Original Article

Phytochemical Screening, Antioxidant, Antibacterial, and Antidandruff Activities of Leaf Extract of *Artemisia indica*

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Abstract

Background: *Artemisia indica* is a traditionally used medicinal plant in the treatment of various conditions such as loss of appetite, abdominal discomfort, antimalarial infection, dermal wound infection, etc. **Objectives:** This study aims to determine the presence of phytochemical content, antioxidant, antibacterial, and antidandruff activity of leaf extract of *A. indica*. **Materials and Methods:** Dried ground leaves were subjected to a cold extraction method using an absolute concentration of methanol, ethanol, and water. Total phenolic, flavonoid, and proanthocyanidin content was estimated by using a linear regression equation from the calibration curve and expressed in terms of gallic acid equivalent (GAE) and rutin equivalent (RE). Antioxidant properties were determined using DPPH (1,1-diphenyl-2-picryl-hydrazyl), nitric oxide, and hydrogen peroxide assay, and their IC_{50} values were calculated. The antibacterial activity was tested using the agar well diffusion method against the common five pathogenic strains, and the zone of inhibition is compared with gentamicin (1 mg/mL) as a positive control. The minimum inhibitory concentration (MIC) value was obtained by the microbroth dilution method. The antidandruff assay was performed on *Malassezia furfur* by the disk diffusion method into Sabouraud dextrose agar overlaid with 1 mL of olive oil, and the MIC value was determined by the microtiter plate method. **Results:** The result showed that the *Artemisia* methanolic extract represents ample content of phenolics (248±3.29 mg/g of GAE), flavonoids (222.33±4.41 mg/g of RE), and proanthocyanidin (222.83±1.62 mg/g of RE equivalent). The antioxidant assay revealed that methanolic extract has the highest radical scavenging activity followed by aqueous extract and then ethanolic extract. The antibacterial activity of leaf extract shows MIC value ranging from 6 to 25 µg/mL against various human pathogenic bacteria. The antidandruff assay showed that MIC value of methanolic extract is lesser than that of ethanolic extract (350<400) mg/mL. **Conclusion:** The results concluded that leaf extract of *A. indica* contains phenolics, flavonoids, and proanthocyanidin and exhibits adequate antibacterial, antidandruff, and antioxidant activity.

Keywords: *Antibacterial, antidandruff, antioxidant, Artemisia indica, phytochemical screening*

Introduction

Traditionally used herbal plant genus *Artemisia* exceeds more than 400 species found in nature and it is named in honor of "Artemis" known as the Greek goddess. The common name includes "Wormwood," which is named due to wide application in the past for the treatment of worms (intestinal helminth). Geographically, *Artemisia* family is worldwide distributed, mainly abundant across the temperate zones of the Northern hemisphere, whereas few species are also found in the Southern hemisphere. *Artemisia* species are pungent in odor and bitter in taste due to the presence of essential oil named terpenoids. Phytochemically, about 839 compounds have been reported from 14 species of wormwood.[1]

Artemisia indica is considered a valuable medicinal plant due to its antihelminthic, antipromastigote, antioxidants, cytotoxic, antimicrobial, antimalarial, and antidiabetic activity.[1-5] The leaf infusion is also considered to enhance the appetite. The juice of the plant is used to treat diarrhea, dysentery, and abdominal discomfort associated with helminth infection.[1] Studies have shown that *A. indica* contains phytochemicals such as saponins, tannins, flavonoids, alkaloids, sterol, phenol, proanthocyanidin, and glycosides with various bioactivities.[6,7] Javid *et al.*[8] in their study have shown the significant antibacterial activity of *A. indica* against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi,* and *Staphylococcus aureus* and antifungal activity against *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus,* and *Fusarium* species. However, evidence for the antidandruff activity of this plant species in literature till

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date is scarce. This study aims for phytochemical screening and investigates *in vitro* antioxidant, antibacterial, and antidandruff activity of this plant.

Materials and Method

Specimen collection and preparation of extracts

The *A. indica* plant was collected from UN Park, Lalitpur district of Nepal. The identification of plant sample was confirmed by the National Herbarium and Botanical Laboratories, Department of Plant Resources located at Godavari, Lalitpur (Reg no.: 051-072/073, Ref no.: 101). Fresh leaf material was washed thoroughly under running tap water, shade dried, and used for extraction. The dried leaf was powdered to a fine powder and stored in airtight bottles. Twenty grams of powdered leaves were weighed and soaked in 200 mL of different solvents for 72 h with intermittent stirring for 30 min every 16–24 h using a magnetic stirrer. The solvents used to obtain extracts were methanol, ethanol, and water. The extracts were then filtered in a Whatman filter paper. The filtrate was taken and dried in rotatory evaporator at 70ºC.[9]

