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Antimicrobial and Antioxidant potential of *Achyranthes aspera* Linn. and Its increased prevalence in Cosmetics

***Manoj Kumar Singh[1] , Anil Kumar Singh[2] and Md. Sarfaraz Ahmad[3]**

[1]Department of Botany, KR College, Gopalganj, Bihar (India)
[2]Associate Professor, Department of Botany, B R A Bihar University, Muzaffarpur, Bihar (India)
[3]Associate Professor, Department of Botany, Gopeshwar College, Hathwa, Gopalganj, Bihar (India)
(Jai Prakash Vishvidyalay, Chapra, Bihar, India)
Email: manojkumarsingh12672@gmail.com

Abstract

Achyranthes aspera Linn. (family: Amaranthaceae) is an indigenous medicinal plant found in Asia, South America, and Africa. It is known to contain active components like tannins, phenol, alkaloids, flavonoids, terpenoids, saponins etc. The study emphasize on the comparison of antioxidant and antibacterial activity among root, stem and leaf extract of *A. aspera.* The evaluation of scavenging activity was screened based on three methods of antioxidant activity, DPPH Assay, FRAP Assay and ABTS Method. *A.aspera* root extract showed higher percentage scavenging activity (66.32%) at 500 (µg/ml). IC50 for for root, leaf and stem extract was found to be 299.4 (µg/ml), 379.75 (µg/ml) and 467.31 (µg/ml). The scavenging activity estimated in the extracts of A.aspera using Ferric reducing-antioxidant power (FRAP) assay showed best results in case of stem extract (184.15%) at 500 (µg/ml). FRAP assay showed comparable percentage inhibition in the root (86.81%), leaf (85.23%) and stem (85.71%) extracts. Out of the three extracts root (R.E), stem (S.E) and leaves (L.E), root extract was observed to possess potent activities with maximum antibacterial activity at highest concentration (187.5mg/ml). FTIR spectra was used to analyze functional groups.

Traditional therapeutic culture mainly constitutes of medicinal plants as the key player to improve the health standards of people. These plants are considered to have the resource of active components that possess drug-like properties. The Multiple drug resistance issue faced by commercial drugs has caused their limited usage for people[24]. Thus, researchers focussed on an alternative source of Medicinal Plants as a potent antimicrobial agent[38]. A range of drugs is available in the market nowadays which has natural source origin[10]. Various surveys conducted by the United States and other countries discuss the usage

of higher plants and their active components against many fatal infectious diseases[19].

A survey conducted by The World Health Organization (WHO) revealed that approx. 80% of Asian and African population is dependent on Herbal medication for primary health issues (WHO Regional Office for the Western Pacific (1993)). The natural compounds synthesized by plants as integral components in their defense mechanism, also work for the welfare of humanity. These natural compounds have various medicinal properties, majorly Antimicrobial and Antioxidants[26]. Thus Ethno-botany, science to study the association of plant and people, is emerging to be an impressive way of combating pathogens and discover effective medicines[11].

All the cells undergo the natural process of oxidation inside it. These oxidation processes cause the formation of extremely reactive free radical species like hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and ozone (O_3) [4]. The reactive free radical species are also generated inside our body as a cause of some external factors and lifestyle like smoking, pollution, pesticide, radiation, etc. [25]. They have a role in neurodegenerative disorders and aging[17]. Free radicals have a fatal effect on genetic material, also they tend to damage membrane, proteins and fat. Thus, loss of property in proteins and enzymes cause premature wrinkling, aging and even develops cancerous growths on the damaged skin[6,17].

Phenols and other phytochemicals are known to possess natural oxidant property which curbs the effect of free radicals and the oxidative damage caused by them[12,17,40]. Achyranthes aspera Linn is an indigenous medicinal plant of Asia, South America, and Africa. It belongs to the family Amaranthaceae and shows the presence of active components like tannins, phenol, alkaloids, flavonoids, terpenoids, saponins and reducing sugars[34,35]. A list of biological activities are reported in this plants such as antimicrobial[22,34], anti hypoglycemic[1], hypolipidemic[21], antihyperglycemic[37], anticarcinogenic[9], anti-inflammatory[14], cardioprotective[16], hepatoprotective[2], immunomodulatory[31] and antiparasitic[3,39] activities. Given the above-mentioned properties possessed by *Achyranthes aspera Linn*, the study is designed to emphasize its antioxidant, antibacterial and antifungal properties. It highlights the comparative evaluation of these activities among root, leaves and stems extracts. The study thus investigates the best candidates out of three to utilize it for the betterment of health and humanity.

