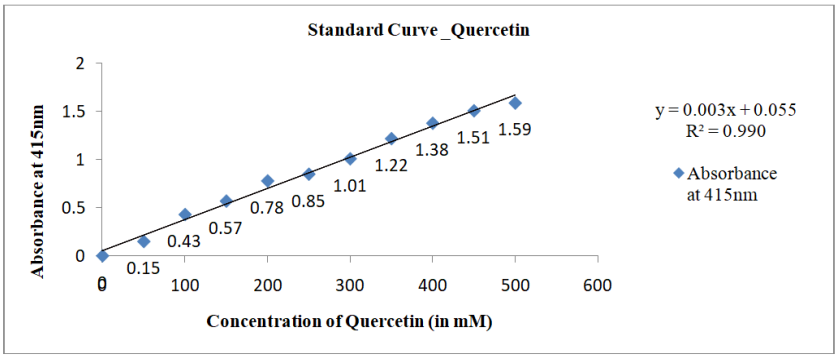

Fig. 5. Quercetin Standard curve (for quantitative estimation of flavonoids).

For estimation of unknown concentration of Flavonoid in sample
Slope y= 0.0032x + 0.055
R^2 = 0.9909
Where y = Measured Absorbance at 415nm; x = Flavonoid Concentration (equivalent to mM Quercetin)
So, the equation for flavonoid content (x)
Flavonoid content (x)=(y-0.055)/(0.0032)
For Quantitative estimation of Flavonoids in the different plant extracts, The Flavonoid content (x) (equivalent mM of Quercetin) was estimated by substituting the value of Absorbance at 415nm (y) in the equation.

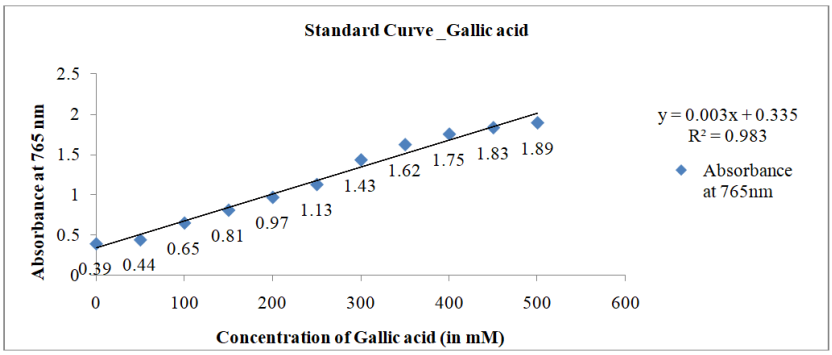

Fig. 6. Gallic acid Standard curve (for quantitative estimation of Phenols).

For estimation of unknown concentration of Phenol in sample
Slope y= 0.0034x + 0.3355
R^2 = 0.9834
Where y = Measured Absorbance at 765nm
x = Phenol Concentration (equivalent to mM Gallic acid)
So, the equation for Phenol content (x)
Phenol content (x)=(y-0.3355)/(0.0034)
For Quantitative estimation of Phenolic in the different extracts, Phenol content (x) (equivalent mM of Gallic acid) was estimated by substituting the value of Absorbance at 765nm (y) in the equation.

Quantitative estimation of Total Flavonoid and Total Phenol in Plant sample: The Total Flavonoid and Phenol content was estimated in the aqueous and hydro-alcoholic leaf extract of *Acyranthus aspera* from the slope equation as indicated in their respective standard curve.
Higher flavonoid content (84.89±1.47 equivalent to mM of Quercetin) was found in Hydro-alcoholic leaf extract (Table 1, Fig. 7). The aqueous extracts contained higher Phenol content (121.89 ±1.39 equivalent to mM of Gallic acid) as compared to Hydro-alcoholic leaf extract (Table 2, Fig. 7).

