



Antioxidant and Antibacterial Activities of Leaf Extract of *Achyranthes aspera* Linn. (Prickly Chaff Flower)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GP and KKV designed and performed the experimental work. Author SSG performed the animal experimental work. Authors CVR and MS did the proof reading. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: The present study was undertaken to establish the potential role of *Achyranthes aspera* Linn for cure of skin diseases.

Study Design: The plant is traditionally used by various tribes for curing a wide range of diseases. A 50% ethanolic extract of the leaves was subjected to phytochemical studies and further investigated for *in vitro* antioxidant and antibacterial activities.

Place and Duration of Study: CSIR-National Botanical Research Institute (NBRI), Lucknow, between December 2012 and November 2013.

Methodology: *In vitro* antioxidant activity was determined by DPPH free radical scavenging assay, hydroxyl radical scavenging activity, β -Carotene-linoleic acid assay and reducing power assay. Antibacterial activity was studied by agar well diffusion method.

Results: The total phenol and flavonoid content was estimated to be 3.363% and 6.36% respectively. The HPTLC analysis showed the presence of oleanolic acid, lupeol and β -sitosterol. The free radical scavenging activity of the extract was concentration dependent

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and IC₅₀ was observed at a concentration of 62.24µg/ml for DPPH free radical scavenging activity and 68.32µg/ml for hydroxyl radical scavenging activity. The extract showed significant total antioxidant activity and reducing power. Antibacterial activity was studied by well diffusion method and the MIC was recorded at 0.75 mg/ml for *S. aureus*, 0.8 mg/ml for *M. luteus*, 2.75 mg/ml for *E. coli* and 0.8 mg/ml for *P. aeruginosa*.

Conclusion: The results obtained from current study demonstrate that the leaf extract of *Achyranthes aspera* L possess significant antioxidant and antibacterial properties. Presence of various classes of phytochemicals e.g. Phenols, flavonoids, saponins, alkaloids etc. contribute highly to its medicinal values, thus indicating its potential for cure of skin diseases.

Keywords: *Achyranthes aspera*; skin diseases; DPPH; antioxidant; antibacterial; reducing power.

1. INTRODUCTION

Medicinal plants used by traditional healers have long served mankind as the primary source of therapeutic aids. In spite of the great march of synthetic products in to modern medicine, half of the world's medicinal compounds are still derived or obtained from plants. Mostly herbs contain secondary metabolites like polyphenols, flavonoids, triterpenoids etc. which have significant antioxidant and antibacterial properties. About 17000-18000 flowering plant species are known in India, out of which about 6000-7000 have been known to be used as folk medicines [1]. Some of the most important drugs which have revolutionized the modern medicare systems have been isolated first from the medicinal plants used by primitive or ancient societies. These wonder drugs include the curare alkaloids, penicillin and other antibiotics, cortisone, reserpine, podophyllotoxin and other therapeutic agents. Drugs or pharmaceutical products isolated from plants are at times found to be less costly than synthetic drugs. Well known examples of such products are atropine, digoxin and morphine [2]. Natural products may also be used as building blocks for the synthesis of 'semi synthetic' drugs; this is the case with plant saponins that can be extracted and easily altered chemically to produce sapogenins for the manufacture of steroidal drugs. Furthermore, the medicinal plants may give the chemical blueprints for the development of related synthetic drugs; for example cocaine from *Erythroxylum coca* which provides the chemical structure for the synthesis of procaine and other related local anaesthetics.

Achyranthes aspera Linn (Prickly chaff flower) (Amaranthaceae) is traditionally known to cure various diseases. It is pungent, antiphlegmatic, antiperiodic, diuretic, purgative and laxative, useful in oedema, dropsy, piles, boils and eruptions of skin etc. Infusion of plant is taken during pneumonia; infusion of roots is a mild astringent during bowel complaints. Plant inflorescence is boiled in water and sieved and taken orally to cure jaundice. Paste of roots in water is used to cure ophthalmia and opacities of cornea [3]. Triterpenoid saponins possessing olealonic acid as aglycone, alkaloids, sterols and phenols are the major class of phytoconstituents present in the plant [4]. An alkaloid achyranthine, amino acids, arginine, histidine, lysine, cystine, threonine, methionine, lucine, isolucine, phenylalanine, tryptophan and carbohydrate, valine, arhamnopyranosyl, β-D gluuronopyranosyl, β-D galactopyranosyl, galactose, xylose, rhamnose and glucose, large amount of potash, hormones, ecdysterone and inokosterone have been reported in the plant [5-8]. The plant shows antibacterial [9], anti-inflammatory [10] and abortifacient activity [11]. *A. aspera* elevates thyroid hormone level and is also reported to have anticoagulant, antiarthritic, antitumor and anti-hepatocarcinogenic activity. The plant is also reported to possess antidepressant, wound healing and antinociceptive activity [12].