Qualitative phytochemical screening

In order to identify the phytochemical derivatives present in a leaf extract of *A. indica*, a standard procedure of phytochemical screening was performed. Presence of alkaloids was tested using Dragendorff's and Meyer's tests, amino acids by ninhydrin test, carbohydrates by Barfoed's and Fehling's tests, flavonoids by $FeCl_3$ and Shinoda's tests, glycosides by Libermann's test, saponin by froth test, tannins by $FeCl_3$, and terpenoids by Salkowski's test.[6,9]

Quantitative phytochemical screening

Determination of total phenolic contents

Total phenol contents in the extracts were estimated using the Folin–Ciocalteu method. Aliquots of 50 µL of 12.5, 25, 50, 100, 200, and 400 µg/mL gallic acid solutions were mixed with 0.1 mL Folin–Ciocalteu reagent (diluted 10-fold) and 0.1 mL (75 g/L) sodium carbonate. Incubation of mixture was done for 30 min and observed in a spectrophotometer at an absorbance of 765 nm. The calibration curve was made by plotting absorbance against concentration. The total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract.[10]

Determination of total flavonoid contents

The total flavonoid content was determined by the Kumaran and Karunakaran method.[11] The rutin calibration curve was prepared by mixing 2 mL of various concentrations of ethanolic solutions of rutin with 2 mL (20 mg/mL) of aluminum trichloride and 6 mL (50 mg/mL) of sodium acetate. The absorbance at 440 nm was read after 2.5 h. The same procedure was used for 2 mL of plant extract (10 mg/mL) instead of rutin solution. All determinations were carried out in duplicates. The content of flavonols was calculated using a standard curve obtained from various concentrations of rutin.

Determination of total proanthocyanidins

In this method, 1 mL of standard solution of concentration 0.5 mL (1 mg/mL) was mixed with 3 mL of 4% vanillin methanol solution and 1.5 mL of HCl: the mixture was allowed to stand for 15 min at room temperature and the absorbance was measured at 500 nm. A standard curve was constructed using rutin as standard. All the measurements were done in triplicates, and the total proanthocyanidin content was expressed as rutin equivalent (RE).[12]

Antioxidant assay

DPPH (1,1-diphenyl-2-picryl-hydrazyl)

The DPPH assay is performed for the assessment of free radical scavenging activity of an antioxidant molecule which is considered as the standard and easy colorimetric process for the scientific evaluation of antioxidant activity of the pure compounds. Five different concentrations of 100, 200, 300, 400, and 500 µg/mL are taken in a test tube. From each concentration, 1 mL of plant extract is mixed with 1 mL aliquot of 0.5 mM DPPH and incubated in the dark at 37°C for 30 min, and the absorbance was noted at 517 nm.[12] Ascorbic acid was taken as positive control.

The percentage of inhibition was calculated using the below formula:

$$\% \text{ of inhibition} = \frac{(A_c - A_s) \times 100}{A_c},$$

where A_c is the absorbance of DPPH solution and A_s is the absorbance of the sample after 30 min of incubation in the dark.

IC_{50} values were determined using GraphPad Prism software after obtaining percentage of inhibition.

Nitric oxide scavenging activity

The nitric oxide scavenging assay was performed according to the procedure done by Awah and Verla.[13] An aliquot of 5 mM of sodium nitroprusside was prepared in phosphate-buffered saline adjusting pH 7.4. At 1 mL of different concentrations (25, 50, 100, 200, 400 µg/mL) of methanolic, ethanolic, and aqueous extract of *Artemisia*, 0.3 mL of previously prepared 5 mM sodium nitroprusside solution was added. The test tubes containing all the above mixtures were incubated at 25ºC for 5 h, and then 500 µL of Griess reagent was used. The absorbance was noted at 546 nm. Quercetin was used as the standard solution to which effect of each extract (methanolic, ethanolic, and aqueous extracts) was compared. NO scavenging activity in terms of percentage was calculated as:

$$\% \text{ NO scavenging activity } = \frac{(A_c - A_s) \times 100}{A_c},$$

where A_C is the absorbance of the control (without extract or standard) and A_S is the absorbance of extract or standard.

Hydrogen peroxide scavenging assay

The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch *et al.*[14] To perform this assay, 43 mM of $H_2O_2$was prepared in phosphate-buffered saline (0.1 M, pH 7.4). *Artemisia* extract (100, 200, 300, 400, and 500 µg/mL) concentration in 3.4 mL of phosphate-buffered saline was added to 600 µL of H_2O_2 solution (43 mM). The absorbance value of the reaction mixture was noted at 230 nm. Sodium phosphate buffer without H_2O_2 served as blank, and quercetin was used as a standard for this assay.