Plant collection and Identification: Fresh whole root, fresh whole stem and fresh whole leaves of Achyranthes aspera were collected from Bihar region during month of March, 2018. Botanical identification of the plant was done by submitting the plant specimen herbarium to College herbarium, Department of Botany, Gopeshwar College, Hathwa, Gopalganj, Bihar.

Extract Preparation :

The three parts (root, leaf, and stem) were collected separately in zipped plastic bags and brought to the laboratory for analysis. The plant material was thoroughly washed under

running tap water, rinsed with distilled water and then dried. The moisture was completely removed from plants by shade drying for 14 days. After complete drying, the plant material was powdered using the mechanical grinder. 10gm of leaf, root and stem powder were weighed separately and extracted sequentially in Hexane, Ethyl acetate and then Methanol (10% w/v) using a Soxhlet apparatus. The final extract was then concentrated using a rotary evaporator at 40°C. The concentrated samples were then dried using lyophilizer and stored at 4°C for subsequent analysis.

Antioxidant Activity :

Determination of antioxidant activity with 2,20 -diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method :

The antioxidant activity of the extracts was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH[8].

Ferric reducing-antioxidant power (FRAP) assay :

This method measures the ability of antioxidants to reduce ferric iron. It is based on the reduction of the complex of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacy-clopenta-1,4-diene chloride (TPTZ) to the ferrous form at low pH. This reduction is monitored by measuring the change in absorption at 593 nm, using a diode-array spectrophotometer. Antioxidant assay can be conducted by the method developed by Benzie and Strain [7].

The 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay :

2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging activity of each crude and partition extract was conducted according to method described by a previous study[13].

Test Organism and Culturing of Isolates:

A total of 3 test organisms were used in the study to assess the antibacterial activity of the Achyranthes aspera root, stem and leaves extract. *S.pyogenes* (ATCC 19615), *S. epidermidis* (ATCC 12228), *P.acne* (ATCC 11827) strains were maintained on Nutrient agar plates (pH7.4) and incubated overnight at 37'"C before use. The stock culture for these isolates was maintained as glycerol stock at -70°C for any future use.

Standardization of Bacterial Suspension :

Antibacterial activity was done by Disk Diffusion method using bacterial suspensions standardized under the CLSI guidelines. The bacterial suspension was grown in MH broth and its turbidity was brought equivalent to 0.5 McFarland solution (1-2 × 108 CFU/mL) before the assay was performed.

Antibacterial Activity :

The antibacterial assay was done by Disk Diffusion method described by Heately (1944) method. All the four isolates were before test grown in Mueller Hinton broth (MHB) at 37°C for 24 hours in a shaker incubator. The incubated bacterial suspension was then taken 100 µl and spread on Muller

hinton agar media plates. These plates were then left to absorb the suspension on the surface, then the disks of the extracts were placed on the surface in a series of increasing concentrations. These plates were then incubated at 37°C for 24 hours, the clear zone around the disk (zone of inhibition) was recorded in mm and compared with standard antibiotics.

