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Original Article

Metabolic Characterization, Antioxidant Capacity, and *In vitro* Antiurolithiatic Potential of *Achillea millefolium* L.

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ABSTRACT

Objectives: The current study investigated the antioxidant and anti-urolithiatic potential of *A. millefolium* along with its metabolic characterization. **Methods:** *A. millefolium* has been collected at vegetative (V) and mature (Inflorescence (I) and Stem and Leaves (S+L)) stages and extracted in ethanol. Antioxidant potential has been determined by total phenolic content, total flavonoid content, ferric reducing antioxidant potential, total antioxidants through phosphomolybdate assay and free radical scavenging assays (1,1-diphenyl-2-picrylhydrazyl and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid). *In vitro* antiurolithiatic activity is determined by turbidity changes in artificial urine, nucleation assay, and aggregation assay. Fourier transform-infrared spectroscopy and gas chromatography-mass spectrometry has been used to characterize the active metabolites of plant *A. millefolium*. **Results:** Maximum antioxidant activity was observed in Inflorescence with values 76.58 mg of GAE/g of extract, 18.82 mg of RE/g of extract, 199.799 mg of AAE/g of extract and 327.95 mg of AAE/g of extract for TPC, TFC, FRAP and total antioxidants through phosphomolybdate assay respectively. Maximum radical scavenging activity was also observed in inflorescence with 86.3% and 69.655 % inhibition against DPPH and ABTS free radicals at 1000µg/ml concentration respectively. Maximum *in vitro* antiurolithiatic potential of plant *A. millefolium* observed in inflorescence i.e. 80 %, 41.84% and 63.41 % inhibition at 1000µg/ml concentration for turbidity changes in artificial urine method, nucleation assay and aggregation assay respectively. FT- IR and GCMS of inflorescence of *A. millefolium* confirmed the presence of major functional groups and active metabolic compounds respectively. **Conclusion:** Inflorescence of plant *A. millefolium* is an excellent source of natural anti-oxidants with significant antiurolithiatic potential.

Key words: *Achillea millefolium* L., Antioxidant activity, Antiurolithiatic activity, Inflorescence, Active metabolites.

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INTRODUCTION

Free radicals are normally generated in the cell like during respiration and have some beneficial role to play in cell but become harmful when produced in excessive amount in response to external stress like UV irradiation, X-rays, gamma-rays, pollutants and any disease condition which leads to oxidative stress. This oxidative stress elicit on set of many diseases like cancer, cardiovascular diseases, asthma, diabetes, stroke, infertility and neurodegenerative diseases, kidney stones. Antioxidants are free radical scavengers and thus convert the oxidants into their less toxic forms and therefore play an important role in curing of various diseases which results in the production of ROS and cause damage to cell. Plants are excellent source of natural antioxidants and produce various secondary metabolites to boost their defense mechanisms.[1] Urolithiasis is the process of formation of kidney stones in the urinary tract. Kidney stones are one of the most painful disorders of global concern. Super saturation of urine with stone forming substances or imbalance between promoters and inhibitors of kidney stones are two major cause of stone formation. Kidney stone formation i.e. urolithiasis occurs in three major steps including super saturation of urine, nucleation and aggregation.[2] Kidney stones vary in composition like calcium oxalate, calcium phosphate, struvite (magnesium ammonium phosphate) and urate with calcium oxalate stones being the most prevailing. Treatment of kidney stones involves various types of clinical procedures including surgical treatment like extracorporeal shock wave lithotripsy (ESWL) and percutaneous nephrolithotomy (PNL) but different plants reported to have therapeutic potential for treatment of Urolithiasis.[3,4] *A. millefolium* commonly known as yarrow is a high altitude plant mainly characterized by numerous white flowers arranged in corymbose cluster with flat headed appearance[5] and reported as diuretic, anti-inflammatory, antispasmodic, antibacterial, haemostatic, and hypertensive.[6] Present study has been design to study the antioxidant and *in vitro* antiurolithiatic potential of plant *A. millefolium* with its metabolic composition.