The percentage of H_2O_2 scavenging and standard compounds was calculated using the following equation:

$$H_2O_2 \text{ scavenging effect } (\%) = \frac{(A_c - A_s) \times 100}{A_c},$$

where A_C is the absorbance of the control (without extract or standard) and A_S is the absorbance of the extract or standard.

Collection of test organisms

The pure cultures of the test organisms of bacteria were collected in a sterile nutrient agar slant from National Public Health Laboratory, Kathmandu, Nepal. The test bacterial organisms used were four Gram-negative bacteria *E. coli* (ATCC 25922), *Proteus mirabilis* (ATCC 15028), *P. aeruginosa* (ATCC 27853), and *Salmonella typhi* (ATCC 14028), and one Gram-positive bacteria *S. aureus* (ATCC 14028) for antibacterial assay. The bacterial turbidity was adjusted to 0.5 MacFarland, and pure culture of one of the dandruff-causing fungi, i.e., *Malassezia furfur*, was inoculated from the Dixon agar and collected in a sterile Sabouraud dextrose agar (SDA) plate.

Antibacterial assay

Bacterial growth inhibition was determined by using the agar well diffusion method. Test bacteria were spread on Muller-Hilton agar medium using a sterile cotton swab. About 6 mm diameter wells were made using a sterile cork borer. Dried methanolic, ethanolic, and aqueous extracts were dissolved in distilled water for making the concentration of 10 mg/mL, and then the required volume of each solvent was drawn with a micropipette and diluted in distilled water to prepare different volumes of each extract (30, 40, 50, and 60 µL) and loaded into the well. Pure distilled water was used as solvent control in the antimicrobial susceptibility test for all three different extracts, and broad-spectrum antibiotic gentamicin (1 mg/mL) was used as a positive control. The plates were incubated for 24 h at 37°C. After incubation, the mean zone of inhibition of four different volumes of same concentration (10 mg/mL) of extracts was measured (in mm). The final data were presented in form of mean ± standard deviation.[15]

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the methanolic, ethanolic, and aqueous extracts was determined by the microbroth dilution method using peptone water. For finding MIC value, 2-fold serial dilutions of plant extract were carried out (200, 100, 50, 25, 12.5, and 6.25 µg/mL). Tubes were incubated at 37°C for 24 h and observed for any visible growth by observing broth turbidity. The bacterial suspensions in peptone water were used as positive control, and uninoculated extracts in broth were used as negative control in a test tube. The MIC was interpreted as the lowest concentration of the extract that inhibited bacterial growth when compared with control tubes that contained only the extracts.[16]

Antidandruff assay

Test organism

The fungal strain was tested with 10% H_2O_2 to investigate the presence of catalase which is an indicator of *Malassezia* spp. The catalase test was performed by using 10% H_2O_2 in a clean test tube in which inoculation of *Malassezia* spp. Results in the formation of oxygen bubbles. Inoculation was done on SDA plates incorporated with 1 mL of olive oil for providing source of lipid, and SDA was added with penicillin and streptomycin combination (PenStrep) in order to inhibit bacterial growth. The plates were incubated for 48 h at 32°C after which the formation of yellowish-creamy colonies which are typically smooth to slightly wrinkled were examined and studied.[17]

Evaluation of antidandruff assay

Methanolic, ethanolic, and aqueous extracts of *A. indica* were subjected to antifungal screening by the agar disk diffusion method. SDA plates which were overlaid with 1 mL of olive oil were inoculated with the test isolate by spreading the standardized inoculum on the surface of the agar plate with sterile swab. Five-millimeter diameter plates were punched onto the sterile filter paper and soaked with 20 µL of each of the extract solutions at 100 mg/mL concentration. Fluconazole disc (40 mg/mL) is used as positive control and 10% dimethyl sulfoxide (DMSO)-containing disk alone was used as a negative control for antifungal assay. The disks were dried for 7 h and placed onto the agar plates. The incubation was done at 32°C for 48 h due to optimum growth temperature for *Malassezia* spp. The antimicrobial activity was observed by measurement of zone of inhibition, and the assay was performed in triplicates to calculate mean and standard deviation.[17]

Determination of MIC

The methanolic, ethanolic, and aqueous extracts from the leaves of *A. indica* were performed using broth microdilution method for determination of MIC values.[18] The 96-well microtiter plate was used and prepared by pipetting 95 µL of SDA broth with 1 mL of olive oil and was left for 15 min before addition of 5 µL of the yeast suspension into each well. The stock solution of *A. indica* extracts at 100% was added into the first well, and then followed by 100 µL of 75%, 50%, and 25% of plant extracts added to the next three wells consecutively. The plate was covered with a sterile plate shaker at 300 rpm for 20 s and then incubated at 32°C for 48 h. At the end of the

incubation period, the plates were evaluated for the presence or absence of growth. MIC is the lowest concentration of the antifungal agent showing no turbidity after 48 h, where the turbidity is interpreted as visible growth of the fungi. The bioassay was performed in triplicates in order to calculate the mean value.[17]

Statistical analysis

Values of quantitative phytochemical screening, antioxidant assay, and antidandruff assays were performed in triplicates and expressed in mean ± standard deviation using regression equation in Microsoft Excel 2013. IC_{50} values were calculated by plotting inhibition % vs. concentration using GraphPad prism.