Table 1: Flavonoid content in Leaf extracts of *Acyranthus aspera.*

Sample (leaf extract)	Absorbance at 415nm			Flavonoid content (equivalent to mM of Quercetin)			Mean Flavonoid content ± S.D.
Aqueous	0.07	0.08	0.08	4.68	7.81	7.81	**6.77 ±1.47**
Hydro-alcoholic	0.32	0.33	0.33	82.81	85.93	85.93	**84.89±1.47**

S.D.- Standard deviation

Table 2: Phenol content in Leaf extracts of *Acyranthus aspera.*

Sample (leaf extract)	Absorbance at 765nm			Phenol content (equivalent to mM of Gallic acid)			Mean Phenol content ± S.D.
Aqueous	0.76	0.75	0.75	124.85	121.91	121.91	**121.89±1.39**
Hydro-alcoholic	0.57	0.55	0.56	68.97	63.09	66.03	**66.03±2.40**

S.D.- Standard deviation

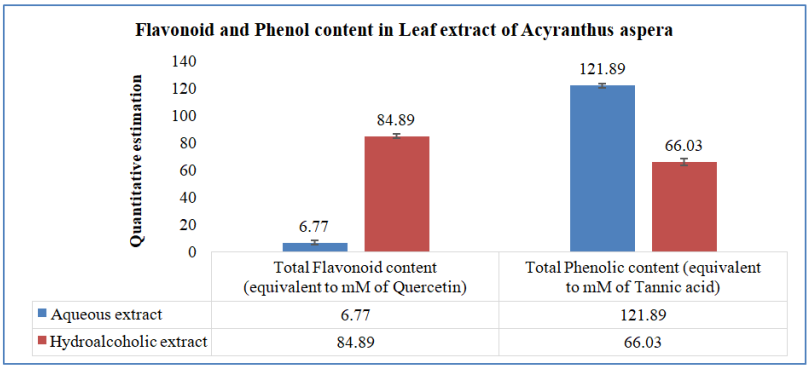

Fig. 7. Quantitative estimation of Flavonoid (equivalent to mM of Quercetin) and Phenolic (equivalent to mM of Gallic acid) in leaf extract of *Acyranthus aspera.*

In-vitro studies:

Antibacterial assay: The aqueous extract of *Acyranthus aspera* had shown better anti-microbial activity against gram-positive bacteria such as *Bacillus* sp., and gram-negative bacteria such as *Pseudomonas* sp., and *Salmonella* sp. The extract didn't show any antibacterial activity against other test organisms such as *Escherichia coli* and *Staphylococcus aureus*. In the hydro-alcoholic extract, the highest activity was shown against pathogenic bacteria such as *Staphylococcus aureus* and also showed positive activity against other bacteria such as *Pseudomonas* sp., *Escherichia coli*, *Salmonella* sp., *and Bacillus* sp. A better anti-bacterial effect was found for Hydro-alcoholic extract against test pathogenic bacteria as compared to aqueous plant extract (Table 3, Fig. 8).

Anti-oxidant assay: The anti-oxidant activity of the plant extract was assessed by the change in colour of the standard DPPH solution from purple to yellow. The percentage of inhibition was calculated to determine the anti-oxidant property quantitatively. Aqueous leaf extract had higher anti-oxidant activity than the hydro-alcoholic leaf extract of *Acyranthus aspera* (Table 4, Fig. 9).

Table 3: Antibacterial assessment of aqueous and hydro-alcoholic leaf extract.

Sr. No.	Pathogenic Bacteria	Zone of inhibition (in mm) Aqueous extract	Zone of inhibition (in mm) Hydro-alcoholic extract
1.	*Escherichia coli*	--	10
2.	*Pseudomonas aeruginosa*	33	18
3	*Staphylococcus aureus*	01	32
4	*Salmonella* sp.	05	--
5	*Bacillus* sp.	20	18

