

Phytochemical Screening, GC-MS Analysis and Biological Activities of Extracts of *Artemisia vulgaris* Linn.

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Highlights

- Aerial part of *Artemisia vulgaris* was extracted in methanol, hexane and chloroform.
- GC-MS analysis showed the presence of 12 different compounds.
- Antibacterial activity showed potent results.
- IC₅₀ value was calculated as 1.50 mg/mL and LC₅₀ value as 0.18 mg/mL.
- TPC (87.75 mg gallic acid equivalent/g) and TFC (47.25 mg quercetin equivalent/g).

Abstract

The aerial parts of *Artemisia vulgaris* were subjected for successive extraction in solvents methanol, hexane and chloroform, respectively, and compounds present in extract were analysed by GC-MS analysis 12 different compounds were found in chloroform extract in which Germacrene was found most abundantly (33.84%). Chloroform extract showed strong antibacterial activity, which was examined against 6 bacteria using the disc diffusion method. The IC₅₀ value of chloroform extract accounted for 1.50 mg/mL. The TPC content of the extract was found to be 87.75 mg gallic acid equivalent/g of dry extract, whereas TFC content was found to be 47.25 mg quercetin equivalent/g of dry extract and exhibited LC₅₀ value of 0.18 mg/mL.

Keywords: *Artemisia vulgaris*, Phytochemical screening, GC-MS, Biological Activity

Introduction

Artemisia vulgaris Linn. is an important perennial medicinal herb that belongs to the family *Asteraceae*, commonly called Mugwort pollen in Hindi and Titepati in Nepali. The perennial plant *A. vulgaris* grows to a height of 50-150 cm, flowers in August, and sets seeds in September. *A. vulgaris* has long been used as a traditional remedy in public health, as well as an edible plant (primarily as a spice) [1]. It is an aggressive and invasive plant that is found growing wild and abundantly all over the temperate and cold temperate zones of the world. It is somewhat toxic, and several pharmacological activities of *A. vulgaris* have been reported [2,3].

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All parts of the plant are found to be anthelmintic, antiseptic, antispasmodic, carminative, cholagogue, diaphoretic, digestive, emmenagogue, expectorant, nervine, purgative, stimulant, slightly tonic, and they are also used to cure women's complaints. The leaves are used as an appetizer, diuretic, hemostatic and stomachic. The leaves of the plant have an antibacterial action, inhibiting the growth of *Staphylococcus aureus*, *Bacillus typhi*, *Shigella dysenteriae*, *Streptococci*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas*. The stem is also antispasmodic, antirheumatic, and stomachic [4,5].

Leaves of *A. vulgaris* were successively extracted in three different solvents, methanol, hexane and chloroform, respectively then different biological tests were carried out to check the potency of extract as an antioxidant, antibacterial and its cytotoxicity, which could be very beneficial to discover new synthetic drugs with less toxicity and side effects.

Materials and Methods

Collection of plant materials

The fresh leaves of *A. vulgaris* were collected from Chovar, Kathmandu, Nepal, in the month of October 2018. The collected leaves were washed, shade dried and grinded to make powder.

Extraction

The dried powdered leaves of *A. vulgaris* were extracted successively in three different solvents methanol, hexane and chloroform, respectively. Firstly, it was dissolved in methanol for about 15 days and was filtered then concentrated using a Rota evaporator. Then concentrated methanol extract was suspended with 1% HCl; then, it was neutralized using NH_4OH . Thus, the prepared solution was subjected for extraction with hexane from which aqueous layers were taken for further extraction with chloroform. After mixing with chloroform, the solution was shaken vigorously in a separating funnel to separate chloroform extract from the aqueous layer. Thus, obtained chloroform extract was concentrated using a Rota evaporator.

Phytochemical Screening

To find the main class of chemical compounds present in the plant extracts, phytochemical screening was carried out on methanol, hexane and chloroform extracts as per the standard methods [6–8].