One of the major aspects of researchers thrust on the plant as a source of an antioxidant agent is that they naturally produce these compounds in abundance. The damages due to the oxidation process are extremely fatal for the cell, ultimately leading to several physiological and neurodegenerative disorders. Plants and microbes both can curb the effect of oxidative damage. Some of the major compounds of plant origin having such properties are phenols, benzoquinones, phenolic acids, polypropenol, flavonoids, isoflavonoids phenylpropanoids, phenolics quinines, lignins, melanins, tannins, etc. . The phytochemicals which play a key role as an antioxidant agent are phenolic and polyphenols. The objective of this study was to emphasize the antioxidant properties possessed by *Achyranthes aspera* as already reported in its leaves extract by Priya *et al.*[29], we compare the property in root, leaves and stem extract using three different methods of antioxidant activity i.e., DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay and FRAP (Fluorescence recovery after photo-bleaching) method. In previous studies, majorly stem and roots have been explored for their antioxidant and antibacterial activities. Kumar *et al.*[23], reported a significant antioxidant activity possessed by root, stem and leaves extracts. The *in vitro* antioxidant activity of crude plant extracts was determined by DPPH radical scavenging activity. The methods DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay and FRAP (Fluorescence recovery after photo-bleaching) method are used here to assess the scavenging activity of *Achyranthes aspera* root, stem and leaves extract.

Table-1. Determination of antioxidant activity with 2,20 -diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

S. No	Concentration (µg/ml)	Scavenging Activity (%)		
		R.E	L.E	S.E
1	100	31.61	22.45	12.18
2	200	37.82	34.13	27.63
3	300	54.92	46.92	34.46
4	400	59.59	53.01	45.03
5	500	66.32	57.74	51.09
IC_{50} (µg/ml)		299.4	379.75	467.31

R.E: Root Extract; L.E: Leaf Extract; S.E: Stem Extract

Determination of antioxidant activity with 2,20 -diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method :

The results of the antioxidant activity of extracts of *A. aspera* roots, stem and leaves were determined using DPPH Assay. The results obtained are shown in Table-1. The dried samples obtained from the extract were dissolved at a 10 different concentration in a range of 100-500 (µg/ml). Antioxidant potential of the extracts was measured by DPPH radical scavenging activity. The results (shown in table-1) are expressed as % inhibition of DPPH and its IC_{50} value.

A. aspera root extract showed higher percentage scavenging activity (66.32%) at 500 (µg/ml) as compared to stem (51.09%) and leaves (57.74%) extract. The DPPH radical scavenging activity was found to be increasing as the dose of the extract increases in all three cases. IC50 for for root, leaf and stem extract was found to be 299.4 (µg/ml), 379.75 (µg/ml) and 467.31 (µg/ml) respectively. Earlier, roots and leaves have been reported to possess DPPH radical scavenging activity with IC50 values 241.86 µg/ml and 129.91 µg/ml respectively, where root extract results were found similar (299.4 µg/ml) to this study while leaf extract showed much elevation in the IC50 concentration. Rao *et al.*[30] observed the radical scavenging activity of seed ethanol and root ethanol extracts using DPPH assay, it showed an increase in percentage inhibition at100 µg/ml, whereas the seed aqueous and root aqueous showed at 500 µg/ml.

Ferric reducing-antioxidant power (FRAP) assay :

The second method used for analysis of the antioxidant property of *A. aspera* root, stem and leaf extracts was Ferric reducing-antioxidant power (FRAP) assay. The method is used to assess plant's ability to reduce the ferric ions. The colorless Fe+3 form is oxidized to blue-colored Fe+2 tri-pyridyltriiazine (TPTZ) compound. This causes shifts in absorbance at 593 nm which was measured as Optical Density (O.D.). This assay shows linear relationship of molar concentration and antioxidant property.

Table-2. Determination of antioxidant activity with Ferric reducing-antioxidant power (FRAP) assay

S. No	Concentration (µg/ml)	Scavenging Activity (%)		
		R.E	L.E	S.E
1	100	21.2	31.4	34.83
2	200	27.41	37.66	59.55
3	300	38.3	46.9	61.79
4	400	51.11	54.57	79.78
5	500	59.26	51.9	89.88
IC_{50} (µg/ml)		405.71	395.28	184.15

R.E: Root Extract; L.E: Leaf Extract; S.E: Stem Extract

The scavenging activity estimated in the extracts of *A. aspera* using Ferric reducing-antioxidant power (FRAP) assay showed best results in case of stem extract (89.88%) at 500 (µg/ml) followed by root extract (51.9%) and lastly leaf extract (59.26%) (Table-2). Sharma *et al.*[36] reported a dose-dependent relationship in the reducing antioxidant power, the power increased as the concentration increased for both parts (root and inflorescences) of *A. aspera*

The 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay :

The result of the determination of antioxidant activity in root, stem and leaf extract of *A.aspera* are demonstrated in the table given below (Table:3). The results observed are quite variable from results obtained by the other two methods of antioxidant activity, DPPH, and FRAP assay. The method showed comparable percentage inhibition in the root (86.81%), leaf (85.23%) and stem (85.71%) extracts. IC50 values of the extract were quite significant at 137.96 (µg/ml), 202.94 (µg/ml) and 104.02 (µg/ml). Although, the pattern of the dose-dependent increase was similar as reported in earlier studies, percentage inhibition is higher.