The current study aims to establish the potential of *A. aspera* for curing skin diseases. Skin disease is a common disorder of predominantly the superficial layers of skin which affects all age groups. Infectious skin diseases like scabies, pediculosis capitis, tinea capitis, contact dermatitis and non-infectious skin ailments like dermatitis are of common occurrence in India due to low socioeconomic status, climatic factors and poor hygiene. An important group of skin pathogens are *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Candida species etc.* Understanding the potential of *A. aspera* as a cure for skin diseases can help in development of cost effective medicine as the plant is abundantly grown and has been traditionally used by various tribes.

2. MATERIALS AND METHODS

2.1 Sample Collection and Extraction

Leaves of *Achyranthes aspera* L were collected from Lucknow district, Uttar Pradesh (India) during the month of January, 2012. The voucher specimen was identified, authenticated and submitted at CSIR-National Botanical Research Institute, Lucknow (Herbarium No. NBRI 98577). Leaves of the collected plants were washed thoroughly with distilled water and shade dried for ten days. A 1000 g dried leaves were ground to a fine powder using mixer grinder and subjected to extraction thrice in 50% ethanol using cold maceration technique. The extract was concentrated in rotary vacuum evaporator and stored at 4°C until further use (yield = 8.24%).

2.2 Phytochemical Analysis

2.2.1 Qualitative analysis of phytochemicals

The *Achyranthes aspera* leaves extract (AALE) was subjected to preliminary phytochemical screening. Presence of alkaloids (Mayer's test), flavonoids (alkaline reagent test), tannins (Braymer's test) carbohydrates (Molisch's test), glycosides (Liebermann's test), saponins (Salkowski test), triterpenoids (Liebermann Burchard test), proteins and amino acids (Ninhydrin test) were tested in AALE.

2.2.2 Identification of phytoconstituents in AALE using HPTLC fingerprinting

The HPTLC plates are coated with high performance silica gel which is of very small and uniform in size (about 5 µm). These high performance silica gels give more efficient and reproducible separation than conventional grades of silica gel. A known quantity of test and standard solutions were applied on a precoated silica gel GF254 plate of uniform thickness (0.2 mm) with the help of LINOMAT 5 applicator attached to CAMAG HPTLC system. The plate was developed in the solvent system toluene: ethyl acetate: formic acid (9:1: 0.1). The plate was scanned densitometrically by CAMAG Scanner 3 by using software WinCats (3.1.1) and fingerprint profile was recorded. Standard peak of the reference marker compounds were scanned for their spectral analysis at the range of 200-700 nm wavelength and λ_{max} was recorded. Identification of all the marker compounds in extract were confirmed by overlaying absorption spectra at three different levels, i.e. peak start, peak apex and peak end position of the spot of the respective marker compounds.

2.2.3 Quantitative analysis of total phenol and total flavonoid content

The total phenol content was determined using Folin-ciocalteau reagent and the total flavonoid content was estimated using aluminium chloride method [13].

2.3 Determination of *in vitro* Antioxidant Activity

2.3.1 DPPH free radical scavenging assay

The free radical scavenging activity of AALE on stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was estimated by method mentioned by Brand-Williams et al. [14]. Briefly, 2 ml of AALE at varying concentrations (50µg/ml to 250µg/ml) was mixed with 2.0 ml of DPPH solution in methanol (0.004% w/v). The mixture was allowed to stand at room temperature in dark for 20 min. Then the mixture was vortexed and absorbance was recorded at 517 nm using spectrophotometer. Ascorbic acid was used as a reference standard and control consisted of DPPH solution without extract. The test was performed in triplicate and percentage scavenging of DPPH free radical by extract was calculated using the equation: $(A_{\text{control}} - A_{\text{test}}) / A_{\text{control}} \times 100$, where A_{control} is the absorbance of control and A_{test} is the absorbance in presence of extract or standard.