Antibacterial Activity

To check the capacity of plant extracts to kill or inhibit the growth of pathogenic micro-organisms, antibacterial activity was carried out. The antibacterial screening of plant extracts was carried out by the agar well diffusion method based on the procedure given by chemists [9, 10]. All the strains of bacteria were grown in nutrient agar at 37 °C and reactivated for further use in nutrient broth. The different extracts of *A. vulgaris* and standard drugs were tested for antimicrobial activity against the test organisms using the agar diffusion method [11].

Mueller Hinton agar media was prepared, and the plates were swabbed for 24 hours with cultures of the bacteria that had been cultured overnight in nutrient broth. 0.5 mL of each extract and Dimethyl sulphoxide (DMSO) were individually impregnated into 8 mm diameter sterile discs. DMSO was used as negative control and discs of standard drugs as a positive control. The plates were then incubated at 37 °C for 24 hours. After the incubation period, each plate was observed for the zone of inhibition and measured using a transparent scale or slide callipers [12].

Antioxidant Activity

Antioxidant activity of plant extract was carried out to check the ability of the extract to kill or scavenge the free radicals, which are responsible for the oxidation reaction. It was examined by using DPPH free radical scavenging method. In this method, first of all, a stock solution of concentration 1mg/mL was prepared by dissolving 1 mg of sample in 1 mL of methanol, then 0.1 mL of these solutions were added to 0.1 mL of 0.1 mM DPPH (prepared in methanol) and was left for 30 minutes in a dark room. After 30 minutes, their absorbance was taken at 517 nm against DPPH, DMSO was used for a blank test and as standard, Quercetin was prepared. The sample with yellow colour (more than 50% inhibition than control) was taken for further testing, which was anticipated to be potent antioxidants [13].

To examine the IC_{50} value against DPPH, extracts with different concentrations were prepared by a two-fold dilution method [14].

The percentage of radical scavenging activity was calculated by using the following formula:

$$\text{Percentage scavenging} = \frac{(A_0 - A_T)}{A_0} \times 100\%$$

Where,

A_0 = Absorbance of the DPPH

A_T = Absorbance of the DPPH free radical solution containing the sample extract.

The concentration of the solution that can inhibit 50% of DPPH free radicals is referred to as the IC_{50} value of the extract, which has an inverse relation with antioxidant activity [15].

Total Phenolic Content

The content of total phenolic compounds in chloroform extracts was determined by Folin–Ciocalteu method. For the preparation of calibration curve 1 mL aliquots of 0.024, 0.075, 0.105 and 0.3 mg/mL ethanolic gallic acid solutions were mixed with 5 mL Folin–Ciocalteu reagent (diluted ten-fold) and 4 mL (75 g/L) sodium carbonate. The absorption was read after 30 minutes at 20 °C at 765 nm, and the calibration curve was drawn. 1 mL chloroform extract (10 g/L) was mixed with the same reagents as explained above, and after 1 hour, the absorption was measured for the determination of plant phenolics. All determinations were performed in triplicate [16]. The total content of phenolic compounds in plant methanol extracts in gallic acid equivalents (GAE) was calculated by the following formula:

$$\text{TPC} = \frac{C \times V}{m}$$

Where,

C = the concentration of gallic acid founded from the calibration curve, mg/mL;

V = the volume of extract, mL;

m = the weight of pure plant chloroform extract, g

Total Flavonoid Content

1 mL of sample (0.1 mg/mL in methanol) was mixed with 1 mL of 2% $AlCl_3$ (dissolved in methanol) and kept for 1 hour, and absorbance was measured at 415 nm against the blank (methanol). Quercetin was used as standard. Total flavonoid content is stated as mg of quercetin equivalents per gram of dried sample [17].

The concentration of total flavonoid content in the sample was determined as milligram of quercetin equivalent by using the following equation:

$$\text{TFC} = \frac{C \times V}{m}$$

Where,

C = concentration of quercetin from curve ($\mu\text{g/mL}$)

V = volume of extract (mL)

m = weight of plant extract (g)

Brine Shrimp Lethality Assay

Brine shrimp (*Artemia salina*, fairy shrimp, or sea monkeys) lethality assay is commonly used to check the cytotoxic effect of bioactive chemicals. It is a rapid (24 hours), inexpensive and straightforward test as no aseptic techniques are required. The nauplii of brine shrimp were exposed to different concentrations of solutions of chloroform extracts of *A. vulgaris* for 24 hours. The number of motile nauplii was calculated that represented the effectiveness of the chloroform extracts.