Results

Phytochemical screening

The results of qualitative phytochemical screening are shown in Table 1.

Estimation of total phenolic content of methanolic, ethanolic, and aqueous extracts of *A. indica*

The content of phenolic compounds in *A. indica* extracts (10 mg/mL) was determined from the regression equation of the calibration curve (y= 0.0003x−0.012, R^2= 0.995). A calibration curve was drawn by using different concentrations of gallic acid and is as shown in Figure 1. The amount of phenolic compounds was expressed as GAE in mg/g dry weight of plant extract and is shown in Table 2. It was found that the total phenolic content was higher in *Artemisia* methanolic extract (AME) than *Artemisia* ethanolic extract (AEE) and *Artemisia* aqueous extract (AQE) with the increase in magnitude of the order of AME (248±3.29)>AEE (189.32±3.29)>AQE (114.17±0.82) mg/g dry extract GAE.

Total flavonoid content in methanolic, ethanolic, and aqueous extracts of *A. indica*

The flavonoid content of the *A. indica* extracts (10 mg/mL) was determined from the regression equation of the calibration curve (y= 0.002x−0.019, R^2= 0.991). A calibration curve was drawn by using different concentrations of rutin and is shown in Figure 2. The amount of flavonoids was expressed as RE in mg/g dry weight of plant extract and is as shown in Table 2. Flavonoids content was also found to be higher in methanolic extract than in ethanolic and aqueous extracts with the corresponding increasing magnitude order of AME (222.33±4.41) > AEE (156.33±6.79) > AQE (153±7.21) mg/g dry extract of RE.

Total proanthocyanidin content in methanolic and ethanolic extracts of *A. indica*

The proanthocyanidin content of *A. indica* extracts (10 mg/mL) was determined from the regression equation of the calibration curve (y= 0.004x−0.041, R^2=0.992). A calibration curve was drawn by using different concentrations of rutin and is shown in Figure 3. The amount of proanthocyanidins was expressed as RE in mg/g dry weight of plant extract and is shown in Table 2. The proanthocyanidin content was also found to be higher in the methanolic extract than in ethanolic and aqueous extracts of *A. indica* with the increase in magnitude of AME (222.83±1.6) > AEE (157.5±2.36) > AQE (198.66±1.8) mg/g dry extract of RE.

Antioxidant activity

DPPH radical scavenging, NO scavenging, and hydrogen peroxide scavenging assay

In-vitro antioxidant activity of methanolic, ethanolic, and aqueous extracts of *A. indica* was measured on the basis of DPPH radical scavenging activity, NO scavenging activity, and hydrogen peroxide scavenging activity using ascorbic acid as standard in DPPH assay and quercetin as standard in NO and H_2O_2 scavenging assays. Using the results of all the three methods, the *Artemisia* methanolic extract had shown the highest antioxidant activity among all different solvent extracts, followed by aqueous extract and ethanolic extract, i.e., AME>AQE>AEE as shown in Figures 4–6.

Antibacterial and antidandruff activity

The antibacterial activity leaf extract of *A. indica* was tested against five human pathogenic bacteria, i.e., *S. typhi*, *S. aureus, P. mirabilis, P. aeruginosa,* and *E. coli*. Antibacterial activity

Table 1: Qualitative test of phytochemicals in methanol, ethanol, and aqueous extracts

Primary and secondary metabolites	Extracts		
	Methanol	Ethanol	Aqueous
Alkaloids	+	+	+
Proteins	+	+	−
Steroids	+	+	+
Anthraquinones	+	+	+
Phenols	+	+	+
Carbohydrates	+	+	+
Glycosides	+	+	−
Saponin	+	+	+
Tanins	+	+	+