2.3.2 Hydroxyl radical scavenging activity

The assay was done as described by Kumar et al. [15]. Hydroxyl radical was generated by the Fe^{3+} -ascorbate-EDTA- H_2O_2 system (Fenton reaction). The reaction mixture contained, in a final volume of 1 ml, 2-deoxy-2-ribose (2.8 mM); KH_2PO_4 -KOH buffer (20 mM, pH 7.4); FeCl_3 (100 µM); EDTA (100 µM); H_2O_2 (1.0 mM); ascorbic acid (100 µM) and various concentrations of AALE and ascorbic acid (50 µg/ml to 250 µg/ml). The reaction mixture was incubated for 1 h at 37°C and 0.5 ml of the mixture was added to 1 ml of 2.8% TCA followed by addition of 1 ml of 1% aqueous TBA and the mixture was incubated at 90°C for 15 min. The absorbance was recorded at 532 nm. Percentage inhibition was calculated according to the formula: $(A_{\text{control}} - A_{\text{test}}) / A_{\text{control}} \times 100$, where A_{control} is the absorbance of control and A_{test} is the absorbance in presence of extract or standard.

2.3.3 β-Carotene–linoleic acid assay

β-carotene bleaching assay was done according to methods mentioned by Wettasinghe and Shahidi [16]. A 1 ml of β-carotene solution (0.2 mg/ml in chloroform) was pipetted into a round bottom flask containing 0.02 ml of linoleic acid and 0.2 ml of 100% Tween 20. The mixture was evaporated in a rotary vacuum evaporator for 10 min to remove chloroform. After that the mixture was immediately diluted with 100 ml of distilled water with vigorous shaking to form an emulsion. Varying concentrations of extract and standard (100 µg/ml to 500 µg/ml) was added to 5 ml of the emulsion in different test-tubes and the mixture was kept at 37°C for 1 h. Absorbance of sample and control was measured at time $t=0$ and $t=60$ min. Total antioxidant activity was calculated based on the following equation:

$$AA = [1 - (A_t - A_t^0) / (A_0 - A_0^0)] \times 100$$

Where AA is antioxidant activity, A_0 and A_0^0 are the absorbance values measured at the initial incubation time for samples and control respectively while A_t and A_t^0 are the absorbance values measured in the samples or standards and control at $t=60$ min.

2.3.4 Reducing power assay

The reducing power of AALE was determined by method mentioned by Jayanthi and Lalitha 2011 [17]. Substances possessing reducing power react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}) which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Varying concentrations of plant extract and standard (50 $\mu\text{g/ml}$ to 250 $\mu\text{g/ml}$) were mixed with phosphate buffer (2.5 ml) and potassium ferrocyanide (2.5 ml). The mixture was kept at water bath at 50°C for 20 min. After cooling 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. The upper layer (2.5 ml) of the resulting solution was mixed with distilled water (2.5 ml) and freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Ascorbic acid at various concentration was taken as standard. Increased absorbance indicated the increased reducing power of extract and standard.

2.4 Determination of *In vitro* Antibacterial Activity

The antibacterial activity was tested using agar well diffusion method according to Lino A et al. [18] and Arshad H et al. [19]. The MTCC cultures were obtained from department of Pharmacognosy, CSIR-NBRI. The AALE was tested for its antibacterial property against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Micrococcus luteus*.

2.4.1 Agar well-diffusion assay

A 1 ml of test culture (10^7 CFU/ml) was inoculated into a sterile plate with 20 ml Muller Hinton agar which was then made to solidify. Three wells of approximately 6 mm diameter were made on the surface of agar plate using a sterile cork- borer. Stock solution of AALE was dissolved in DMSO at varying concentrations (0.25 to 1 mg/ml). A 50 μl extract of each concentration was pipetted in the well. A 50 μl DMSO served as negative control and 10 μg of streptomycin served as positive control respectively. The plates were then incubated at 37°C for 24 h and the zone of inhibition was recorded.

2.4.2 Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was estimated by using various concentrations of the extract (0.5-5 mg/ml) to record the lowest concentration that inhibited the growth of bacteria giving visible zone of inhibition after incubation of 24 h [20].