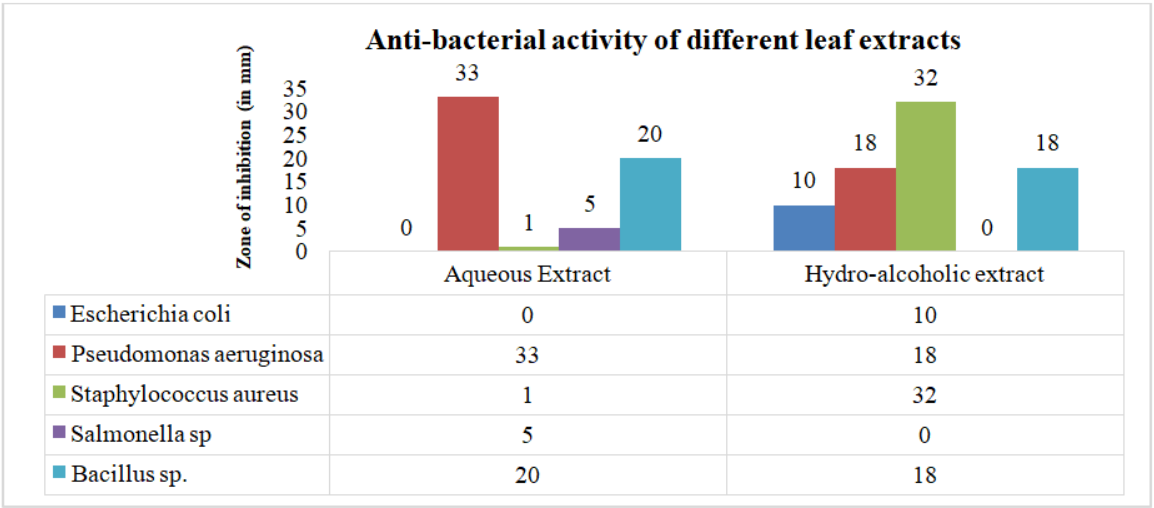

Fig. 8. Antibacterial assessment of aqueous and hydro-alcoholic leaf extract of *Acyranthus aspera.*

Table 4: Anti-oxidant study (DPPH method) for Aqueous and Hydro-alcoholic leaf extract of *Acyranthus aspera*.

Sr. No.	Samples	Test (in triplicates)	Absorbance at 517nm	Radical Scavenging activity	Mean Radical Scavenging activity±S.D.
1.	Blank	-	0.49	-	-
2.	Ascorbic Acid (0.1mg/ml)	-	0.12	75.51	75.51
2.	Aqueous Extract (0.1mg/ml)	1/3	0.21	57.14	56.46 ±0.96
		2/3	0.22	55.10	
		3/3	0.21	57.14	
3.	Hydro-alcoholic Extract (0.1mg/ml)	1/3	0.38	22.45	23.81 ±0.97
		2/3	0.37	24.49	
		3/3	0.37	24.49	

S.D.- Standard Deviation

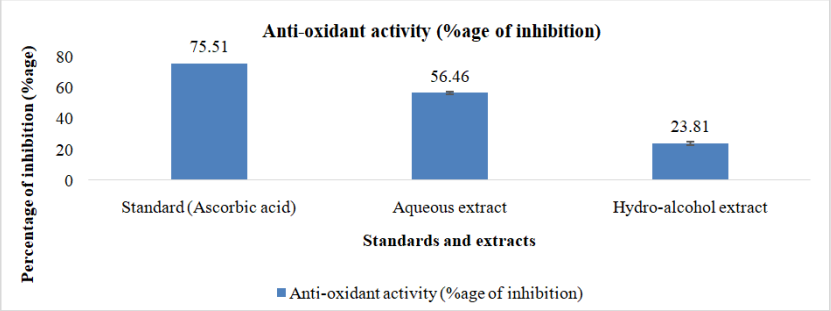

Fig. 9. Anti-oxidant study (DPPH method) for Aqueous and Hydro-alcoholic leaf extract of *Acyranthus aspera.*

Anti-inflammatory activity: The anti-inflammatory activity can be studied by extract capable of inhibition of protein denaturation. The hydro-alcoholic extract had shown greater anti-inflammatory activity as compared to the standard indomethacin. The aqueous extract didn't exhibit any such activities (Table 5, Fig. 10).

Screening for the potential of plant extracts for green synthesis of silver nanoparticles: The different plant extracts (Aqueous and Hydro-alcoholic) were then screened for the production of silver nano-particle from molecular silver nitrate solution. Both the solutions had shown positive for their capabilities for the production of silver nanoparticles (Fig. 11, 12). Qualitatively the aqueous extract produced more nano-particles as compared to hydroalcoholic extracts due to increased intensity of reddish brown coloration in the screening solution.

