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Evaluation of antioxidant potential and phytochemical characterization using GCMS analysis of bioactive compounds of *Achillea filipendulina* (L.) Leaves

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Abstract
Achillea filipendulina (L.) is an important medicinal herb of family Asteraceae was investigated with aim to the screening of bioactive compounds present in its leaves. The qualitative test reveals the presence of alkaloids, flavonoids, terpenoids, glycosides, steroids, saponin and tannin. 31 bioactive compounds were identified from GCMS analysis. Among the identified compounds, 13-Docosenamide, (Z) and 9-Octadecenamide was found to be the major compound. *In vitro* antioxidant scavenging activity was performed by DPPH assay and hydrogen peroxide assay showing the highest % inhibition of 92.98% and 82.02% as compared to standard respectively. Analysis of total phenolic and flavonoid content exhibited the presence of significant amount of phenolic content i.e. 261.77±0.54 mg/g and flavonoid 54.51±0.01 mg/g. Since the plant possess high content of phenols and flavonoids and also significantly high antioxidant scavenging activity it may have large number of pharmacological values. The results suggested the possible use of *A. filipendulina* for the development of highly potent, safe and novel antioxidant compounds.

Keywords: *Achillea filipendulina*, DPPH, antioxidant, phenols, methanolic extract, 13-Docosenamide

Introduction
The plant diversity is a treasure house of potential drugs and there has been an increasing awareness in the recent years about the importance of medicinal plants. Drugs from the plants are easily available, safe, less expensive, and efficient and rarely have any side effects. For thousands of years, in traditional treatment medicinal plants are used to cure variety of diseases. Humans have been using many medicinal plants as a source of medicine for different ailments and curing diseases since time immemorial. The demand for herbal medicine is increasing in both developed and developing countries since they have few or no side effects. A great number of medicinal plants contain some chemical constituents that exhibit antioxidant properties. Activities of antioxidants are primarily related to plant phenolic that may found in all parts of plants (Mathew and Abraham 2006) [18]. Phenolic compounds have received much attention and are of interest as potential chemo preventive agents. Plants may be an attractive alternative to currently available commercial antioxidants (Kayano *et al.*, 2002; Borra *et al.*, 2013) [13, 3]. As there is restriction on the synthetic antioxidants i.e. butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroqunone (TBHQ) which is widely used in the food industry because of their toxic effect to liver and their carcinogenicity (Grice, 1986) [8]. Therefore, the utilization and development of plant based phenolic for more effective antioxidants are desired. Plant based antioxidant offers good potential for providing important fundamental benefits to public health as they play an important role in cancer chemoprevention, delaying, inhibiting or even reversal of the process of carcinogenesis (Shureiqi *et al.*, 2000 and Tsao *et al.*, 2004) [27, 30]. Since plant-derived antioxidants are generally considered to be multifunctional, accordingly attention is focused on the protective biochemical functions of naturally occurring antioxidants. The genus *Achillea* belongs to the family Asteraceae (Compositae). It is represented by about 85 species mostly found in Europe and Asia and a few in North America. It has great medicinal values like antioxidant, antidiabetic, antimicrobial and anti-inflammatory properties (Saedinia *et al.*, 2011 and Stojanovic *et al.*, 2005) [23, 29]. Presently studied species *Achillea filipendulina* L. (Fernleaves Yarrow) is a perennial, herbaceous and aromatic herb. The stem is thickened at base and it grows 80 - 100 cm. The leaves are green, pinnate, hairy and feathered shape. It flowers from June to August and flowers grow in cluster arranged in corymbs and of rich gold colour.

Literature showed that there is not much study on medicinal uses of *Achillea filipendulina*, but this plant is traditionally used as emmenagogue, expectorant and antitussive (Mosayebi *et al.*, 2008) [20]. To the best of our knowledge, information concerning the *in vitro* antioxidant features of methanolic extract of *Achillea filipendulina* has not been found in the literature. The present study was designed to screen the bioactive phytochemicals present in plant using GCMS analysis and to evaluate the methanolic plant extract for its antioxidant potential. The conclusion drawn from this work may add to the overall value of the medicinal potential of the herb.

Materials and Methods

Plant collection and identification

Plant was collected in the month of August from Mashobra, Himachal Pradesh and identified from Regional Horticultural Research and Training Section, Mashobra. This identification was later confirmed with the help of diagnostic keys and morphological descriptions given in various floras.

Plant extract preparation

Leaves of *A. filipendulina* was washed thoroughly with running tap water and with distilled water then shade dried for 2-3 days then oven dried and grounded into course powder using pestle-mortar/ grinder. 10g of fine powder then subjected to extraction with 20 ml of methanol solvent in the orbital shaker for 24 hrs. The extract was filtered through Whatman paper and the filtrate was evaporated at room temperature until a very concentrated extract was obtained. The residue remaining was used as plant extract and again re-suspended in methanol to make 1mg/ml stock solution and was stored at 4°C for further use.