+ = present; − = absent

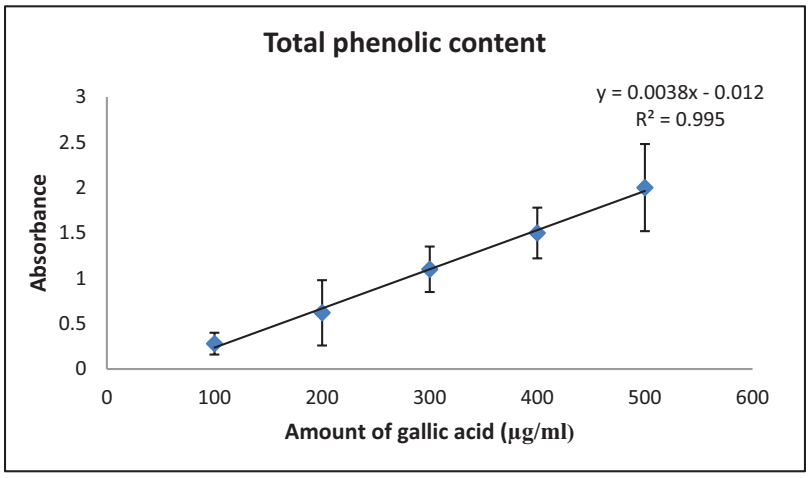

Figure 1: Standard calibration curve for quantification of total phenolic content

Table 2: Summary table of total amount of phenol, flavonoid, and proanthocyanidin content in methanolic, ethanolic, and aqueous extracts of *A. indica*

Extract	Total phenolic content (TPC) (mg of GAE/g of dried extract)	Total flavonoid content (TFC) (mg of RE/g of dried extract)	Total proanthocyanidin content (TPAC) (mg of RE/g of dried extract)
AME	248±3.29	222.33±4.41	222.83±1.62
AEE	189.32±3.29	156.33±6.79	157.5±2.36
AQE	114.17±0.82	153±7.21	198.66±1.8.

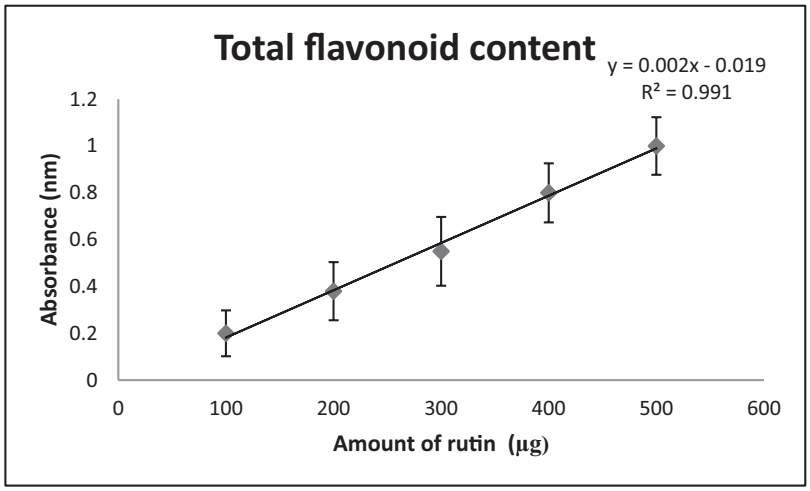

Figure 2: Standard calibration curve for quantification of total flavonoid content

of leaf extract of *A. indica* against Gram-positive and Gram-negative bacteria are shown in Table 3.

Leaf extract of *A. indica* also showed the antidandruff potential against dandruff causing fungi. The antidandruff activity of *A. indica* against the test organism *M. furfur* is shown in Table 4.

Table 3 shows that the significant zone of inhibition was observed in methanolic and ethanolic extracts of *A. indica* for *P. aeruginosa* and *E. coli* on comparison with other pathogens. The methanolic extracts of *A. indica* also showed high antimicrobial potency against *S. aureus* and *P. mirabilis* when compared with ethanolic extracts.

Figure 7 shows that the lowest inhibitory concentration was found to be in the positive control gentamicin, followed by methanolic and ethanolic extracts of *A. indica*. MIC values of the aqueous extract were not determined due to lack of antimicrobial activity of aqueous extract in the well diffusion assay. MIC values revealed that the minimum concentration to inhibit growth of pathogens was observed in the methanolic extract when compared with ethanolic extract.