Table 5: Anti-inflammatory study (Albumin denaturation assay) for Aqueous and Hydro-alcoholic leaf extract of *Acyranthus aspera.*

Sr. No.	Samples	Test (in triplicates)	Absorbance at 680nm	Anti-inflammatory	Mean anti-inflammatory activity±S.D.
1.	Blank	-	0.54	-	-
2.	Indomethacin (0.1mg/ml)	-	0.41	14.81	**24.07**
2.	Aqueous Extract (0.1mg/ml)	**1/3**	0.58	-	-
		2/3	0.59	-	
		3/3	0.59	-	
3.	Hydro-alcoholic Extract (0.1mg/ml)	**1/3**	0.39	27.78	**28.39 ±0.87**
		2/3	0.39	27.78	
		3/3	0.38	29.62	

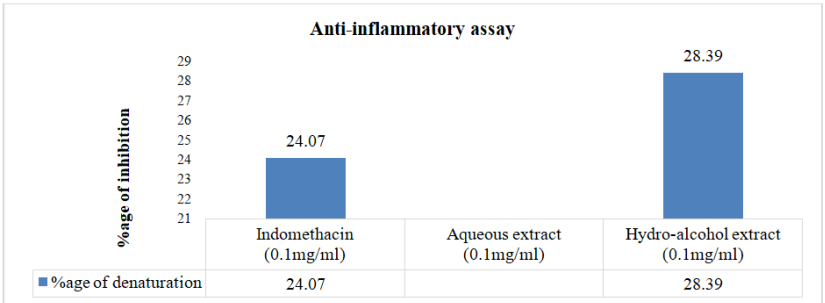

Fig. 10. Anti-inflammatory study (standard albumin denaturation assay) for Aqueous and Hydro-alcoholic leaf extract of *Acyranthus aspera.*

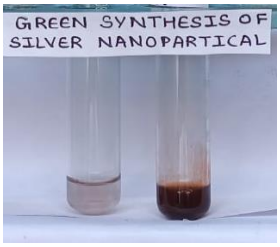

Fig. 11. Screening for production of Silver nanoparticles (AgNP's) from molecular silver nitrate solution by Aqueous extracts of *Acyranthus aspera*.

Fig. 12. Screening for production of Silver nanoparticles (AgNP's) from molecular silver nitrate solution by Hydro-alcohol extracts of *Acyranthus aspera*.

Purification of nano-particles by centrifugation. The solutions were first centrifuged, and the pellet was collected and washed with distilled water. The process was repeated for repeatedly for 10-15 times to get purified nano-particle.

Characterization of silver nano-particles. The Characterization of silver nanoparticles was proposed with Absorbance Peaks observed in the spectral analysis from 420nm-450nm that corresponds to the formation of Silver nanoparticles. The highest spectrum peaks for aqueous and hydroalcoholic extract were found at 420nm and 440nm respectively (Fig. 13, 14).

The purified silver nanoparticles were analysed for their morphology by Transmission Electron Microscope (TEM). The Morphology of the synthesized Silver nanoparticles is highly variable. The assemblies were found to be aggregated of Silver nano-particle (Ag-NP) in the range 15nm-20nm (aqueous extract) and 100-150nm (hydroalcoholic extract) respectively.

Antimicrobial assay of purified silver nanoparticles (AgNP) and extract + silver nanoparticles (Extract + AgNP): The antimicrobial assay was carried out for the purified silver nanoparticles (AgNP) and extract + silver nanoparticles (Extract + AgNP). The purified AgNP have better antimicrobial property against all test pathogenic bacteria. The association of AgNP with silver nanoparticles enhanced the activity of plant extracts against pathogens with an increased zone of inhibition as compared to the plant extracts only. The plant extracts also gained some antimicrobial properties may be due to the presence of active silver nanoparticles (AgNP). The purified nanoparticles exhibited almost similar functionality that has been synthesized from both extracts (Table 6, Fig. 15).

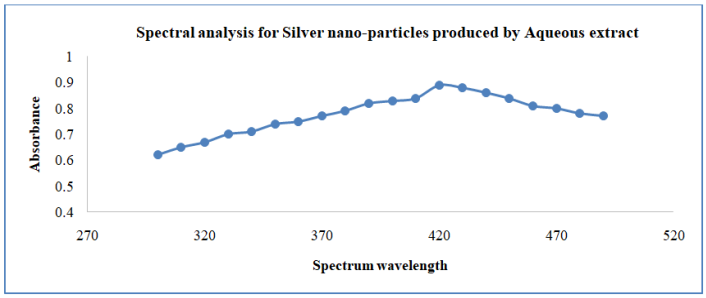

Fig. 13. Spectrum peak for nanoparticles produced by aqueous extracts.