Preliminary qualitative phytochemical analysis

Qualitative phytochemical analysis was carried out with different extracts *viz.*, aqueous, methanol, ethanol and chloroform. These phytochemical were selected because of their importance in pharmaceuticals industry. Phytochemicals along with their standard confirmatory test were given below.

- **Alkaloids:** Manu and Kuttan, 2009 [17].
- **Flavonoids:** Njoku and Odi, 2009 [21].
- **Glycosides:** Siddiqui and Ali, 1997 [28].
- **Terpenoids:** Siddiqui and Ali, 1997 [28].
- **Steroids:** Siddiqui and Ali, 1997 [28].
- **Tannins:** Njoku and Odi, 2009 [21].
- **Saponins:** Njoku and Odi, 2009 [21].

Gas Chromatography Mass Spectrum analysis

GCMS technique was used to analyzed the composition of the methanolic leaves extract. GC/MS analyses were carried out using a thermo Trace 1300GC coupled with Thermo TSQ 800 Triple Quadrupole MS fitted with RT-5 (Resteck). Capillary column TG 5MS (30m X 0.25mm) with 0.2 µm film thickness was used.

Characterization of Components

Interpretation on mass spectrum of GCMS was done using the database of National Institute of Standard and Technology (NIST). The fragmentation patterns of the mass spectra of unknown compound were compared with those of the known compounds stored in the (NIST) research library. The retention time, name, molecular weight and molecular formula and properties of the components of the test materials were mentioned (Table 1 and Fig.1).

Determination of *In Vitro* Total Antioxidant Activity

DPPH radical scavenging assay

The free radical scavenging activity was determined by DPPH (diphenyl- 2-picrylhydrazyl) assay (Harini *et al.*, 2012) [10]. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

IC_{50} represents the level where 50% of the radicals were scavenged by test samples.

Hydrogen peroxide radical activity

Hydrogen peroxide scavenging potential of the plant extract was determined by the method used by Jayaprakasha *et al.*, (2004) [12].

The percentage of H_2O_2 scavenging by the plant extract was calculated as follows

$$\text{\% Scavenged (Hydrogen peroxide)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

IC_{50} represents the level where 50% of the radicals were scavenged by test samples

Determination of Total Phenolic Content (TPC)

Total phenolic content (TPC) of the plant extracts was determined by using the Folin-Ciocalteu reagent (Habila *et al.*, 2010) [9].

Determination of Total Flavonoids Content (TFC)

TFC of the plant extracts was determined by using $AlCl_3$ colorimetric assay as proposed by (Chang *et al.*, 2002) [4].

Results

Qualitative Phytochemical Analysis

The preliminary phytochemical analysis of leaves was carried out in four different extracts i.e. aqueous, methanol, ethanol and chloroform. *A. filipendulina* leaves revealed the presence of alkaloids, glycosides, flavonoid, phenols, saponins, tannins, terpinoids, and steroid in aqueous, methanol and ethanol extracts tested except for tannins in aqueous and steroid in ethanol. Only alkaloid and steroids were detected in the chloroform extract. Results for the same presented in Table 1.

Table 1: Preliminary Qualitative analysis of phytochemical in various leaves extracts of *Achillea filipendulina*

S. No	Phytochemical tests	Aqueous extract	Methanol extract	Ethanol extract	Chloroform extract
1.	Alkaloids	+	+	+	+
2.	Flavonoids	+	+	+	-
3.	Glycosides	+	+	+	-
4.	Terpenoids	+	+	+	-
5.	Steroids	+	+	-	+
6.	Tannins	-	+	+	-
7.	Saponin	+	+	+	-

(+) indicate presence, (-) indicate absent

Gas Chromatography Mass Spectroscopy

From GCMS analysis, 31 active components were detected from the methanolic leaves extract of *A. filipendulina*. The identification of phytochemical compounds was based on retention time, molecular formula, peak area, molecular weight and medicinal activity were presented in Table 2. Among the identified compounds, 13-Docosenamide, (Z) and 9-Octadecenamide was found to be the major compound attained the largest peak (86.89%) with the retention time (19.66 min) which was followed by 3,4-Hexanediol, 3,4-bis (4hydroxyphenyl) and 1,4-Dimethoxy 2,3-dimethylbenzene (7.22%). Another compound Heneicosane, 11-(1-ethylpropyl)-, Undecane and decane showed the peak area of 2.69%. The compound 1-Heptatriacotanol, cis-Vaccenic acid, Z -4-Nonadecen1-ol-acetate, 11,13-Dimethyl 12-tetradecen1-ol-acetate and Ethanol, 2-(9, 12 octadecadienyloxy), (Z,Z) showed the peak area of 0.84%. 6,9,12-Octadecatrienoic acid methyl ester (*gamma*-Linolenic acid), Trilinolein, 6,9,12,15-Docosatetraenoic acid, methyl ester, Dasycarpidan-1methanol, acetate (ester) and Ethyl iso-allocholate showed the peak area of 0.52% with the retention time 15.04 min. The other compounds showing less prominent peak presented in Fig. 1.