Table 4 shows that the methanolic extract was found to exhibit highest susceptibility (12±2) mm with MIC (300 mg/mL) over ethanolic extract having zone of inhibition (8±3) mm and MIC (400 mg/mL) on known standard strain of *M. furfur*, whereas the aqueous extract did not show any activity and hence MIC values were not determined. Here 10% $DMSO_4$-containing disk was used as a negative control and fluconazole served as a positive

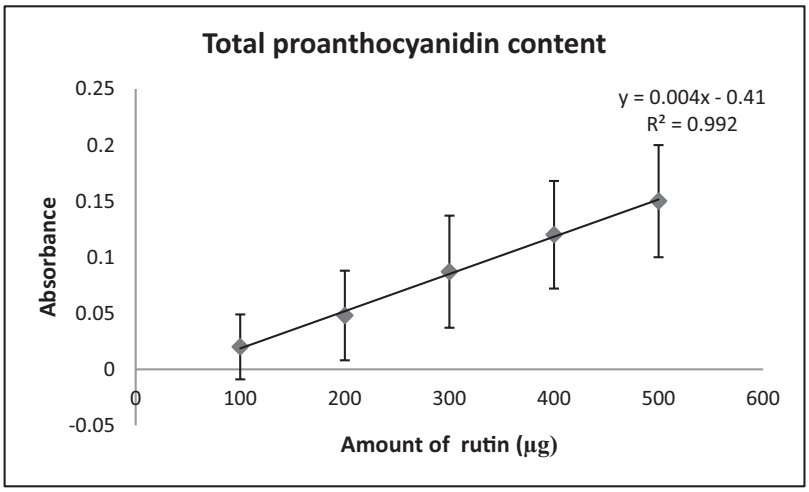

Figure 3: Standard calibration curve for quantification of total proanthocyanidin content

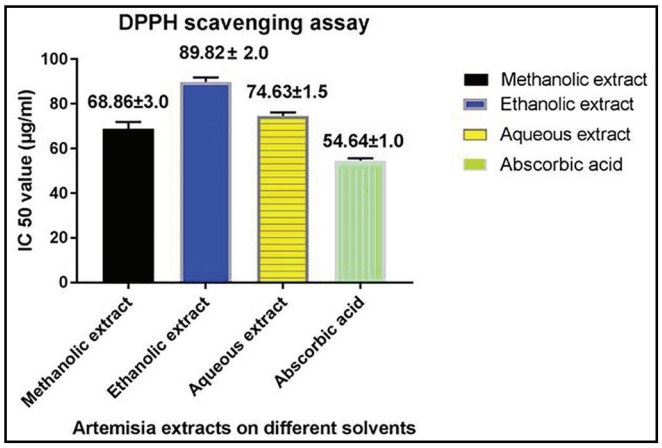

Figure 4: IC_{50} value of *A. indica* by DPPH assay with respect to percentage of inhibition measured in triplicates

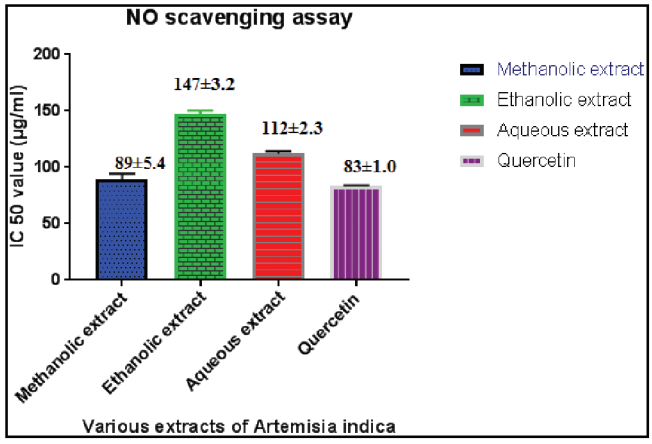

Figure 5: IC_{50} value of *A. indica* by NO scavenging assay with respect to percentage of inhibition measured in triplicates

control representing mean zone of inhibition and standard deviation, i.e., (16±2) mm with MIC values of 35 mg/mL.

Discussion

The presence of secondary metabolites in *A. indica* extracts such as saponins, alkaloids, tannins, sterols, triterpenoids, phenolic, flavonoids, glycosides, and many enriched phytochemicals exhibits biological activity and serves as the preliminary evidence for the synthesis of plant-derived medicines. Qualitative phytochemical screening results in this study determined the presence of alkaloids, flavonoids, saponin, tannin, glycosides, phenol, and anthroquinononones. Similar findings have been observed in a study done in India on qualitative screening in *A. indica* extracts.[6] Quantitative phytochemical analysis in our study measured the total content of phenol, flavonoid, and proanthocyanidin. Our qualitative screening was further supported by work done by Pandey *et al.*[19] in *Artemisia vulgaris*. In our study, the methanolic extract of *Artemisia* (AME) exhibited the highest yield of phenol followed by AEE and least in the AQE per gram of GAE. Similarly, the results of total flavonoid content and proanthocyanidin content also revealed the highest yield in methanolic extract when compared with ethanol and aqueous solvent extracts. The study conducted by Ruwali *et al.*[6] in *A. indica* and Kumar *et al.*[20] in *Artemisia pallens* had also shown that majority of phytochemicals such as phenol, alkaloids, terpenoids, flavonoids, etc. were extracted using methanol solvent. This demonstrates methanol as excellent polar solvent for obtaining *Artemisia* extract. DPPH is commonly used as a substrate for the fast evaluation