Antibacterial Activity :

In this study, we reported the antimicrobial screening of root, stem and leaf extract of *A. aspera* against skin infection causing isolates. The antimicrobial activity analysis was done using Disk diffusion method against some of the bacterial species which have their major role in causing skin diseases viz., *S.pyogenes* (ATCC 19615), *S. epidermidis* (ATCC 12228), *P.acne* (ATCC 11827). The plant extract was extracted consequently in solvents as mentioned earlier in methodology. The screening showed some significant results at higher concentrations of the extract. A series of concentrations were used for the assay ranging between 11-187.5 µg/µl.

Out of the three extracts root (R.E), stem (S.E) and leaves (L.E), root extract was observed to possess potent activities (table-4) with maximum antibacterial activity at highest concentration (187.5mg/ml). The extract showed pronounced inhibitory action with clear

Table-3. Antioxidant capacity by The 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay of different extracts of spices extract:

S. No	Concentration (µg/ml)	Scavenging Activity (%)		
		R.E	L.E	S.E
1	100	47.25	31.82	51.43
2	200	57.14	57.95	58.09
3	300	62.64	59.09	63.8
4	400	76.92	77.27	77.14
5	500	86.81	85.23	85.71
IC_{50} (µg/ml)		137.96	202.94	104.02

R.E: Root Extract; L.E: Leaf Extract; S.E: Stem Extract

zone of 11mm *(S. pyogenes)*, 16mm (*P. acne*) and 14mm (*S. epidermidis*). Significant activity demonstrated as compared to the standard drug levofloxacin (500ppm) with zones 21mm, 23mm and 24mm respectively. Other extracts of stem and leaf exerted only a weak or moderate effect against the tested organism at the tested concentrations.

Fig 1: Bacteria: *P. acne*; Results obtained in the disc diffusion assay; antibacterial activity is expressed as clear zone of inhibition by the extract and its diameter around the discs is mentioned in the table. Size of inhibition zones were including the sterile blank discs 6 millimeter (mm) in diameters. Absence of bacterial inhibition indicates (-), Positive control:500ppm levofloxacin antibiotics; Negative control:DMSO (100%)

Table-4. Antibacterial activity of A.aspera root, leaf and stem extract against *Propionibacterium acne*

S. No.	Name	Zone of Inhibition mm		
		L.E.	S.E.	R.E.
1	(-) control	-	-	-
2	23.438mg/ml	11	12	12
3	46.875mg/ml	11	13	13
4	93.75mg/ml	13	14	14
5	187.5mg/ml	15	15	16
6	(+) control	23	23	23

*Positive control:500ppm levofloxacin antibiotics; Negative control:DMSO (100%)

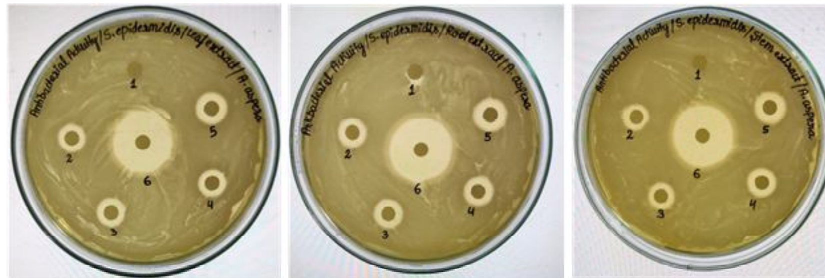

Fig 2: Bacteria: *S. epidermidis*; Results obtained in the disc diffusion assay; antibacterial activity is expressed as clear zone of inhibition by the extract and its diameter around the discs is mentioned in the table. Size of inhibition zones were including the sterile blank discs 6 millimeter (mm) in diameters. Absence of bacterial inhibition indicates (-), Positive control:500ppm levofloxacin antibiotics; Negative control:DMSO (100%).

