JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)

An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Phytochemical determination, Antioxidant properties and Antibacterial activity of Ethanol extract of *Artemisia vulgaris*

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Abstract

In the present study, the ethanol extract of *Artemisia vulgaris* leaves and their phytochemical components determination, antioxidant properties and its antibacterial activity. The phytochemical screening analysis showed the presence of various bioactive compounds such as Proteins, phenol, saponins, flavonoids, alkaloids, glycosides and triterpenoids in ethanol extract of *A. vulgaris*. The plant extract and ascorbic acid comparatively tested different concentrations for scavenging activity of DPPH and reducing power activity. This study reveals that in percentage inhibition of the scavenging activity of DPPH (64.06%) by ethanol extract of *A. vulgaris* when compared with ascorbic acid (88%) at highest concentration (50 µg/ml) and the reducing power assay was result showed that the reducing power increases with the increase of concentration of both plant extract and ascorbic acid. In addition, we studied that low amount of ethanol extract of *A. vulgaris* inhibited activity of the bacterial microorganism. The ethanol extract of *A. vulgaris* was very successful in inhibiting growth of bacterial pathogens. Finally we conclude that the ethanol extract of *A. vulgaris* leaves has good antioxidant properties and antibacterial agents.

Keywords: *Artemisia vulgaris;* Ethanol extract; Antioxidant; Antibacterial activity.

Introduction

Plants have several vital phytochemicals such as alkaloids, phenols, flavonoids, essential oils, tannins and saponins, which can be used as therapeutically important compounds with lesser or no side effects. Based on the medicinal properties of the various phytoconstituents many attempts have been made using

phytoconstituents as therapeutic drugs. Natural bioactive compounds extracted from plant sources have been reported to exert potent antioxidant properties compared to the chemically synthesized compounds.

Flavonoids are vital metabolites complicated in defense against stress (temperature and oxidative), one of the phytoconstituents (Mierziak *et al*., 2014). Phenolic acids are the secondary metabolites, a major bioactive phytoconstituents, contributing for greater antioxidant activity (Wojdyło *et al*., 2007). Saponins are known to show protective effects on blood cholesterol, anti-cancer activity and also known to show antiviral property (Vickers *et al*., 2006). Alkaloids are the compounds that show cytotoxic effect, and hence some of the alkaloids are used as anticancer agents. The theoretical and practical understanding of medicinal plants has diversely considered cultural changes, function of plant habitat collection as well as biochemical and ecological aspects (Goulletquer *et al*., 2014).

The genus Artemisia belongs to one of the largest and most widely distributed genera of the family Asteraceae (Compositae). It is a diverse and economically important genus and it has more than 500 species. Most plants within this genus have a great importance as medication, foodstuff, ornamentals or soil stabilizers, some are allergic or toxic, and some are weeds growing in the fields (Hayat *et al*., 2010). *Artemisia vulgaris* L., commonly known as mugwort, has a long history of its use in traditional systems of medicine in different parts of the world. A native plant in temperate Europe, Asia, Northern Africa, Alaska and North America.

The whole plant is traditionally used in the treatment of various ailments such as anti-leishmanial activity, anti-malarial, anti-bacterial, antifungal, anti-diabetic, anti-epileptic, anti-helminthic, antiseptic, anti-nociceptive and anti-inflammatory activities. In ancient China, extracts of *Artemisia vulgaris* (L.) were used to control stored-product insect pests (Ding *et al*., 2000). The active components identified in *A. vulgaris* includes flavonoids, sesquiterpene lactones, alkaloids (Bamoniri *et al*., 2010) and many phenolic compounds which are important mainly for their antioxidant and health benefits (Melguizo-Melguizo *et al*., 2014).

The present study aimed to investigate the ethanol extract of *Artemisia vulgaris* plant leaves analysis of the Preliminary phytochemical screening analysis, antioxidant properties and also assessed on the antibacterial properties of ethanol extract of *Artemisia vulgaris* plant leaves against *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* using the agar disk diffusion methods.