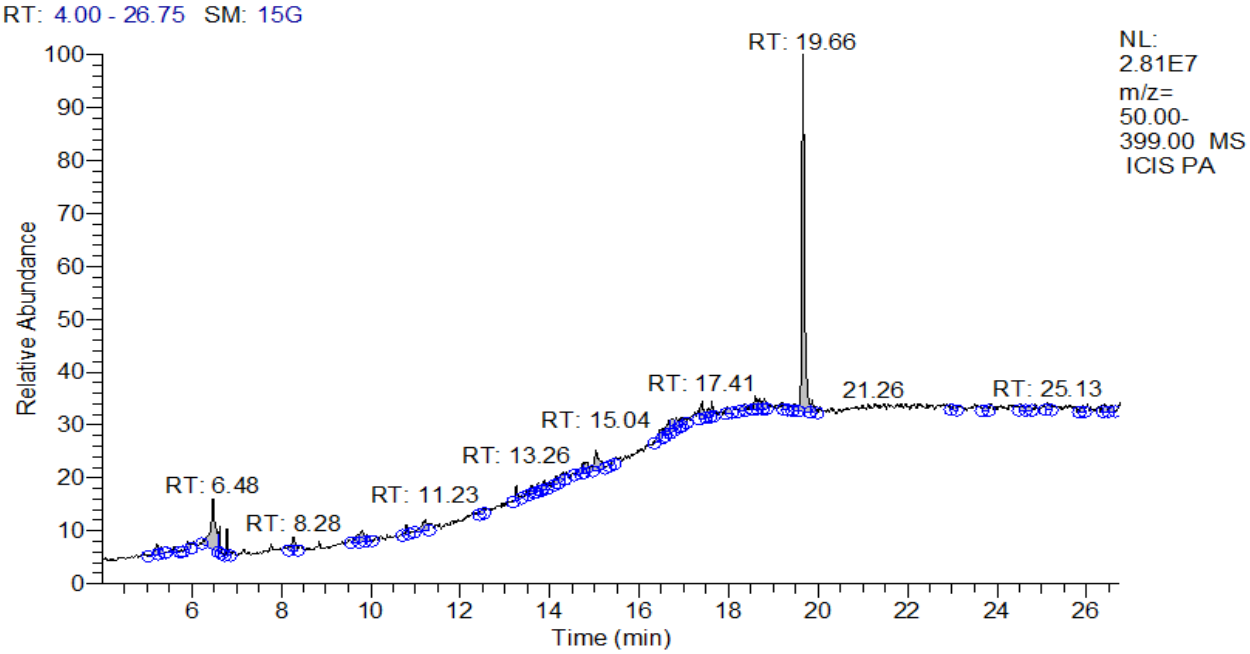


Fig 1: GCMS Chromatogram of methanolic leaves extract of *Achillea filipendulina*

Table 2: Bioactive Components identified and their activity in methanoic leaves extract of *Achillea filipendulina* using GCMS analysis

S. No.	RT	Name of the compound	Molecular formula	MW	Peak Area %	Compound nature	Medicinal Activity
1	5.23	Doconexent	$C_{22}H_{32}O_2$	614	1.26	Fatty acid	Support brain development, protect neurological function
2	5.61	Cinnamic acid, 4-hydroxy-3-methoxy-, {5-hydroxy-2-hydroxymethyl-6-[2-(4-hydroxy-3-methoxyphenyl)ethoxy]-4-(6-methyl-3,4,5-trihydroxytetrahydropyran-2-yloxy) tetrahydropyran-3-yl} ester	$C_{31}H_{40}O_{15}$	652	1.03	Ester	Flavoring agent, used in pharmaceutical, used in perfumery industries
3	5.78	Dodecanoic acid	$C_{32}H_{50}O_6$	530	1.03	Fatty acid	Increase total serum cholesterol, increase good blood cholesterol
4	5.91	Desoximetasone	$C_{22}H_{29}FO_4$	376	1.20	Steroid	Strong corticostreroid, Treat eczema, dermatitis, allergies, rashes
5	6.48	3,4-Hexanediol,3,4-bis-(4hydroxyphenyl)	$C_{18}H_{22}O_4$	302	7.22	Phenol	Antioxidant, Anti-cancer, Anti-inflammatory