Extracts having LC_{50} value less than 1 mg/mL are considered as bioactive in toxicity evaluation by Brine shrimp lethality assay [18,19].

Results and Discussion

Phytochemical Screening

Phytochemical screening was performed in all three extracts, methanol, hexane and chloroform extracts of *A. vulgaris*. More polar extracts showed the presence of a large number of compounds, whereas very few compounds were found in the less polar extract.

Table 1: Phytochemical screening of plant extracts

Constituents	Methanol	Hexane	Chloroform	Presence
Alkaloids	+	+	+	Alkaloids were present in all three extracts
Flavonoids	+	+	+	Flavonoids were present in all three extracts
Glycosides	+	+	+	Glycosides were present in all extracts
Phenols	+	-	+	Phenols were present in methanol and chloroform extracts
Quinones	-	-	-	Quinones were absent in all extracts
Tannins	+	-	+	Tannins were present in methanol and chloroform extracts
Saponins	+	-	+	Saponins were present in methanol and chloroform extracts
Carbohydrates	+	-	+	Carbohydrates were present in methanol and chloroform extracts
Protein	+	+	+	Protein was present in all extracts
Emolin	+	-	+	Emolin were present in methanol and chloroform extracts

'+' indicates the presence and '-' the absence.

GC-MS spectra analysis of chloroform extract

GC-MS chromatogram of chloroform extract showed the presence of 12 major compounds, which are presented below:

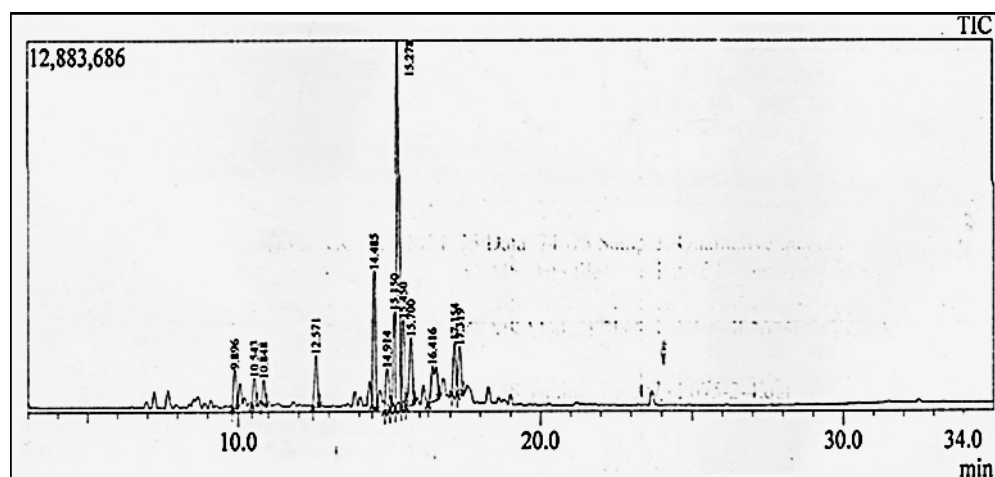


Fig1: Chromatogram of chloroform extract of *A. vulgaris*

Table 2: Compounds present in the chloroform extract

S.N.	Name of the compound	Retention time	Molecular formula	Molecular weight	Area (%)
1	Germacrene	15.275	C ₁₅ H ₂₄	204	33.84
2	Isocaryophyllene	14.483	C ₁₅ H ₂₄	204	11.67
3	Ledol	16.417	C ₁₅ H ₂₆ O	222	10.61
4	γ-Elemene	15.45	C ₁₅ H ₂₄	204	7.89
5	β-Himachalene	15.15	C ₁₅ H ₂₄	204	7.38
6	β-Sesquiphellandrene	15.7	C ₁₅ H ₂₄	204	7.28
7	Geraniol ester	12.567	C ₁₂ H ₂₀ O ₂	196	4.83
8	α-Muurolol	17.158	C ₁₅ H ₂₆ O	222	4.66
9	α-Caryophyllene	14.917	C ₁₅ H ₂₄	204	4.08
10	Chrysanthone	9.9	C ₁₀ H ₁₆ O	152	3.33
11	Camphor	10.542	C ₁₀ H ₁₆ O	152	2.34
12	Borneol	10.85	C ₁₀ H ₁₈ O	154	2.09