2.5 Statistical Analysis

All the data were presented as mean \pm SEM and analyzed by Wilcoxon Sum Rank Test [21] and unpaired Student's t-test for the possible significant interrelation between the various groups. A value of $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Phytochemical Studies

Preliminary phytochemical estimation showed the presence of sugars, proteins, alkaloids, tannins, saponins, glycosides, triterpenoids, flavonoids and phenols. Quantitative analysis (Table 1) showed significant flavonoid, phenol and sugar content while the starch and tannin

content was comparatively low. The HPTLC profile reveals the presence of oleanolic acid, β -sitosterol and lupeol at R_f 0.25, 0.40 and 0.57 respectively (Fig. 1).

Table 1. Quantitative analysis of *Achyranthes aspera* leaves (% content in dry leaf powder)

Physicochemical parameters	Percentage content
Sugar	3.49±0.277
Starch	0.173±0.015
Tannin	0.501±0.043
Phenols	3.363±0.158
Flavonoids	6.36±0.052

Values are mean \pm SD of three determinations

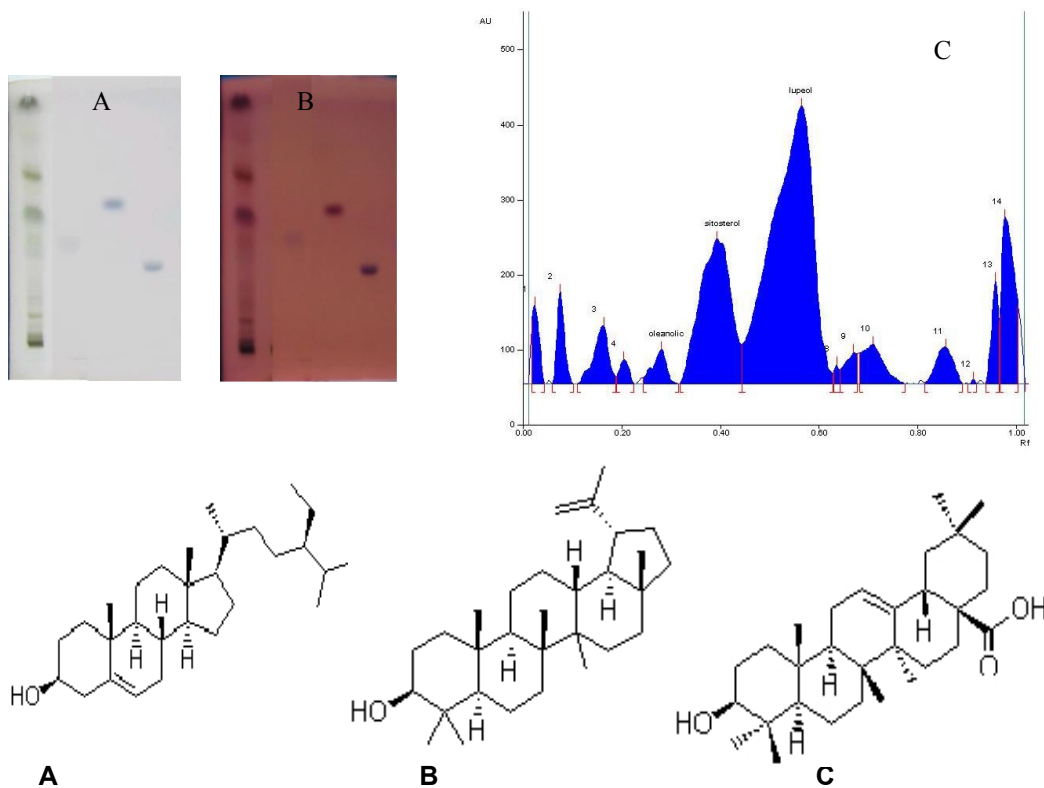


Fig. 1. HPTLC fingerprint profile of *Achyranthes aspera* leaves (A) under visible light after derivatization (B) UV 366 nm after derivatization [Lanes: 1-50% ethanolic extract; 2- β -Sitosterol; 3-Lupeol; 4-Oleanolic acid]; (C) densitometric scanning profile of extract along with oleanolic acid, β -sitosterol and lupeol marker components; (D) β -Sitosterol; (E) Lupeol structure; (F) Oleanolic acid structure