MATERIALS AND METHODS

Collection and Preparation of ethanol extract from *Artemisia vulgaris leaves*

Artemisia vulgaris fresh leaves were collected from Nilgiri District, Tamilnadu, India. The collected leaves were washed thoroughly with running tap water followed by sterile distilled water. The leaves were then kept in the shade to dry at room temperature for two weeks, and then were ground into a powder using a kitchen blender. The leaf broth solution was prepared by combining 100 g of the final powder sample and 99.8% ethanol by cold maceration technique. The liquid extract obtained was filtered by using Whatman filter paper 1 and allowed to evaporate in a rotary evaporator so as to get the plant extract in the crude

form. The extract was then filtered and collected in an Erlenmeyer flask and stored properly in the refrigerator at 4 °C for further used.

Figure 1: Ethanolic extract of *A. vulgaris*

Preliminary phytochemical screening

The detection of phytoconstituent present in the ethanolic extract of *A. vulgaris* leaves was carried out by standard methods (table l)

Table: 1

S. No	Phytochemical Test	Test	Procedure EEAV- ethanolic extract of *A. vulgaris* leaves
1	Protein $RCH(NH_2)COOH$	Xanthoproteic	In 2 ml EEAV add 2 ml of concentrated HNO_3 observation of orange colour indicates the presence of proteins (Godghate *et al*., 2012)
2	Carbohydrates $C_6H_{12}O_6$	Benedict's	In 2 ml EEAV add 2 ml of Benedict's reagent and boiled. Formation of orange red precipitate indicates the presence of carbohydrates (Godghate *et al*., 2012)
3	Alkaloides $C_{18}H_{25}NO_5$	Mayer's test	In 2 ml EEAV, drops of Mayer's reagent were slowly added by the side of the test tube, formation of a white or creamy precipitate indicates the presence of alkaloids (Kumar *et al*., 2012)
4	Phenolics C_6H_6O	Phenol test	0.5 mL of FeCl3 (w/v) solution was added to 2 ml of EEAV solution, formation of an intense colour indicated the presence of phenolics (Gibbs, 1974)

5	**Flavonoids** **C_6-C_3-C_6**	**alkaline reagent**	2 ml of 2% sodium hydroxide solution was added to 2 ml of EEAV. Appearance of yellow colour precipitation indicates the presence of flavonoids (Kumar *et al*., 2012)
6	**Triterpenoids** **C_30H_{48}**	**Salkowski's**	In 2 ml EEAV add 1 ml of chloroform followed by a few drops of concentrated H_2SO_4 on the side of the test tube and shaken well, formation of yellow colour at the lower layered indicates the presence of triterpenoids (Kumar *et al*., 2012)
7	**Saponins** **$C_{58}H_{94}O_{27}$**	**frothing**	2 ml EEAV was diluted with 10 ml of distilled water in a test tube and shaken for 5 mins, observation of stable foam indicates the presence of saponins (Auwal *et al*., 2014)
8	**Tannins** **$C_{76}H_{52}O_{46}$**	**ferric chloride**	2 ml EEAV was mixed with few drops of 10% ferric chloride solution, the change of colour into dark blue or green indicates the presence of gallic tannins and catechol tannins (Auwal *et al*., 2014)
9	**Glycosides** **C-glycosyl**	**Keller-Kilani**	A mixture of 4 ml of glacial acetic acid and 1 drop of 2.0% FeCl3was added to 10 ml EEAV followed by the addition of 1 ml of concentrated H2SO4. Formation of brown ring between the layers indicates the presence of cardiac glycosides (Gul *et al*., 2017)

In-vitro antioxidant assay

DDPH free radical scavenging activity

Radical scavenging activity of ethanolic extract of *A. vulgaris* leaves was determined by Calorimetric assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a source of free radical according to the procedure of Blois with a slight alteration (Blois, 1958; Olugbami *et al*., 2015). The collected solutions of ethanolic extract of *A. vulgaris* leaves extract were prepared at a concentration of 1 mg/ml and for test sample; it was serially diluted to make the concentrations of 10, 20, 30, 40 and 50 µg/ ml respectively.