Antibacterial Screening Analysis

Chloroform extract showed antimicrobial activity against different microorganisms. However, the antimicrobial activity varied according to species of microorganisms.

Table 3: Antimicrobial activity shown by chloroform extract of *A. vulgaris* in diameter (mm) of inhibition zone

Test Organism (80μl)	Zone of inhibition	
	<i>Artemisia vulgaris</i> (15μl)	Control (DMSO) (15μl)
<i>Klebsiella pneumoniae</i>	8mm	No activity
<i>Bacillus subtilis</i> KACC 17047	6mm	No activity
<i>Micrococcus luteus</i> KACC 13377	15mm	No activity
<i>Enterobacter cloacae subsp. disolvens</i> KACC 13002	13mm	No activity
<i>Pseudomonas</i> sp.	No activity	No activity
<i>Staphylococcus aureus</i> KACC 10768	No activity	No activity

Antioxidant Activity

The antioxidant capacity of plants is associated with the activity of “free radical scavenging enzymes” (superoxide dismutase, catalase, peroxidase, etc.) and the contents of antioxidant substances, mainly phenolic compounds, carotenoids, tocopherol, and ascorbic acid. The antioxidant potential has an inverse relation with IC₅₀ value, which can be calculated from the logarithmic regression of the % inhibition versus antioxidant activity. Lower the IC₅₀ value higher is the antioxidant activity. All the calculations are based on the standard method [20]. Absorbance was measured at 517 nm.

The absorbance of each solution was measured and recorded as follows

Table 4: Antioxidant activity of Chloroform extract of *A. vulgaris* sample by DPPH method

Concentration (mg/mL)	Percentage Scavenged
0.25	15.709
0.5	24.918
1	41.495
1.5	46.912
2	62.838

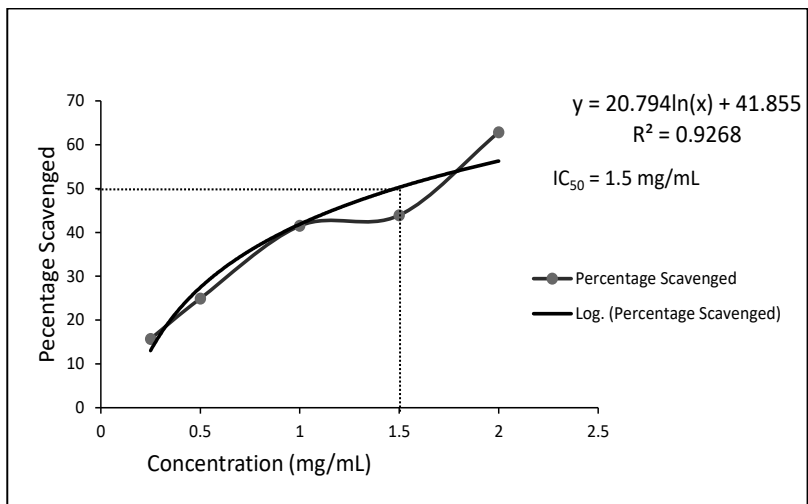


Fig 2: Graphical representation of the DPPH assay of the chloroform extract

According to the Food and Drug Administration (FDA), IC₅₀ represents the concentration of a drug that is required for 50 % inhibition *in vitro*. The IC₅₀ value of chloroform extract of *A. vulgaris* was found to be 1.50 mg/mL. This study shows that chloroform extract is more effective in inhibiting a specific biological or biochemical function.