MATERIALS AND METHODS

Chemicals

All chemicals (Folin-Ciocalteu reagent, gallic acid, ethanol, sodium nitrite solution, aluminum chloride, rutin, TPTZ solution, acetate buffer, ferric chloride solution, chemicals for phosphomolybdate reagent, $ABTS^{\bullet+}$, potassium persulfate, potassium phosphate buffer, chemicals for AU, Tris, NaCl, calcium chloride, sodium oxalate) used were of good quality and analytical grade. DPPH was procured from Sigma Aldrich.

Collection, identification, and extraction of *A. millefolium*

Plant *A. millefolium* was collected from Poonch District, Jammu and Kashmir at two different growth stages i.e. vegetative (V) and mature separated into stem + leaves (S+L) and inflorescence (I). For identification of plant herbarium specimens were submitted in the (RHMD) NISCAIR with reference ID's NISCAIR/RHMD/consult/2017/3084-33 and

NISCAIR/RHMD/consult/2018/3293-94. Extraction of plant samples was done by soxhlet method in ethanol.

Total phenolic content

Total phenolic content (TPC) of plant extract was determined according to the method of Slinkard *et al.*[7] To 300 µl of plant extract (1mg/ml), 1 ml ethanol, 3.16 ml distilled water and 200 µl Folin-Ciocalteu reagent was added. 600 µl of 10% sodium carbonate solution added after 8 min of incubation at RT, test tubes were wrapped using aluminum foil and incubated at 40°C for 30 min in water bath. For blank equal volume of ethanol was used instead of plant extract. The absorbance value was taken at 765 nm using UV visible spectrophotometer. The standard curve was obtained using gallic acid. TPC was determined in terms of mg of gallic acid equivalent (GAE) per g of extract.

Total flavonoid content

The total flavonoid content (TFC) was determined according to method of Ahmed *et al.*[8] To 300 µl extract (1mg/ml), 3.4 ml aqueous ethanol (30%), 150 µl of 0.5 M aq. sodium nitrite solution and 150 µl of 0.3 M aluminum chloride solution were added. After 5 min, 1 ml of 1 M sodium hydroxide solution was added, shaken well and absorbance was recorded at 506 nm using UV visible spectrophotometer. For blank equal volume of ethanol was used instead of plant extract. Standard curve was obtained using rutin and TFC was determined in terms of mg of rutin equivalent (RE) per g of extract.

Ferric reducing antioxidant potential

FRAP was determined according to the method of Benzie and Strain.[9] For FRAP reagent 25 ml acetate buffer (30 mM; pH 3.6), 2.5 ml TPTZ solution (10 mM in 40 mM HCl) and 2.5 ml ferric chloride solution (20 mM) were prepared, mixed and incubated for 15 min at 37°C. Standard curve was obtained using Ascorbic acid (vitamin C). 2.85 ml of FRAP reagent was taken in a test tube and 150 µl of plant sample (1mg/ml) or standard was added, and this mixture was incubated in the dark for 30 min, and absorbance value was taken at 593 nm. For blank equal volume of ethanol was used instead of plant sample. FRAP was determined in terms of mg of ascorbic acid equivalents (AAE) per g of extract.

Phosphomolybdate assay for total antioxidant capacity

Total antioxidant capacity of extracts was determined by phosphomolybdate assay proposed by Prieto *et al.*[10] the procedure described by Jan *et al.*[11] and Mashwami *et al.*[12] with slight modification. To 300 µl plant extract (1mg/ml), 3 ml of phosphomolybdate reagent (28 mM sodium phosphate, 0.6 M sulfuric acid, and 4 mM ammonium molybdate) was added. Test tubes were wrapped using aluminum foil and were incubated for 90 min at 95°C. Reaction mixture allowed to cool at room temperature and its absorbance were recorded at 765 nm. Standard was obtained using ascorbic acid. For blank equal volume of ethanol is used instead of plant sample. TAC was determined in terms of mg of ascorbic acid equivalents (AAE) per g of extract.