0.1 mM DPPH solution was prepared by dissolving 3.94 mg in 100 ml of ethanol and then 1 ml of this solution was mixed with 150 µl ethanol, which was used as control. The above mentioned concentration of ethanolic extract of *A. vulgaris* leaves was taken and mixed with 1 ml DPPH solutions to each test tube. After 15 min of incubation, the absorbance of control and test sample was measured at 517 nm in UV- visible spectrophotometer and the compound ascorbic acid was used as a reference. The percentage inhibition and IC_{50} were calculated. The free radical scavenging activity (% inhibition activity) was calculated using the given formula-

$$\text{Inhibition activity (\%)} = \frac{\text{Control absorbance - Sample absorbance}}{\text{Control absorbance}} \times 100$$

Reducing power by $FeCl_3$

Reducing power of ethanolic extract of *A. vulgaris* leaves was measured by the method of Oyaizu with small modifications (Oyaizu, 1986). Samples of ethanolic extract of *A. vulgaris* were prepared at a

concentration of 1 mg/ml and the test sample concentrations as 10, 20, 40, 60, 80 and 100 µg/ml were prepared by serial dilution. Here, 2 ml of above concentration were mixed thoroughly with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% $K_3 Fe (CN)_6$ solution. These mixtures were heated up in the water bath at a constant temperature of 50°C for 20 min and then allowed for cooling, added 2.5 ml of 10% trichloroacetic acid to the mixture and centrifuged for 10 min at 1000 rpm. After centrifugation, 2.5 ml of supernatant part was taken and mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride and incubated for 10 min. Then, absorbance at 700 nm was measured in a spectrophotometer. Control value was also recorded with the same process without the ethanolic extract of *A. vulgaris*. The standard compound Ascorbic acid was used as reference materials

Antibacterial activity of ethanolic extract of *A. vulgaris* leaves

Experimental bacteria were purchased from Microbial facilities at GBIMT, Chandigarh, India. The antibacterial activity of ethanolic extract of *A. vulgaris* leaves against harmful pathogens such as *P. aeruginosa, E. coli* and *K. pneumonia* was tested by the standard Kirby–Bauer disk diffusion method (Bauer *et al.*, 1959). To achieve this method, Mueller-Hinton agar was autoclaved solution transfer to the Petri plates. Those plates were sterilized under UV light. Bacterial cultures were swabbed into MHA plates and sterilized filter paper disks 6 mm were placed on MHA plates. Then the disks were loaded with distilled water as a control, ethanolic extract of *A. vulgaris* leaves (different sample dose 50µg/ml, 100µg/ml and 150µg/ml) were separately placed on the media. The bacterial inoculated plates were incubated at 37°C for 24 h. The diameters of the zones of bacterial growth inhibition surrounding were measured using Hi-Media antibiotic zone scale.

Results and Discussion

Primary phytochemicals screening of ethanol extract of *A. vulgaris* leaves

Primary phytochemicals screening test of ethanol extract of *A. vulgaris* leaves revealed the presence of various bioactive compounds such as Proteins, phenol, saponins, flavonoids, alkaloids, glycosides and triterpenoids shown in Table 2. The present in bioactive compounds of *A. vulgaris* such as flavonoids, sesquiterpenoids, essential oils, tannins, phenols, and saponins may produce an anti-inflammatory response by inhibiting the activity of prostaglandins synthesizing enzyme (Ashok and Upadhyaya, 2013).