Total Phenolic Content Analysis

Phenolic compounds are ubiquitous secondary metabolites in plants. They are known to have antioxidant activity, and the activity of these extracts is likely due to these compounds[21]. The total phenolic content was determined as a milligram of gallic acid equivalent using the calibration curve of gallic acid.

The absorbance of each solution as measured and recorded as follows:

Table 5: Absorbance of gallic acid

Concentration (mg/ml)	Average Absorbance (nm)
0.01	0.03
0.02	0.117
0.04	0.266
0.06	0.429
0.08	0.6
0.1	0.789

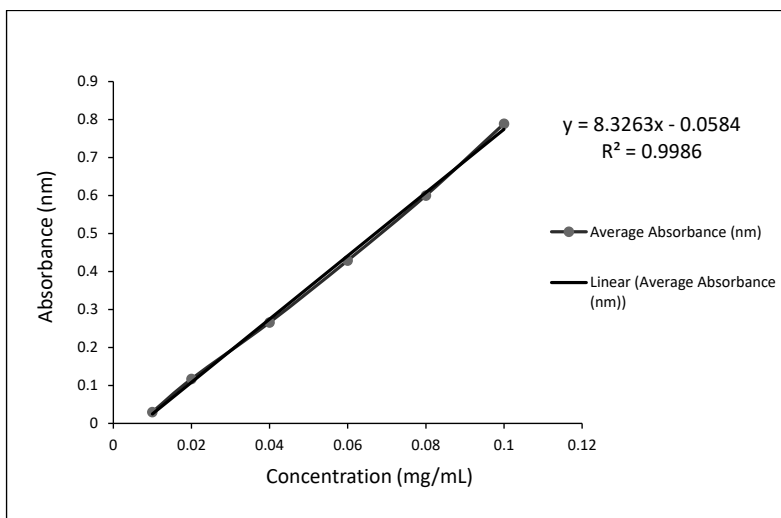


Fig 3: Calibration curve of gallic acid for total phenolic content determination

The total phenolic content with necessary data is shown in the following table.

Table 6: Total phenolic content in chloroform extract of *A. vulgaris*

Sample Concentration (mg/mL)	Weight of extract per mL m (g)	Absorbance	GAC, C (mg/mL)	TPC as GCE $= \frac{c \times v}{m}$ (mg/g)
1	0.001	0.67	0.08775	87.75

Based on table 6 and figure 4, the total phenolic content of chloroform extract was tested as gallic acid equivalent by reference to a standard curve ($y = 0.0083x - 0.0584$, $R^2 = 0.9986$). The total phenolic content in the chloroform extract of *A. vulgaris* was found to be 87.75 mg gallic acid equivalent /g of dry extract, which is shown in table 6.

Total Flavonoid Content Analysis

Total flavonoid content was expressed as mg of quercetin equivalents per gram of dried sample [22].

The absorbance of each solution was measured and recorded as follows:

Table 7: Absorbance of quercetin

Concentration (mg/ml)	Average Absorbance (nm)
0.005	0.06
0.01	0.122
0.02	0.425
0.04	0.96
0.08	1.901

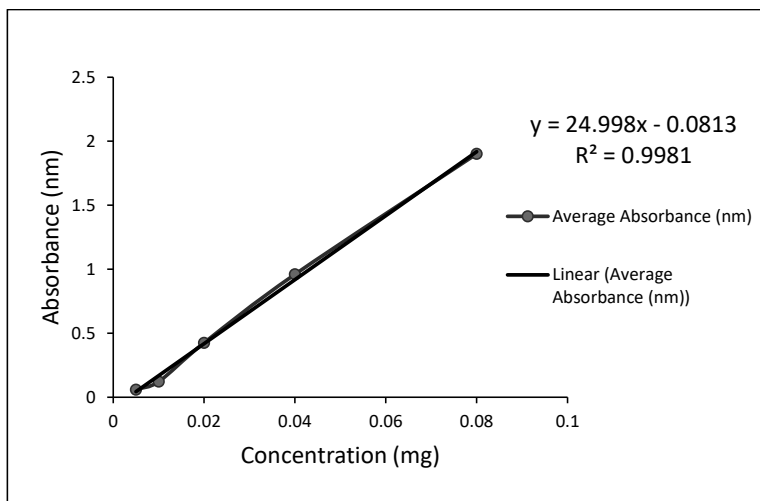


Fig 4: Calibration curve of Quercetin for total flavonoid content determination

The total flavonoid content with necessary data is shown in the following table:

Table 8: Total flavonoid content in chloroform extracts of *A. vulgaris*

Sample Concentration (mg/mL)	Weight of extract per mL m (g)	Absorbance	QC, C (mg/mL)	TFC as OE $= \frac{c \times v}{m}$ (mg/g)
1	0.001	1.109	0.047252	47.252

Based on table 8 and figure 5, the total flavonoid content of chloroform extract was tested as quercetin equivalent by reference to a standard curve ($y = 0.025x - 0.0813$, $R^2 = 0.9981$). The total flavonoid content in the chloroform extract of *A. vulgaris* was found to be 47.25 mg quercetin equivalent /g of dry extract, which is shown in table 8.

Brine Shrimp Lethality Analysis

Meyer et al. (1982) used the Brine Shrimp Cytotoxicity Test (BSCT) to determine the lethality of a test sample in a basic zoological organism such as the shrimp (*Artemia salina*).

The result of the Brine Shrimp Lethality assay are given in the table below:

Table 9: Effect of chloroform extract of *A. vulgaris* in brine shrimp

Concentration (C) (mg/mL)	Percentage Mortality
0.05	14.95
0.1	28.57
0.2	82.98
0.4	93.19
0.8	100

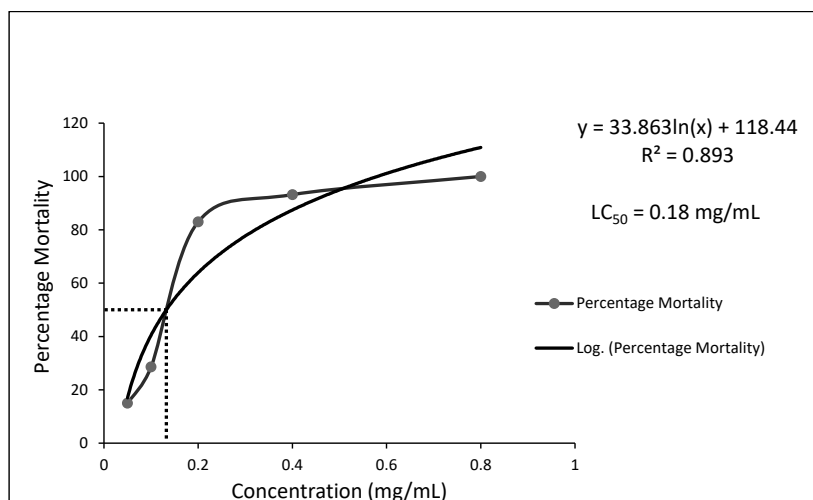


Fig 5: Plot of the concentration of chloroform extract of *A. vulgaris* versus percent shrimp mortality after 24 hours of exposure

The LC_{50} value of chloroform extracts of *A. vulgaris* was found to be 0.18 mg/mL, which is considered bioactive in toxicity evaluation.

Conclusions

The presented results revealed that extract of aerial parts of the *A. vulgaris* plant possesses various bioactive agents. From the preliminary phytochemical screening of the methanolic extract, the various phytoconstituents like flavonoids, phenols, saponins, tannins, glycosides, carbohydrates protein, emolins were identified. The GC-MS analysis of the chloroform extract of *A. vulgaris* showed 12 major compounds; among them Germacrene (33.84%) was found abundantly. It showed the widest range of zone of inhibition against *Micrococcus luteus* (15 mm). The IC_{50} and LC_{50} values of chloroform extract were found to be 1.50 mg/mL, and 0.18 mg/mL, respectively, whereas total phenolic and total flavonoid content, were calculated as 87.75 mg gallic acid equivalent/g of dry extract and 47.25 mg quercetin equivalent/g of dry extract respectively.

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