of antioxidant activity because of its stability in the radical form and simplicity of the assay. The principle behind this assay in the color change of DPPH solution from purple to yellow as the radical is quenched by the antioxidant. The color changes are measured quantitatively by UV–Vis spectrophotometer absorbance at 517 nm. DPPH inhibition percentage in this study finds AME>AEE>AQE with lower IC_{50} value (68.86±3) µg/mL. These findings correlate with another study conducted by Ruwali *et al.*[21] in *A. indica*, in which antioxidant activities in different concentrations show AME>AEE with comparatively lower IC_{50} value (83.8±1.25) µg/mL in concentration similar to the methanolic extract. Alternatively, antioxidant properties investigated in South Korea by Lee[22] in different *Artemisia* species such as *Artemisia selengensis*, *Artemisia japonica,* and *Artemisia capillaries* revealed to have high antioxidant potential (>90% inhibition) with lowest value ranging from 19 to 27 µg/mL. The degree of discoloration indicates the scavenging potential of the antioxidant extract, which is due to free radical scavenging activity. More reduction of the DPPH radical is related to the high scavenging activity of the extract and lower concentration of IC_{50} values. Similarly, nitric oxide is an unstable compound under the aerobic condition. It reacts with oxygen to produce the stable product nitrates and nitrite by means of intermediates such as NO_2, N_2O_4, and N_3O_4. It is estimated by the application of Griess reagent. In the NO scavenging assay, NO acts as a scavenger where the level of nitrous acid decreases, whereas in the hydrogen peroxide scavenging assay, hydrogen

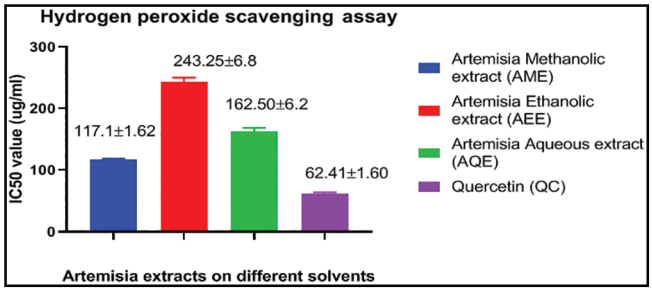

Figure 6: Bar diagram representing IC_{50} value of *A. indica* by H_2O_2 scavenging assay

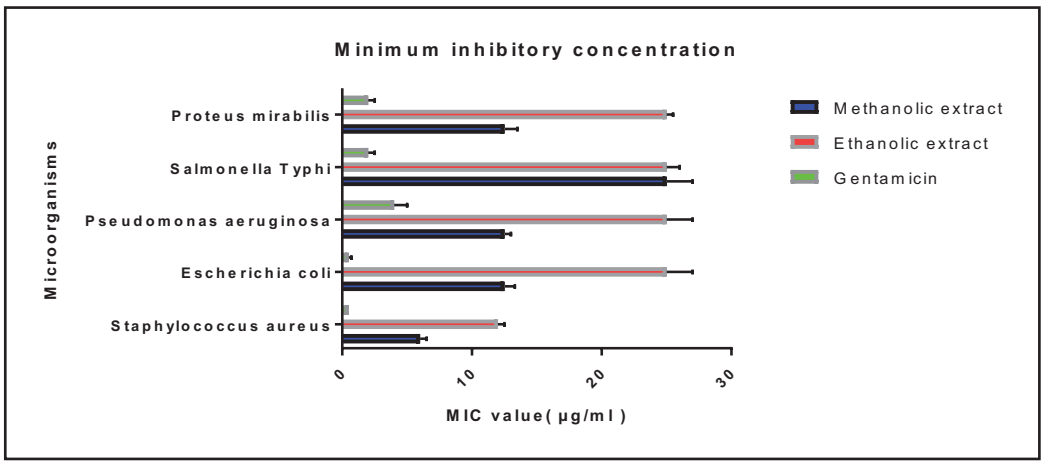

Figure 7: Determination of MIC value of *A. indica* and gentamicin against test organisms

Table 3: Results of antibacterial activity of leaf extracts of *A. indica*

Organism	Gentamicin (1 mg/mL)	Zone of inhibition (ZOI) in mm ± standard deviation		
		Negative control (distilled water)	Methanolic extract (10 mg/mL)	Ethanolic extract (10 mg/mL)
S. aureus	24±0.0	0	13.75±1.70	12.25±0.53
E. coli	17±0.0	0	14±3.91	15.5±4.79
P. aeruginosa	20±0.0	0	15.5±2.64	16±3.65
S. typhi	18±0.0	0	12±3.55	11.25±2.98
P. mirabilis	22±0.0	0	16.5±5.44	12.25±2.98