*Chemical structures sourced from www.chemblink.com

3.2 *In vitro* antioxidant activity

The *in vitro* antioxidant activity was tested by four models and the results suggested significant antioxidant activity of AALE. The free radical scavenging activity of the extract was concentration dependent and IC₅₀ was observed at a concentration of 62.24µg/ml for DPPH free radical scavenging activity and 68.32µg/ml for H₂O₂ free radical scavenging activity. The DPPH free radical and hydroxyl radical scavenging activity of AALE was comparable to the standard ascorbic acid (Fig. 2). Total antioxidant activity estimated by β-carotene bleaching assay was found to be significantly high in AALE as compared to ascorbic acid (Fig. 3). The reducing power of the plant extract increases with increasing concentration which shows that the antioxidant compounds are electron donors and can oxidize intermediates of lipid peroxidation process (Fig. 4).

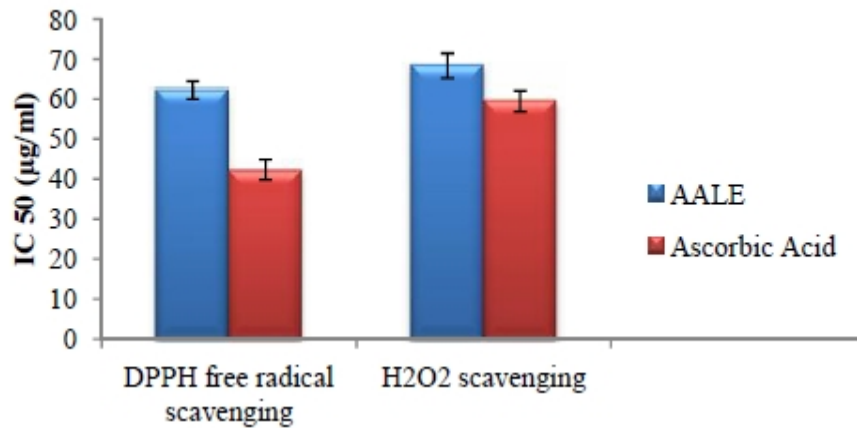


Fig. 2. The effects of AALE and ascorbic acid (standard) on scavenging of DPPH and H₂O₂ free radical. The activity is presented as concentration of AALE and ascorbic acid required for 50% inhibition of DPPH and H₂O₂ free radicals.

Values are mean ± SD of three determinations.

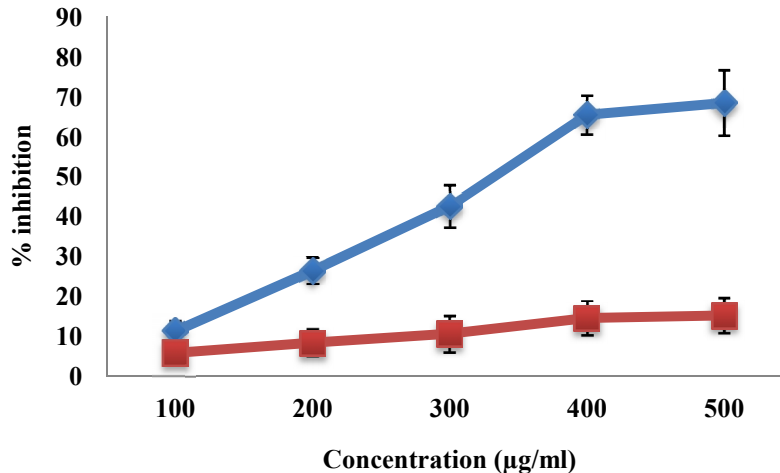


Fig. 3. The effect of varying concentrations of AALE and ascorbic acid on β-carotene bleaching assay.

Values are mean ± SD of three determinations.