Table-5. Antibacterial activity of *A. aspera* root, leaf and stem extract against *S. epidermidis*

S. No.	Name	Zone of Inhibition mm		
		L.E.	S.E.	R.E.
1	(-) control	-	-	-
2	23.438mg/ml	9	10	11
3	46.875mg/ml	10	11	12
4	93.75mg/ml	11	12	12
5	187.5mg/ml	13	13	14
6	(+) control	24	24	24

*Positive control:500ppm levofloxacin antibiotics; Negative control:DMSO (100%)

Fig 3: Bacteria: *S. pyogenes*; Results obtained in the disc diffusion assay; antibacterial activity is expressed as clear zone of inhibition by the extract and its diameter around the discs is mentioned in the table. Size of inhibition zones were including the sterile blank discs 6 millimeter (mm) in diameters. Absence of bacterial inhibition indicates (-), Positive control:500ppm levofloxacin antibiotics; Negative control:DMSO (100%).

Table-6. Antibacterial activity of A.aspera root, leaf and stem extract against *Streptococcus pyogenes*

S. No.	Name	Zone of Inhibition mm		
		L.E.	S.E.	R.E.
1	(-) control	-	-	-
2	23.438mg/ml	9	9	9
3	46.875mg/ml	9	10	9
4	93.75mg/ml	10	11	10
5	187.5mg/ml	11	12	11
6	(+) control	21	21	21

*Positive control:500ppm levofloxacin antibiotics; Negative control:DMSO (100%)

Prasad *et al.* showed in their study, antibacterial properties of leaves and callus extract of this plant in various solvents[28] Prabhat *et al.*(2005) reported that methanolic extracts possess antimicrobial activity while Khan *et al.*[20] reported that the ethanol and chloroform extracts of the seeds of *A. aspera* show mild-to-moderate antibiotic activity against *Bacillus subtilis*, *E. coli*, and *Pseudomonas aeruginosa*.

FTIR :

The *A. aspera* root, leaves and stem extract was also used to identify their functional group using FTIR Spectra. These functional group thus helped to identify the active compounds of the extract using peak values from IR region[15]. The characteristic absorption peaks of root, stem and leaves extract are shown in table 7.

Table-7. FTIR peak values and functional groups in root, leaf and stem extract of A.aspera

S.No.	Sample/extract	Wavenumber (cm^{-1})	Functional group
1 (a)	Root extract	2365.39	O=C=O (Carbon dioxide) stretching
		2324.44	
		2108.9	
1 (b)	Root extract	2365	Isocyanate, nitrile, alkyne, thiocyanate
		2324	
1 (c)	Root extract	1541.3	Nitro compounds
		1516.20	
2	Leaf extract	522.68	Conjugated halides
		488.60	
3	Stem extract	3289.79	Carboxylic Acid
		1636.68	Conjugated alkene
		500.700	Conjugated halides

The FTIR spectrum (Fig 4) and the wavenumber (cm^{-1}) mentioned in the table (table 7) shows the presence of functional groups in three extracts of *A. aspera*. The root extract shows the presence of band at 2365.39 cm^{-1}, 2324.44 cm^{-1} and 2108.9 cm^{-1} indicating O=C=O (Carbon dioxide) stretching. This extract also showed band at 2365 cm^{-1} and 2324 cm^{-1} confirming the presence of Isocyanate, nitrile, alkyne, thiocyanate, bands at 1541.3 cm^{-1} and 1516.20 cm^{-1} for nitro functional groups. In the case of Leaf extracts, bands were observed at 522.68 cm^{-1} and 488.60 cm^{-1} indicating Conjugated halides. The band at 3289.79 cm^{-1}, 1636.68 cm^{-1} and 500.700 cm^{-1} showing the presence of functional groups Carboxylic Acid, Conjugated alkene and Conjugated halides in stem extract.