Gentamicin and distilled water serve as positive and negative controls, respectively. Pure distilled water and its solution of aqueous extract for all above microorganisms exhibited zero zone of inhibition

Table 4: Results of antidandruff activity of leaf extracts of *A. indica*

Plant	Fraction	Zone of inhibition (mm)	MIC value (mg/mL)
A. indica	Methanol (100 mg/mL)	12±2	350
	Ethanol (100 mg/mL)	8±3	400
	Aqueous (100 mg/mL)	No activity	Not applicable
Standard drugs	Fluconazole (40 mg/mL)	16±2	35
Negative control	10% $DMSO_4$	No activity	Not applicable

peroxide itself is an unreactive compound that releases hydroxyl radical. According to our study, the nitric acid scavenging assay and hydrogen peroxide scavenging assay were used to evaluate antioxidant potential and found that the highest scavenging assay was shown by methanolic extract when compared with ethanolic and aqueous extracts. However, due to very limited data source for antioxidant activity on *A. indica* by these methods, we have compared our similar studies with another research work on antioxidant studies conducted by Ruwali *et al.* on *A. indica*,[21] in which methanolic extract was found to be superior by all these employed methodologies. Subsequently, a study conducted in India by Bora and Sharma[18] in another species of *Artemisia* (*Artemisia absinthium*) elucidated the significant hydrogen peroxide and nitrite scavenging activity in animal models.

The antibacterial activity of *A. indica* is observed in five human pathogens, which include *S. aureus, P. aeruginosa, S. typhi, E. coli*, and *P. mirabilis*. A broad-spectrum antibiotic gentamicin (1 mg/mL) is used as standard drugs (control). Our study finds that 10 mg/mL of the ethanolic and methanolic extracts of *A. indica* for *P. aeruginosa* and *E. coli* with MIC of 25 µg/mL determined potent antimicrobial action. MIC is defined as the least concentration which inhibits bacterial growth. It varies based on morphology, physiology, and nature of bacteria. Surprisingly, the MIC value was low against Gram-positive bacteria (*S. aureus*) tested on methanolic extract. The aqueous extract has no action against all five pathogens in all four tested concentrations as aqueous extract had low phenolic yield (14.17±0.82) per gram of GAE. This finding is supported by Ouerghemmi *et al*.,[23] in which the authors have shown role of phenolic compounds in natural products for antimicrobial activities and yield of phenol from the extract can be correlated with antimicrobial activity. The MIC ranged from 6 to 25 µg/mL that designates the lower dose required for inhibiting bacterial growth. MIC plays a vital role in dose management to prevent side effects. Koul *et al*.[1] in their review had mentioned that lower dose intakes of all *Artemisia* species are considered relatively safe. However, few reports have been found on side effects specific to *A. indica* extracts, such as occurrence of hypoglycemia when consumed in high concentrations.[24] Another study conducted in China on *Artemisia* species depicts induction of allergic rhinitis and *Artemisia* hay fever among patients allergic to mugwort plants.[25]

Moreover, this forms the first antidandruff study of *A. indica* since no other report of the same is available in the public domain. However, Javid *et al*.[8] have shown antifungal activities of *A. indica* against filamentous fungi. In our study, methanolic extract was found to be superior over ethanolic extract, i.e., AME (12±2)>AEE (8±3) in mm on strain of *M. furfur* with MIC values for methanolic extract less than those of ethanolic extract (350 <400) mg/mL, whereas aqueous extract had no susceptibility against dandruff-causing fungus probably due to low yield of phytochemicals such as phenol. Fluconazole was used as a positive control and DMSO as a negative control. This finding shows beneficial effects of *A. indica* extract, which can be used as a herbal remedy for prevention and control of dandruff. Furthermore, different topical creams and soaps enriched with these phytochemicals can be introduced as one of the major ingredients.

Conclusion

Phytochemically *A. indica* is a rich source of glycosides, phenols, sterols, flavonoids, alkaloids, and tanins. *Malassezia* zone of inhibition and lower MIC values shown by the leaf extract of *A. indica* in bacterial pathogen demonstrate effective antibacterial property. The free radical scavenging potential also enriches its medicinal values. The presence of antimicrobial and antioxidant properties in *A. indica* contributes in the synthesis of plant-derived drugs following their uses on the basis traditional medicines. The antidandruff potential against *M. furfur* also signifies that the leaf extract is also effective against dandruff caused by *Malassezia* species.

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Conflicts of interest

There is no conflict of interest.

Data availability

All data generated or analyzed during the study are included in this article.

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