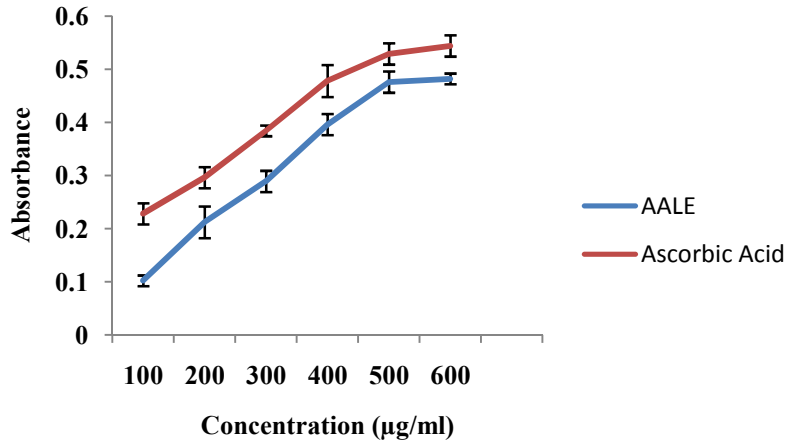


Fig. 4. The reducing power of AALE and ascorbic acid (standard).The absorbance was plotted against concentration of samples.

Values are mean \pm SD of three determinations.

3.3 Antibacterial Activity

AALE showed effective antibacterial activity against all four bacterial strains. Highest activity was observed against *S. aureus* and lowest activity was observed against *E. coli* (Table 2). The extract inhibited the growth of *S. aureus* at a concentration of 1 mg/ml, thus to calculate the MIC, the antibacterial effect of extract was observed at 0.25, 0.5 and 0.75 mg/ml. AALE was found to inhibit the growth of *S. aureus* at 0.75 mg/ml which was hence recorded as the MIC. Similarly, the effect of AALE against *M. luteus* was observed at 0.2, 0.4, 0.6 and 0.8 mg/ml, the growth was inhibited at 0.8 mg/ml. AALE did not inhibit the growth of Gram negative *E. coli* up to a concentration of 2 mg/ml, however the extract was effective at a concentration of 3 mg/ml. Thus the effect of extract on growth inhibition of *E. coli* was observed at varying concentrations (2.25, 2.5 and 2.75 mg/ml). The minimum inhibitory concentration was recorded at 2.75 mg/ml. The MIC of extract against *P. aeruginosa* was observed at a concentration of 0.8 mg/ml (Figs. 5 and 6).

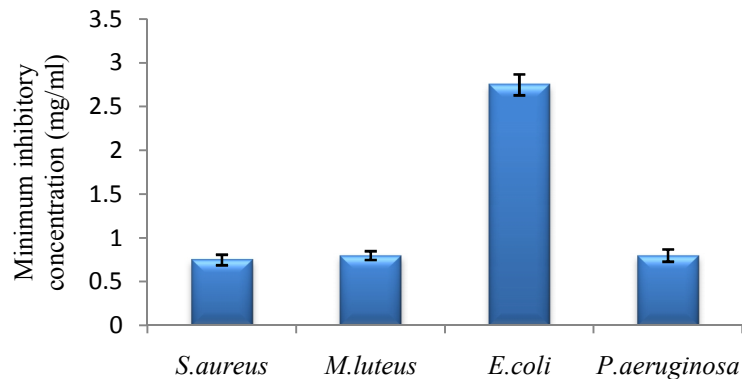


Fig. 5. Antibacterial activity of AALE recorded in terms of minimum inhibitory concentration of AALE required for growth inhibition of bacteria