1, 1-diphenyl-2-picrylhydrazyl radical scavenging assay

The DPPH free radical scavenging assay was performed according to the method of Brand-Williams *et al.*[13] with slight modification. Stock of DPPH radical was prepared by mixing 2.4 mg DPPH in 10 ml of ethanol and was stored in a refrigerator for further use. The working solution of the radical was obtained by diluting the stock with ethanol until an absorbance value of 0.98 (±0.02) at 517 nm was observed.[14] To 3 ml of DPPH working solution, 100 µl plant extract (100µg/ml to 1mg/ml) was added and incubated for 30 min at RT in dark. Absorbance value was taken at 517 nm.

2, 2′-azino-bis-3-ethylbenzotiazolin-6-sulfonic acid radical scavenging assay

$ABTS^{\bullet+}$ decolorization assay was done by method of Re *et al.*[15] The working solution of $ABTS^{\bullet+}$ radical having ABTS (7 mM, 9.5 ml) and potassium persulfate (100 mM, 245 µl), distilled water was added to raise the volume up to 10 ml. This solution was stored in dark at RT for 18 h, and diluted with potassium phosphate buffer (0.1 M, pH 7.4) to an absorbance of 0.70 (±0.02) at 734 nm. 100 µl of sample (100-1000 µg/ml) and 2.90 ml of ABTS radical working solution were mixed thoroughly and absorbance value was taken at 734 nm.

The percent DPPH/ABTS radical scavenging activity was calculated using the following formula:

$$\% \text{ Antioxidant activity} = [(Abs_c - Abs_s)/Abs_c] \times 100.$$

Where Abs_c and Abs_s are absorbance of control and sample respectively. For control 100 µl of ethanol was added instead of sample and ascorbic acid (AA) was used as standard.

Turbidity changes in artificial urine

The artificial urine (AU) was prepared according to the method of Burns and Finlayson[16] and had the following composition: sodium chloride 105.5 mmol/l, sodium phosphate 32.3 mmol/1, sodium citrate 3.21 mmol/l, magnesium sulfate 3.85 mmol/1, sodium sulfate 16.95 mmol/1, potassium chloride 63.7 mmol/1, calcium chloride 4.5 mmol/1, sodium oxalate 0.32 mmol/1, ammonium hydroxide 17.9 mmol/1, and ammonium chloride 0.0028 mmol/1. The AU was prepared fresh each day and pH adjusted to 6.0.

Study without inhibitor: A volume of 2 ml of AU was transferred into the cell and 1 ml of distilled water added to it and blank reading was taken 1 ml of 0.01 M sodium oxalate was added, to the previous volume, and the measurement is immediately taken for a period of 420 sec.[17]

Study with inhibitor: Extract was suspended in distilled water, filtered and used at a final concentration of 100, 250, 500, 750 and 1000 µg/ml. A mixture of 2 ml of AU and 1 ml of plant extract solution is versed in the cell. A blank reading was taken and then volume of 1ml of 0.01M sodium oxalate was added and the measurement is immediately taken for a period of 420 sec.[17]

$$\% \text{ Inhibition} = \{(\text{Abs. Control- Abs. Sample})/ \text{Abs. Control}\} * 100$$

Nucleation assay

For nucleation assay method of Hennequin *et al.*[18] was used with some minor modifications. Solutions of calcium chloride and sodium oxalate were prepared at a final concentration of 3mmol/L and 0.5mmol/L, respectively, in a buffer containing Tris 0.05mol/l and NaCl 0.15mol/l at pH5.5. 1.9 ml of calcium chloride solution was mixed with 200µl of the herb extract at different concentrations and incubated for 30 minat 37°C in water bath. Crystallization was started by adding 1.9 ml of sodium oxalate solution. The OD of the solution was monitored at 620 nm for 420 s. % Inhibition = {(Abs. Control- Abs. Sample)/ Abs. Control} * 100.