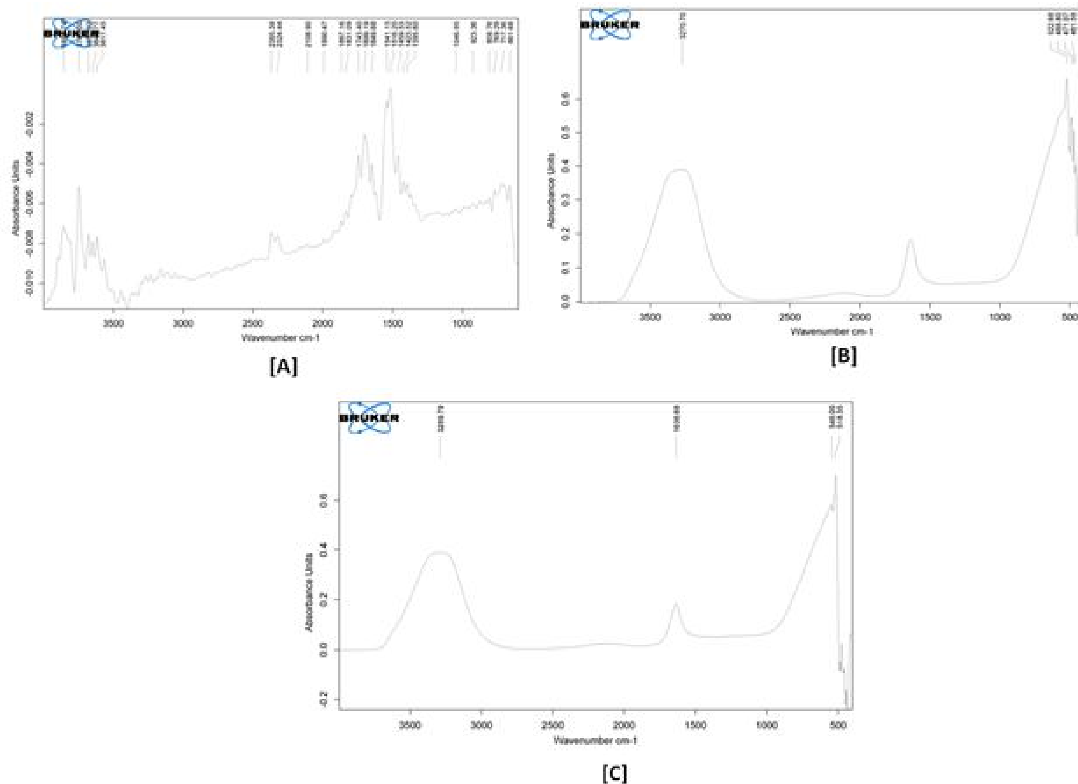

Fig 4: FTIR Spectrum of *A. aspera* extract [A]Root Extract [B] Leaf Extract [C]Stem Extract

This study investigated *A. aspera* plants for its antioxidant and -antibacterial activities. The crude extract of three parts i.e., root, stem, and leaf was extracted using Hexane, Ethyl acetate and then Methanol in Soxhlet. The antioxidant activity were tested using three methods DPPH Assay, FRAP, and ABTS method, the results were compared to assess the most potential scavenging agent among the three. Antibacterial activity was tested against *S. pyogenes*, *S. epidermidis,* and *P. acne*. The root extract exhibited the highest inhibitory action among three with the highest zone of inhibition. The DPPH radical scavenging activity was found to be increasing as the dose of the extract increases in all three cases. The scavenging activity estimated in the extracts of A. aspera using Ferric reducing-antioxidant power (FRAP) assay showed the best results in the case of stem extract at 500 µg/ml followed by root extract and lastly leaf extract. Out of the three extracts root (R.E), stem (S.E) and leaves (L.E), root extract was observed to possess potent activities with maximum antibacterial activity at the highest concentration. The extracts were also screened for their active components by analyzing the functional groups using their FTIR Spectrum. The information can be further used in deep studies of *A. aspera* to

utilize their antioxidant and antibacterial potency for social benefits.

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