Values are mean \pm SD of three determinations

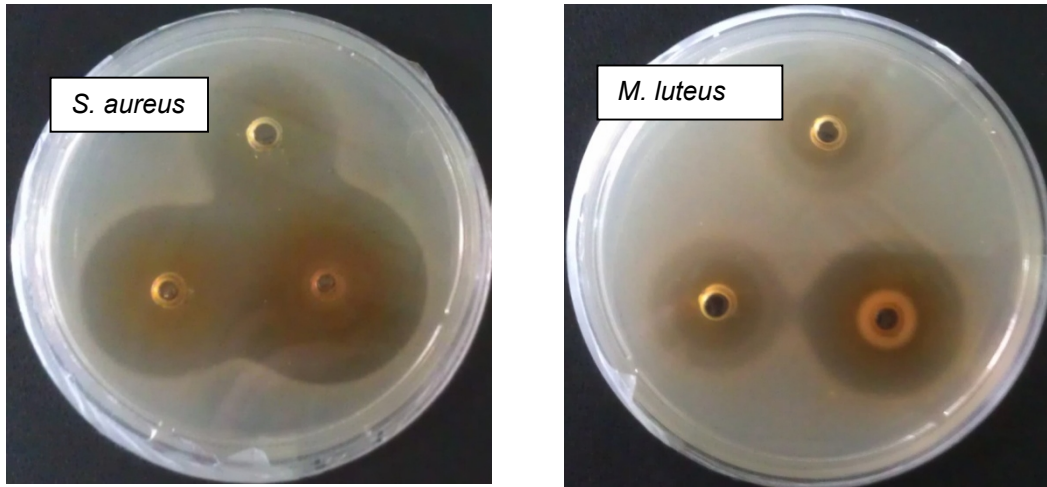


Fig. 6. Antibacterial activity of AALE against *S. aureus* at (a) 3 mg/ml; (b) 4 mg/ml; (c) 5 mg/ml and *M. luteus* at (a) 1 mg/ml; (b) 2 mg/ml; (c) 3 mg/ml by well-diffusion method

Table 2. Antibacterial effect of AALE against different bacteria

	Conc (mg/ml)	Zone of inhibition (mm)
<i>S. aureus</i>	1	8.2±0.52
	2	11.3±1.24
	3	16.4±2.28
	4	20.4±2.86
	5	21.3±2.65
<i>M. luteus</i>	1	7.1±1.24
	2	10.2±1.37
	3	14.2±2.36
	4	16.3±3.24
	5	18.2±2.13
<i>E. coli</i>	1	0±0
	2	0±0
	3	6.4±1.32
	4	8.7±1.56
	5	10.3±1.86
<i>P. aeruginosa</i>	1	6.5±1.25
	2	8.3±1.1
	3	11.3±2.43
	4	13.5±2.18
	5	15.6±3.24
		Streptomycin (10µg)
<i>S. aureus</i>		14.2±0.3
<i>M. luteus</i>		13.6±0.6
<i>E. coli</i>		11.5±0.8
<i>P. aeruginosa</i>		16.4±0.3

4. DISCUSSION

At present, the study of ethnomedicinal plants has become increasingly valuable in the development of health care and conservation programs in different parts of the world. Medicinal plants used by traditional healers have long served mankind as the primary source of therapeutic aids. The herbal extracts are known to exert their medicinal effect by synergistic action of their several constituents acting at single or several target sites [22]. This study provided bases to the folkloric use of *Achyranthes aspera* leaves for cure of skin diseases. Over past decades the prevalence of atopic dermatitis and allergic or irritant contact dermatitis has increased significantly among the general population, causing considerable economic costs and decreased quality of life [23-25]. Synthetic corticosteroids used as topical applications have been the first choice therapy for treatment of these inflammatory skin diseases such as eczema, atopic dermatitis and psoriasis. While effective in many patients, this form of therapy carries the concern for local and systemic adverse effects and may induce skin atrophy, especially after long use [26-27]. Triterpenoids represent a very large class of plant secondary metabolites which have been demonstrated to exhibit a variety of biological activities including anti-inflammatory activities. Phenols, flavonoids and tannins are established antioxidants and contribute to human health.