Aggregation assay

The method used was similar to that described by Hess *et al.*[19] with some minor modifications. `Seed' CaOx monohydrate (COM) crystals were prepared by mixing calcium chloride and sodium oxalate at 50mmol/L. Both solutions were equilibrated to 60°C in hot water bath for 1h and then cooled to 37°C overnight. The crystals were harvested by centrifugation and then evaporated at 37°C. COM crystals were used at a final concentration of 0.8mg/ml, buffered with Tris 0.05mol/l and NaCl 0.15mol/l at pH 5.7. 1 ml extract was taken in test tube to which 3 ml COM crystal solution was added and incubated 37°C and reading were recorded at different time interval of 30, 60, 90, and 120 min.

*% Inhibition = {(Slope Control- Slope Sample)/ Slope Control} * 100*

Metabolic characterization of *A. millefolium*

Fourier transform infrared (FT-IR) spectrum of inflorescence of plant *A.millefolium* was obtained using spectrometer (Shimadzu) in the range of 400–4,000 cm^{-1} and gas chromatoraphy- mass spectrometry was performed by using GCMS program GCMS_QP2010 Ultra with following working conditions: column oven temperature 60°C, injection temperature 260°C, injection mode split, flow control mode linear velocity, pressure 73.3kPa, flame thermionic detector, ion sources temperature 230°C and carrier gas saver off.

Statistical analysis

Performed by calculating ± SEM and strong regression (r^2) value using Microsoft excel 2007.

RESULTS

Plant *A. millefolium* showed maximum TPC, TFC, FRAP and total anti-oxidants through phosphomolybdate assay in inflorescence i.e. 76.58 mg of GAE/g of extract, 18.82 mg of RE/g of extract, 199.799 mg of AAE/g of extract and 327.95 mg of AAE/g of extract respectively followed by stem and leaves (S+L) and lowest activity in vegetative stage (V) (Table 1). Plant *A. millefolium* showed significant radical scavenging activity (DPPH and ABTS) with maximum % activity in inflorescence (I) i.e. 86.3% and 69.655% at 1000µg/ml concentration followed by stem and leaves (S+L) and lowest activity in vegetative stage (V). Plant *A. millefolium* showed increasing activity with increasing concentration i.e. 100 µg/ml to 1000 µg/ml of *A. millefolium* (Table 2).

Plant *A. millefolium* showed excellent % inhibition of formation of calcium oxalate crystals in artificial urine with maximum activity in inflorescence (I) followed by stem and leaves (S+L) and lowest activity in vegetative stage (V) with 80%, 55% and 34% inhibition at 1000 µg/ml respectively (Figure 1 (A)). Plant *A. millefolium* showed significant % inhibition against nucleation of calcium oxalate stones with maximum activity in inflorescence followed by stem and leaves (S+L) and lowest activity in vegetative stage (V) with 41.84%, 36.45 % and 33.92 % inhibition at 1000 µg/ml concentration respectively (Figure 1 (B)). Plant *A. millefolium* showed excellent activity against aggregation of nuclei with similar order i.e. maximum in inflorescence (I) followed by stem and leaves (S+L) and lowest activity in vegetative stage (V) with 63.41%, 29.26% and 19.51% inhibition at 1000 µg/ml concentration respectively (Figure 1 (C)). In aggregation assay absorption at 620 nm decreases with time due to aggregation of calcium oxalate nuclei as observed plant *A. millefolium* with maximum slope in inflorescence (Figure 1 (D)).

FT- IR and GC- MS has been used to study the metabolic composition of inflorescence (I) of *A. millefolium* in order to reveal the possible metabolites for its excellent antioxidant and *in vitro* antiurolithiatic activity. FT- IR of inflorescence of plant *A. millefolium* revealed the presence of major functional groups including C-Cl, S=O, C-H, C-N, N-O, C=C, C=O, C-H and N-H (Table 3 and Figure 2). GC- MS of inflorescence of plant *A. millefolium* revealed the presence of total 55 compounds with many compounds having different activities (Table 3 and Figure 3).

Table 1: TPC, TFC, FRAP and phosphomolybdate assay of *Achillea millefolium* L.