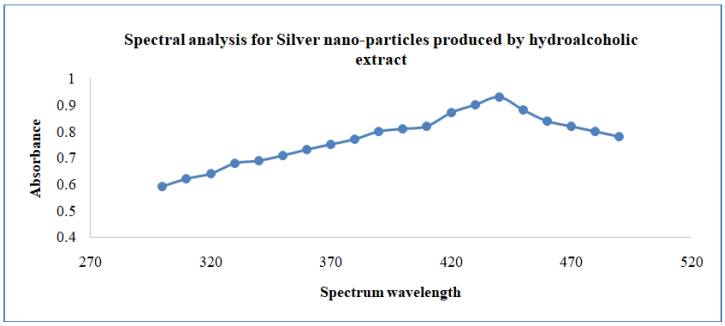

Fig. 14. Spectrum peak for nanoparticles produced by Hydro-alcohol extracts.

Table 6: Antibacterial assessment of aqueous and hydro-alcoholic leaf extract.

Sr. No.	Pathogenic Bacteria	Zone of inhibition (in mm)			
		Aqueous extract + AgNP	Purified AgNP (Aqueous extract)	Hydroalcoholic extract + AgNP	Purified AgNP (Hydroalcohol extract)
1.	*Escherichia coli*	10	14	11	13
2.	*Pseudomonas aeruginosa*	37	26	20	23
3	*Staphylococcus aureus*	08	33	32	34
4	*Salmonella* sp.	06	13	06	14
5	*Bacillus* sp.	22	25	19	26

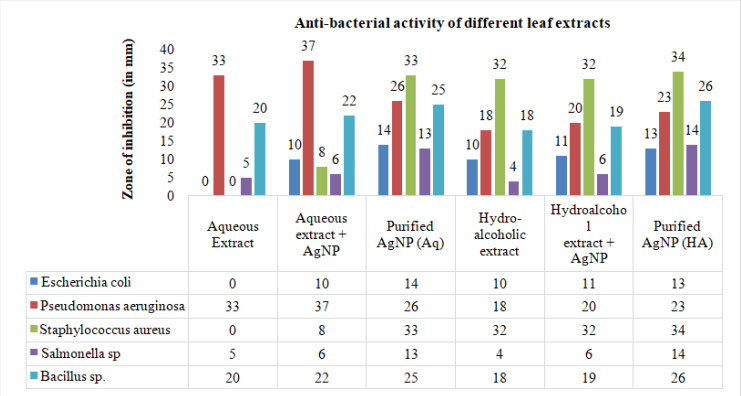

Fig. 15. Comparative study for Antimicrobial assay of purified silver nanoparticles (AgNP) and extract + silver nanoparticles (Extract + AgNP).

DISCUSSION

Sharma *et al.* (2013) studied the phyto-chemistry of different sequential extracts Aqueous, Ethanol, Petroleum ether, Chloroform, Ethyl acetate, and Benzene) of inflorescence and roots of *Achyranthes aspera.* All the extracts had shown positive for the presence of Alkaloids, Tannins, Cardiac glycosides, Reducing sugar, and saponins while proteins were absent in all extracts. Steroids were absent in chloroform, ethanol, and ethyl acetate extract. Terpenoids and Flavonoids were found in all extracts except the benzene extract. Bhosale *et al.* (2012) studied that the aqueous extract of leaf and whole plant contained majorly Alkaloids, Flavonoids, Terpenoids, and Saponins. Sharma *et al.* (2013) also studied that the aqueous extract had the highest yield of 1.71 % for roots and 8.04 % for inflorescences while for ethyl acetate, roots had the lowest yield of 0.08 % and 0.24 % for inflorescences. This research study has also confirmed the presence of different phytochemicals such as Alkaloids, Carbohydrates, Glycosides, Phenol, Amino acid, Saponin, Steroids, Tannin, Terpenoids, and Coumarins in aqueous extracts and Carbohydrates, Flavonoids, Phenol, Quinones, and Coumarins in hydro-alcohol extract.

Datta *et al.* (2019) found out maximum extraction of Flavonoids and Phenolic compounds in 70% ethanol and methanol extract with Total flavonoid content of 20.793±0.122 (Rutin equivalent, mg/g Dry Extract) and 22.019± 0.020 (Rutin equivalent, mg/g Dry Extract) respectively and Total Phenolic content of 74.831± 0.243 (Gallic acid equivalent, mg/g Dry Extract) and 37.276±0.321 (Gallic acid equivalent, mg/g Dry Extract) respectively. The highest anti-oxidant assay was found in 70% ethanol followed by methanol extract. This study showed higher flavonoid content (84.89±1.47 equivalent to mM of Quercetin) was found in Hydro-alcoholic leaf extract as compared to aqueous extract whereas aqueous extracts contained higher Phenol content (121.89 ±1.39 equivalent to mM of Gallic acid) as compared to Hydro-alcoholic leaf extract.