Table 2: The primary phytochemicals screening of the ethanol extract of *Artemisia vulgaris*

SL.No	Phytochemical	Resuts
1	proteins	+
2	carbohydrates	-
3	alkaloids	+
4	Phenolics	+
5	Flavanoids	+
6	Triterpenoids	+
7	Saponins	+

8	tannins	-
9	glycosides	+

+ Present and – Absent

In-vitro antioxidants activity

DPPH free radical scavenging activity

Antioxidant activity of ethanol extract of *A. vulgaris* was analysed based on their ability to scavenging free radicals. In this study showed high DPPH radical scavenging activity was obtained for ethanol extract of *A. vulgaris.* The different concentration of ethanol extract of *A. vulgaris* and Ascorbic acid (standard) comparatively tested for antioxidant activity. In this results Figure 2 shows the ethanol extract of *A. vulgaris* was found in the percentage inhibition values of 11.05 to 64.32% which was lower than ascorbic acid (13.22 to 88.43%) at different concentration (10, 20, 30, 40 and 50 µg/ml) and the IC_{50} value of 3.9 (extract) and 2.5 respectively (Table 3). Recently, Thangjam *et al*. (2020) reported that the methanol extract of *A. vulgaris* leaves showed the higher DPPH scavenging activities. Similarly, Pandey *et al*. (2017) studied that crude extract of *A. vulgaris* was found to be effective in scavenging the DPPH radicals.

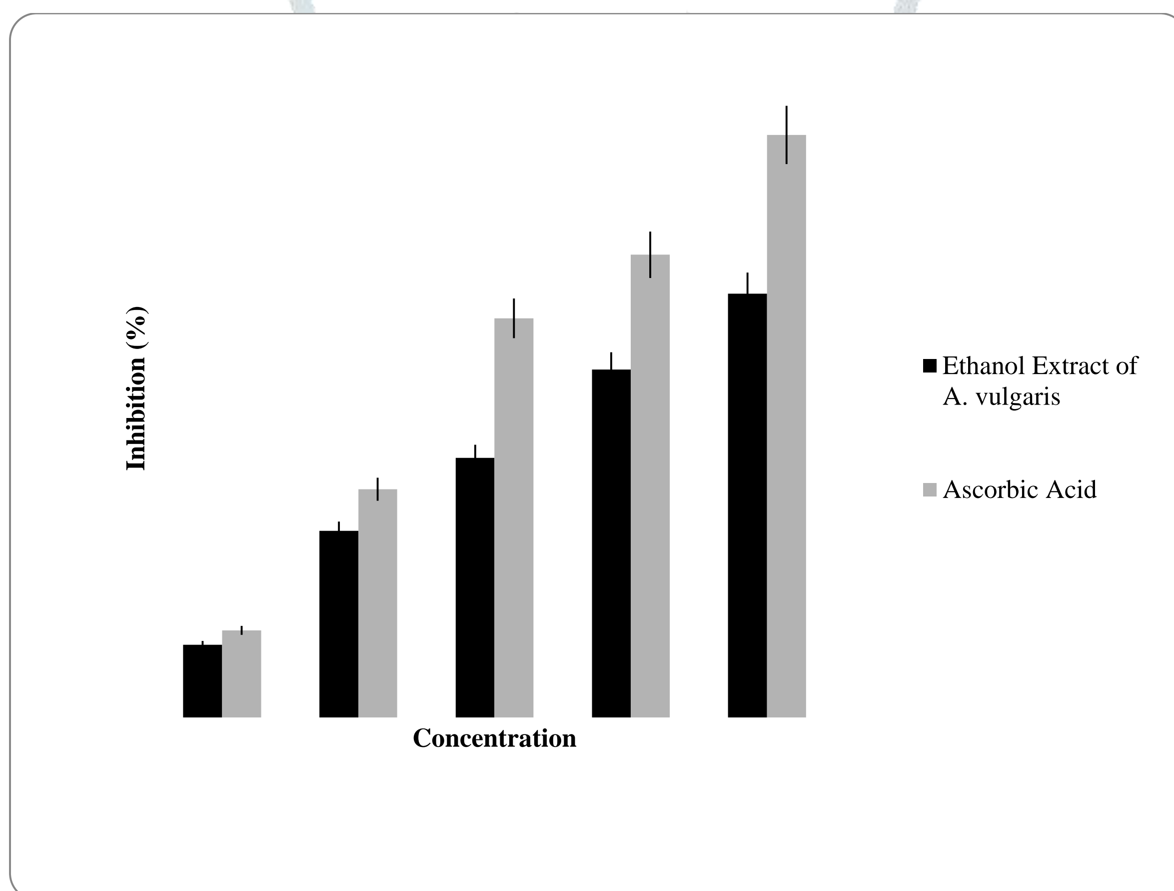

Figure 2: DPPH scavenging activity of ethanol extract of *A. vulgaris* leaves in comparison with ascorbic acid (standard)