S. No.	Plant Sample	TPC (mg of GAE/g)	TFC (mg of RE/g)	FRAP (mg of AAE/g)	Phosphomolybdate Assay (mg of AAE/g)
1.	V	5.08 +0.05^c	4.27 +0.04^c	49.4977 +0.25233^c	85.29533+ 0.645297^c
2.	S+L	32.21333 +0.089505^b	6.17 +0.111505^b	65.5993 +0.91454^b	207.03 + 0.32078^b
3.	I	76.57667 +0.52667^a	18.82333 +0.84667^a	199.799 +4.48545^a	327.9467+ 0.534208^a

V= vegetative *A. millefolium*, S+L= stem and leaves and I= inflorescence of mature *A. millefolium,* different superscript in increasing order of mean values denotes the statistically significant difference determined by one way ANOVA followed by t- test

Table 2: Radical scavenging activity (DPPH and ABTS) of *Achillea millefolium* L.

Concentration (µg/ml)	% Inhibition + SEM (DPPH)			
	AA	V	S+L	I
100	57.86 +0.16^a	33.76+0.24^d	36.235+0.235^c	57.09+1.06^b
250	90.385+0.015^a	38.39+0.21^d	41.51+0.49^c	63.94+0.94^b
500	92.73+0.47^a	39.735+0.035^d	53.06+0.34^c	75.575+0.125^b
750	93.365+0.035^a	4034+0.34^d	63.615+0.015^c	83.26+0.26^b
1000	93.7+0.03^a	41.905+0.0475^d	70.475+1.2375^c	86.3+0.00^b
	% Inhibition + SEM (ABTS)			
100	36.996+ 0.096^a	2.71+ 0.09^d	12.095+ 0.095^c	22.155+ 0.355^b
250	78.67+ 2.33^a	5.4+ 0.4^d	16.12+ 0.18^c	30.35+ 0.15^b
500	89.95+ 0.35^a	7.65+ 0.15^d	18.43+ 0.33^c	60.39+ 0.39^b
750	90.74 +0.06^a	10.155+ 0.155^d	30+ 0.2^c	61.125+ 0.225^b
1000	91.19+ 0.1955^a	28.555+ 0.255^d	34.025+ 0.075^c	69.655+ 0.245^b

AA= ascorbic acid, V= vegetative A. millefolium, S+L= stem and leaves and I = inflorescence of mature A. millefolium, different superscript in increasing order of mean values denotes the statistically significant difference determined by one way ANOVA followed by t- test

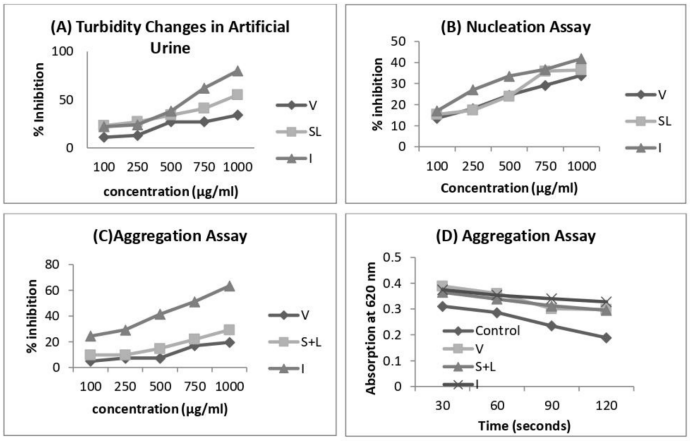

Figure 1: Represents Antiurolithiatic activity by Turbidity changes in Artificial Urine assay (A), Nucleation assay (B) and Aggregation assay (C and D) of plant *A. millefolium* (V= vegetative plant, S+L= stem and leaves, I= Inflorescence).