Vijay Kumar *et al.* (2009) reported that the alcohol root extract of *Acyranthus aspera* showed potential In-vivo anti-inflammatory activity against the carrageenan-induced paw edema method (acute inflammatory model) and cotton pellet granuloma technique (chronic inflammatory model) in Wister rats. This research has revealed an in-vitro analysis of anti-inflammatory activity by albumin denaturation assay where the hydro-alcohol leaf extract showed better activity as compared to the aqueous leaf extract with indomethacin taken as the standard reference drug.

Kaur *et al.* (2005) found that Chloroform and Methanol extract of root and shoot extract showed positive antimicrobial activity against *Klebsiella* sp. whereas Petroleum ether root extract showed positive for *Bacillus subtillis* and *Fusarium* sp. Yadav *et al. (*2016) studied the potential of aqueous extract of root and stem against *Streptococcus mutans* that are the causative organism for Dental caries and periodontal diseases. Ndhlala *et al.* (2015) evaluated the potential of aqueous and acetone extract for anti-microbial activity against *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis, Staphylococcus aureus, Candida albicans and Caenorhabditis elegans.* Present studies showed a better anti-bacterial effect for Hydro-alcoholic leaf extract against test pathogenic bacteria as compared to the aqueous plant extract.

Elumalai *et al.* (2016) studied the potential of leaf extract of *Acyranthus* for green synthesis of silver nanoparticles that were very stable and had shown potential Larvicidal activity against *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi.* Durga Praveena and Vijay Kumar (2014) also studied the potential of the Aqueous extract of *Acyranthus* for silver nanoparticles production and subsequently, the Chitosan-coated AgNP's were produced and analyzed for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The chitosan-coated AgNP had shown positive antimicrobial activity against pathogenic bacteria. Le *et al.* (2020) found that the production of silver nanoparticles from leaf extract was characterized by a peak at 433nm in spectral analysis by UV-Vis Spectrophotometer and analysis by Transmission electron microscope that showed that the silver nano-particles were variable in shape and size varied from 8-52nm. These nanoparticles have strong antifungal activity against *Aspergillus niger, Aspergillus flavus,* and *Fusarium oxysporum.* These plant extracts along with silver nanoparticles can act as potential entities in healthcare and agricultural fields. This current research

also demonstrated that the leaf extracts (Aqueous and Hydro-alcoholic) had the potential for green synthesis of silver nanoparticles that were analyzed by the presence of the highest peak (~420nm-440nm) in the spectral analysis by UV-Vis Spectrophotometer. These nanoparticles were further characterized by the Transmission Electron microscope (TEM) where the nanoparticles were variable in shape with the size varying from 20nm-200nm. Better activity was found for Extract-AgNP mixture as compared to the extract and purified AgNP alone. This might be due to combined and more effective ways of delivering drug phytochemicals to target pathogens.

CONCLUSIONS

Acyranthus aspera, a plant species rich in secondary metabolites, possesses immense potential across various scientific domains. Its widespread distribution in India, its ethnobotanical significance, and the diverse range of bioactive compounds the plant contains make it a compelling subject for further research. The exploration and utilization of plant extracts from *Acyranthus aspera* can contribute to the development of novel drugs, herbal remedies, and sustainable agricultural practices while providing unique solutions to the challenges faced in different industries.

FUTURE SCOPE

Plants have been studied for the presence of many chemical compounds that have medicinal potential. These molecules can be used for the development of new drug molecules that can have potential pharmacological value and remedial potential. The present study suggested that an elaborate study needs to be conducted in order to harness the potential of cancer research and research in other acute and chronic diseases related to vital organs such as the liver, kidney, gastrointestinal tract, etc. With scientific development in different techniques and procedures of separation, characterization, and identification of biometabolite present in plant samples, more research can be enabled to exploit these resources for pharmaceutical and healthcare purposes. Further in-vitro, in-vivo, and in-silico studies can be conducted to validate the potential of chemical compounds for target suitability, drug interaction, drug likeliness, pharmacodynamics, and pharmacokinetics study. Scientific validations can meet reliability to develop a more efficient, safe alternative to API drugs.

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