HPTLC studies of AALE indicate the presence of β -sitosterol, lupeol and oleanolic acid. β -sitosterol is a natural antioxidant. It is one of the most common dietary phytosterols found and synthesized exclusively in plants. Lupeol, a pentacyclic triterpene, is a biologically active constituent that has received much attention due to its wide spectrum of medicinal properties, most importantly, strong anti-inflammatory effects. Lupeol has been extensively studied for its inhibitory effects on inflammation under *in vitro* studies and *in vivo* models of inflammation [28]. The anti-inflammatory potential of lupeol could be assessed from the observation that lupeol pre-treatment significantly reduced prostaglandin E₂ (PGE₂) production in A23187-stimulated macrophages [29]. Results of these studies support the view that lupeol present in AALE may play a significant role in curing skin ailments particularly those related to skin allergy and inflammation. Oleanolic acid was also observed in AALE; acetate derivative of this phyto-constituent inhibits atopic dermatitis and atopic contact dermatitis, thus suggesting that it may be effective in treating allergic skin disorders [30]. Plant extracts containing oleanolic acid are useful in skin care and have been a constituent of many skin care products. The collagen fibre structure damaged on exposure to UV-B can be restored by topical application of oleanolic acid [31]. The effect of antioxidants on DPPH free radical scavenging is thought to be due to their hydrogen-donating ability. The presence of phenols and flavonoids indicate significant antioxidant potential of the plant. DPPH is a stable free radical with deep purple colour; after receiving proton form a proton donor, such as phenolic compounds, it loses its chromophore and becomes yellow [32]. The β -carotene bleaching assay is based on loss of the yellow colour of β -carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion. The rate of beta carotene bleaching can be slowed down in the presence of antioxidants. The principal was used in antioxidant activity evaluation of AALE. Though ascorbic acid is well known polar antioxidant but it showed low antioxidant activity in the β -carotene assay. This could be explained by the phenomenon of "polar paradox" which illustrates the paradoxical behaviour of antioxidants in different media and rationalizes the fact that polar antioxidants are more effective in less polar media while non-polar antioxidants are more effective in relatively polar media [33-34]. The polar antioxidants remaining in the aqueous phase of emulsion are more diluted in lipid phase and are thus effective in protecting linoleic acid [35].

Reducing power can serve as an indicator of antioxidant potential of the plant. Compounds possessing reducing power are electron donors and can reduce the oxidized intermediates of lipid peroxidation, thus acting as both, primary and secondary antioxidants. In this assay the yellow colour of test solution changes to various shades of green and blue, depending upon the reducing power of compound. Presence of reducer converts Fe^{3+} /ferricyanide to ferrous form and its concentration is recorded at 700 nm. A high content of phenols and flavonoids as confirmed by the phytochemical analysis supports the significant antioxidant activity of *Achyranthes aspera* leaves. Phenolics are believed to be capable of acting in redox-sensitive signalling cascades to inhibit DNA damage. The significant antioxidant potentials of AALE also substantiate its role as an anticancer agent [36-37].

The antibacterial activity showed that the extract was most effective against *S. aureus* followed by *M. luteus*, (Gram positive) *P. aeruginosa* and *E. coli* (Gram negative). The lipopolysaccharides on the outer membrane of Gram negative bacteria are usually impermeable to most antibacterial compounds [38]. This explains the low activity of AALE against *P. aeruginosa* and *E. coli*. Greater sensitivity of Gram positive bacteria towards AALE could be due to the presence of an outer peptidoglycan layer instead of LPS which is an ineffective permeability barrier. Thus the plant extract can enter more easily in the Gram positive bacterial cell and disturb the cytoplasmic membrane, the proton motive force (PMF), electron flow and active transport [19]. β -sitosterol is known to possess moderate to good antibacterial activity against *S. aureus*, *M. luteus*, *P. aeruginosa*, *E. coli* [39]. Triterpenoids (eg. lupeol and oleanolic acid) and flavonoids are known to cure skin diseases mainly due to their antibacterial activity [12]. The antimicrobial mechanisms of flavonoids can be classified as inhibition of nucleic acid synthesis, cytoplasmic membrane function and energy metabolism [40]. Antimicrobial action of phenolic compounds has also been reported. The action involves the alteration in permeability of cell membrane that could result in the uncoupling of oxidative phosphorylation, inhibition of active transport and loss of pool metabolites due to membrane damage [41]. The presence of hydroxyl group in phenolic compounds might influence their antimicrobial effectiveness by binding to active sites of enzymes, from hydrogen bonds with enzymes and alter their metabolism, and also the lipid solubility and steric hindrance of phenolic compounds might determine their antimicrobial activity [42].

5. CONCLUSION

The results obtained from current study demonstrate that the leaf extract of *Achyranthes aspera* L possess significant antioxidant and antibacterial properties. Presence of various classes of phytochemicals e.g. Phenols, flavonoids, saponins, alkaloids etc. contribute highly to its medicinal values, which could one of the reasons for its use in curing a wide range of diseases. The present study was done with an objective to provide scientific support to the traditional claims of various tribes regarding curative effects of *A.aspera* leaves on skin ailments and it provides a lead for further exploring the herb for drug development.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that the protocols were duly approved by Institutional Committee for Ethical use of Animals and Review Board (106/IAEC/RB/7-11), CSIR-NBRI, Lucknow.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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