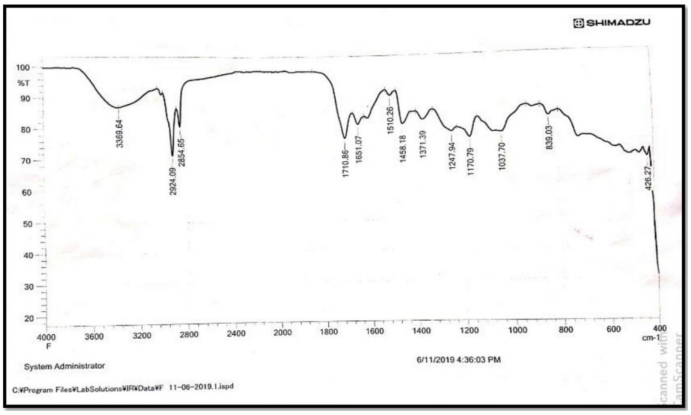

Figure 2: Represents FT- IR Graph of inflorescence of plant *A. millefolium.*

DISCUSSION

Farhadi *et al.*[20] studied the TPC and TFC of *A. millefolium* at different growth stages with maximum activity in flowering stage. Georgieva *et al.*[21] studied the total polyphenolic concentration of the water extracts of *A. millefolium* with 7.92 ± 0.09 mg GAE/g dw comparable to current value of 6.12 mg GAE/g dw in ethanolic extract of inflorescence. Adam *et al.*[22] have also reported total phenolic compounds of *A. millefolium* of leaf of water: acetonitrile (70: 30) extract with 0.645 mg quercetin/g leaf which is much lower than the current value. Keser *et al.*[23] have studied water and ethanolic extract of flower, leaf and seed of *A. millefolium* and found maximum flavonoid content in water extract of leaves i.e. 0.979 mg/ml of rutin equivalent/ g of dw which is lower than the current value of 1.50 mg QE/g dw in ethanolic extract of inflorescence. Georgieva *et al.*[21] also studied FRAP activity of plant with maximum value 76.41 ± 0.53 µM TE/g dw. Plant *A. millefolium* showed maximum total

Table 3: FT- IR and GC- MS analysis of inflorescence of plant *A. millefolium*.

FT- IR		
Peak (cm^{-1})	**Stretching type**	**Functional Groups**
839.03	C-Cl stretching	halo compound
1037.70	S=O stretching	Sulfoxide
1170.79	C-H stretching	Alkane compound
1247.94	C-N stretching	Amine
1510.26	N-O stretching	Nitro compound
1651.07	C=C stretching	Alkene
1710.86	C=O stretching	aliphatic ketone, carboxylic acid, conjugated aldehyde
2854.65	C-H stretching	Alkane
2924.09	C-H stretching	Alkane
3369.64	N-H Stretching	aliphatic primary amine
GC- MS		
S. No.	**% Area**	**Compound name**
1.	0.80	2-methoxy-4-vinylphenol
2.	0.46	Phenol, 2,6-dimethoxy
3.	0.62	Cyclohexanemethanol, 4-ethenyl-α,α,4- trimethyl-3-(1- methylethenyl)-, [1R- (1α,3α,4β)]-
4.	0.65	1H-cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene
5.	0.30	Guaiol
8.	0.46	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydro-. alpha.,. alpha.,4a,8-tetramethyl-, (2R-cis)-
9.	1.84	.tau.-cadinol
10.	7.22	2-Naphthalenemethanol, decahydro-. alpha.,. alpha., 4a-trimethyl-8-methylene-, [2R-(2. alpha.,4a. alpha.,8a. beta.)]-
11.	0.54	Tetradecanoic acid
12.	13.78	n-hexadecanoic acid
13.	2.90	Hexadecanoic acid, ethyl ester
14.	0.44	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)-
15.	0.59	9,12-octadecadienoic acid (Z,Z)-, methyl ester
16.	0.94	Phytol
17.	20.95	9, 12-octadecadienoic acid (Z,Z)-
18.	8.10	Linoleic acid ethyl ester
19.	1.67	Ethyl Oleate
20.	0.61	Santamarine
21.	0.94	Reynosin
22.	0.28	n-tetracosanol-1
23.	1.13	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
24.	0.54	Vitamin E
25.	1.43	Stigmasterol
26.	3.22	.gamma.-sitosterol
27.	0.38	.alpha.-amyrin
28.	0.56	Lupeol