Table 3. Inhibition (%) of ethanol extract of *A. vulgaris* leaves and ascorbic acid (standard)

Concentration	Inhibition (%) (Ethanolic extract of *A.vulgaris*)	Inhibition (%) (Ascorbic Acid)
10 µg/ml	11.05	13.22
20 µg/ml	28.32	34.64
30 µg/ml	39.42	60.58
40 µg/ml	52.8	70.23
50 µg/ml	64.32	88.43
IC_{50} µg/ml	3.9	2.5

Reducing power by $FeCl_3$

Moreover, this study carried out the ethanol extract of *A. vulgaris* was analyzed by reducing power assay for confirmation studies of antioxidant activities. In this study was found that the absorbance at 700 nm was increased with the increase in the concentration of the ethanol extract of *A. vulgaris* as 0.15, 0.31, 0.47, 0.67, 0.79, and 0.92 in comparison with the ascorbic acid (standard) with 0.22, 0.49, 0.74, 0.90, 0.98, and 1.12 at different concentration of 10, 20,40,60,80 and 100 µg/mL respectively (Fig. 3 and Table 4). Similarly, Thangjam *et al*. (2020) found that the Methanol extract of *A. vulgaris* has the ability to transform the ferric ion ($Fe3^{+}$) to ferrous ion (Fe^{2+}) as reducing power with the increase of concentration of the extract.