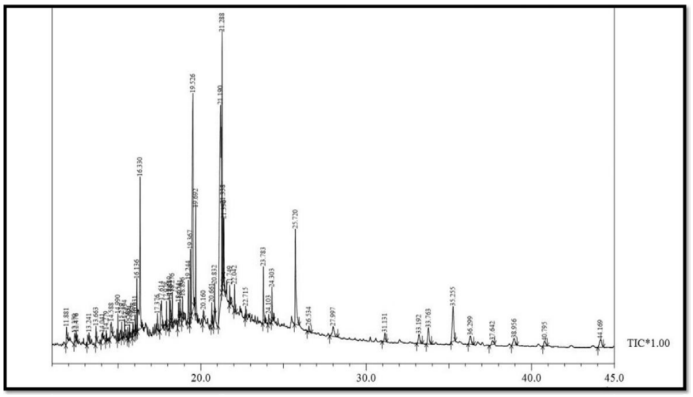

Figure 3: Represents GC- MS graph of inflorescence of plant *A. millefolium*.

antioxidants in inflorescence also by phosphomolybdate assay. Similar results have been obtained for radical scavenging activity of plant *A. millefolium* with maximum activity in inflorescence. Adam *et al.*[22] found maximum DPPH radical scavenging activity of 18.31 % in *A. millefoium* leaf extracted in water: acetonitrile (70:30). Keser *et al.*[23] have found maximum ABTS radical scavenging activity in crude ethanolic extract of flower with 97.40 % inhibition and maximum DPPH radical scavenging activity in the crude ethanolic extract of flower with 91.03 % inhibition. Abdossi & Kazemi[24] also reported the DPPH activity in *Achillea* sp. Plant *Achillea millefolium* L. showed excellent antiurolithiatic potential against calcium oxalate kidney stones with significant % inhibition. Vamsi *et al.*[25] have studied the antiurolithiatic potential of methanolic extract of *Mucuna pruien*s through turbidity changes in artificial urine method and reported maximum activity at 250 µg/ml concentration with 63.1 % inhibition whereas Vennila *et al.*[26] reported maximum activity at 100 mg/ml concentration with 98% inhibition in *Melia dubia* leaves. Similar assay has also been performed by Kumar *et al.*[27] and Khare *et al.*[28]. Galani & Panchal[29] reported the nucleation and aggregation activity of seeds of plant *Centratherum anthelminticum* with maximum % inhibition at 1000 µg/ml concentration i.e. 27.7 % and 70.04 % inhibition respectively. Similar assay of nucleation and aggregation has also been performed by Atmani & Khan[30] on *Herniaria hirsuta*, by Binu and Vijayakumari[31] on *Strychnos potatorum* and by Chandirika and Annadurai[32] on *Lantana camara*.

FT- IR of inflorescence of *A. millefolium* revealed presence of major functional groups including halogen, alkane, alkene, alcohol, amine and nitro compounds (Table 3) which provide antibacterial, antioxidant activity and other pharmacokinetic properties to the compound or drug molecule as reported by Marc and Robinson[33] and Pillai & Nair[34]. GC- MS revealed santamarine and reynosine which are principle identifying compounds of family asteraceae reported by Coronado-Aceves *et al.*[35] GC- MS revealed many compounds reported to have antioxidant activity like 2-methoxy-4-vinylphenol,[36] gamma.-sitosterol,[37] tetradecanoic acid,[38] stigmasterol,[39] n-hexadecanoic acid,[40] linoleic acid ethyl ester, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester,[41] alpha.-amyrin,[42] hexadecanoic acid, ethyl ester, vitamin E,[43] phytol,[44] n-tetracosanol-1.[45] Major antiurolithiatic compounds were also revealed including Lupeol,[46] Stigmasterol,[47] alpha. amyrin,[48] and gamma. sitosterol.[49]

CONCLUSION

A. millefolium showed increased antioxidant and *in vitro* antiurolithiatic activity from vegetative stage to mature stage and maximum activity was found in inflorescence. Hence, *A. millefolium* is an excellent source of natural antioxidants and have capacity to neutralize the damage cause by ROS generated in the cell during various diseased conditions including kidney stones formation. Inflorescence of plant *A. millefolium* showed excellent potential to be used as herbal drug to cure Urolithiasis.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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