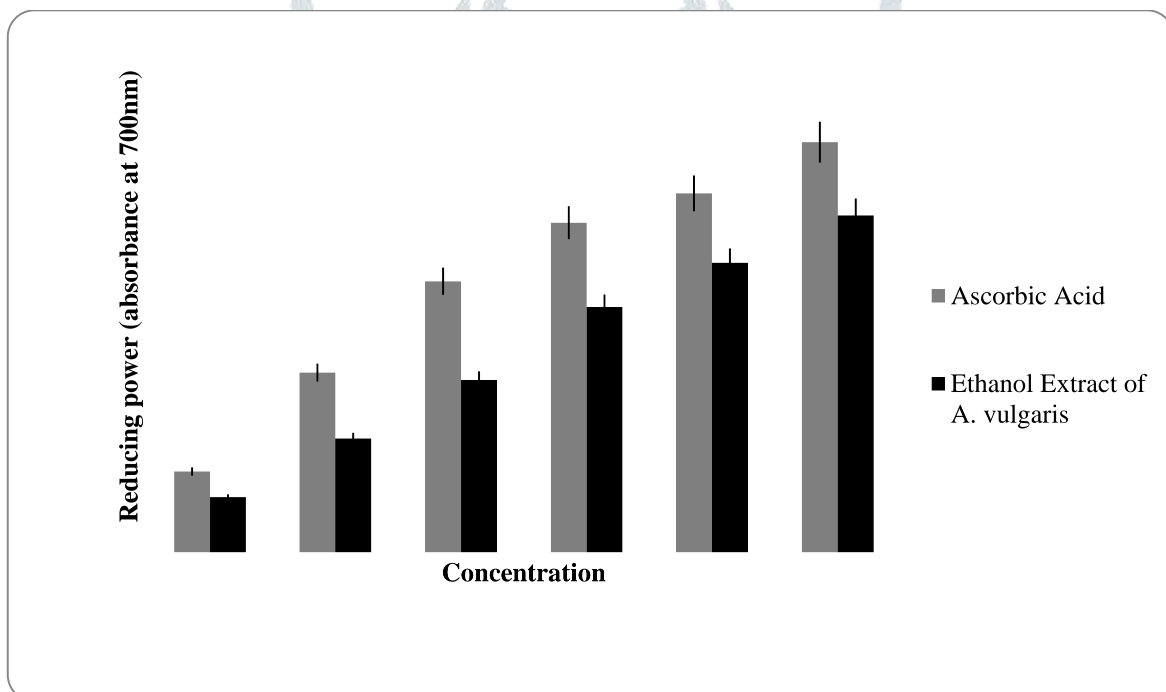

Figure 3: Reducing power of ethanol extract of *A. vulgaris* leaves and Ascorbic acid (standard) antioxidants

Table 4: Reducing power of ethanol extract of *A. vulgaris* leaves and ascorbic acid (standard) antioxidants

Concentration	Absorbance of Ascorbic Acid (Mean ± SD)	Absorbance of ethanolic extract of *A.vulgaris*(Mean ± SD)
10 µg/ml	0.22 ±0.01	0.15 ±0.01
20 µg/ml	0.49 ±0.00	0.31 ±0.00
40 µg/ml	0.74 ±0.00	0.47 ±0.00
60 µg/ml	0.90 ±0.01	0.67 ±0.01
80 µg/ml	0.98 ±0.00	0.79 ±0.01

Antibacterial properties

The antibacterial activities of the ethanol extract of *A. vulgaris* leaves were performed against *P. aeruginosa, E. coli* and *K. pneumonia* by disk diffusion assays. The diameter of the inhibition zone of Control 50µg/ml, 100µg/ml and 150µg/ml is shown in Table 5 and figure 4 (A, B and C)

Figure 4: Antibacterial activity of ethanolic extract of *A. vulgaris* leaves against (A) *P. Aeruginosa* (B) *E. coli* and (C) *K. pneumonia*.

Table 5: Inhibition zone induced by ethanol extract of *A. vulgaris* leaves against bacterial pathogens

The mean diameter of inhibitory zone (mm)				
Bacteria	**Control**	**Ethanolic extract of *A. vulgaris* leaves**		
		(50 µg/ml)	(100 µg/ml)	(150 µg/ml)
E. coli	-	13.9±0.09	14.3±0.33	14.6±0.24
P. aeruginosa	-	6.8±0.47	9.6±1.02	12.5±0.40
K. pneumonia	-	11.2±0.20	14.5±0.40	15.3±0.21

Values are mean ± standard deviation of three replicates

This result indicates that no antibacterial activity was observed with Control against three bacterial strains and high concentration of 150 µg/ml exhibited the highly inhibitory action opposition to *P. aeruginosa*, *E. coli* and, *K. pneumonia* was 14.6 mm, 12.5 mm, and 15.3 mm respectively, when compared to low concentration. In our results agreement with methanol extract of the *A. vulgaris* and *G. fragrantissima* was good antibacterial activity of bacterial pathogens such as *Enterococcus* sp, *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumonia* (Pandey *et al*., 2017). Furthermore studies reported that *Artemisia dubia* extract showed antibacterial potential (Ihsan-ul-Haq *et al*., 2012). In addition, Different parts of *A. vulgaris* were reported to have broad biological activities such as antimicrobial, antihypertensive, antispasmodic and bronchodilator, hepatoprotective, antidepressant, xanthine oxidase inhibitor, and antioxidant (Marasini *et al*., 2020).

Conclusion

Over all in this research concludes ethanol extract of *A. vulgaris* leaves were detection and confirmed the presence of bioactive compounds such as Proteins, phenol, saponins, flavonoids, alkaloids, glycosides and triterpenoids and also, we have found ethanol extract of *Artemisia vulgaris* leaves were high antioxidant properties. Furthermore, our studies found that ethanol extract of *A. vulgaris* leaves proved to be a good antibacterial activity agent. Finally, ethanol extract of *A. vulgaris* leaves were potential utilization in the field of such as cosmetic, pharmaceutical and chemical industries.

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