6	6.48	1,4-Dimethoxy, 2,3-dimethylbenzene	$C_{10}H_{14}O_2$	166	7.22	Benzene	Perfumes, soaps, pharmaceutical
7	6.79	Heneicosane, 11-(1-ethylpropyl)-	$C_{26}H_{54}$	366	1.61	Pheromone	Major component of safflower flower essential oil
8	6.79	Undecane	$C_{11}H_{24}$	156	2.69	Alkane	Antimicrobial agents, transducer for immunosensor and its method of production. Used as carcinogens, enzyme inhibitors, solvents
9	6.79	Decane	$C_{10}H_{22}$	142	2.69	Alkane	Used for industrial purpose or as a type of hydrocarbon solvent.
10	8.28	Decalin-8a-ol-7-one, 4a,8-dimethyl-2-[2-(t-butyldimethylsilyloxy)-1-methyleneethyl]-	$C_{21}H_{38}O_3Si$	366	1.86	Hydrocarbon	Aromatic
11	9.72	Dihydroxanthin	$C_{17}H_{24}O_5$	308	1.16	Carotenoid	Food additive
12	10.79	.psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	$C_{42}H_{64}O_2$	600	0.75.	Carotene	Antioxidant, Anti-asthama, Anti-cancer, immune enhancement, arthrosclerosis prevention, gingivitis
13	11.23	Calcitrol	$C_{27}H_{44}O_3$	416	2.47	Steroid	Treatment of hypocalcaemia, treat osteoporosis, prevention of corticosteroid
14	12.48	Gibberellic acid	$C_{19}H_{22}O_6$	346	0.70	Sequesterpene	Stimulate rapid stem and root growth, increase seed germination, induce mitotic division
15	12.48	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498	0.70	Ether, Steroid	Antimicrobial, Antioxidant, Anti-inflammatory, Anti-arthritic Antiasthma, Diuretic
16	13.26	1-Heptatriacotanol	$C_{37}H_{76}O$	536	1.15	Fatty alcohol	Anti-hypercholeaterolemic
17	13.26	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	1.15	Fatty acid	Lowered total cholesterol, triglyceride level, Shows Anti-carcinogenic properties, inhibition of telomerase enzyme
18	13.26	Z -4-Nonadecen1-ol-acetate	$C_{21}H_{40}O_2$	194	0.84	Acetate compound	Used to make surfactants, lubricating oils, pharmaceuticals
19	13.26	11,13-Dimethyl 12-tetradecen1-ol-acetate	$C_{18}H_{34}O_2$	282	0.84	Acetate compound	No activity reported
20	13.26	Ethanol, 2-(9,12-octadecadienyloxy), (Z,Z)	$C_{20}H_{38}O_2$	310	0.84	Alcoholic compound	Antimicrobial activity
21	13.58	Rhodopsin	$C_{40}H_{58}O$	554	1.05	Carotenoid	Antioxidant
22	13.75	Ursodeoxycholic acid	$C_{24}H_{40}O_4$	392	0.99	Bile acid, alcohol	Treat cholestatic liver disease
23	14.67	Androstane	$C_{29}H_{43}NO_3Si$	481	0.81	Steroid	increase strength, lower body fat
24	15.04	6,9,12-Octadecatrienoic acid methyl ester (gamma-Linolenic acid)	$C_{19}H_{32}O_2$	346	3.72	Fatty acid	Anti-tumor, Anti-oxidant, Anti-inflammatory, Anti-diabetic, Anti-obesity, treat eczema
25	15.04	Trilinolein	$C_{57}H_{98}O_6$	879	0.52	Fatty acid	Antioxidant, Anti-ischemic, Antiarrhythmic, and Antioxidant
26	15.04	6,9,12,15-Docosatetraenoic acid, methyl ester	$C_{23}H_{38}O_2$	346	1.84	Unsaturated fatty acid ester compound	Cardio protective, Hypocholesterolemic
27	15.41	Dasycarpidan-1methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326	0.79	Ester	Anti-inflammatory, Anti-bacterial, Anti-fungal, Anti-diabetic, Anti-cancer
28	15.04	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	3.72	Steroid	Antioxidant, Anti-inflammatory, Anti-arthritic, Antiasthma, Diuretic, Antifungal for plant and human pathogenic fungi
29	19.66	13-Docosenamide, (Z)	$C_{22}H_{43}NO$	337	32.75	Amide compound	Antimicrobial, Anti-nociceptive
30	19.66	9-Octadecenamide,(Z)	$C_{18}H_{35}NO$	281	32.75	Amide compound	Antimicrobial
31	19.66	Cis-11-Eicosenamide	$C_{20}H_{39}NO$	309	32.75	Amide compound	-

(RT: Retention Time, MW: Molecular Weight)

In Vitro Total Antioxidant Activity

The antioxidant activity of *A. filipendulina* methanolic leaves extract was examined by comparing it to the activity of known antioxidant ascorbic acid by the following *in vitro* assays

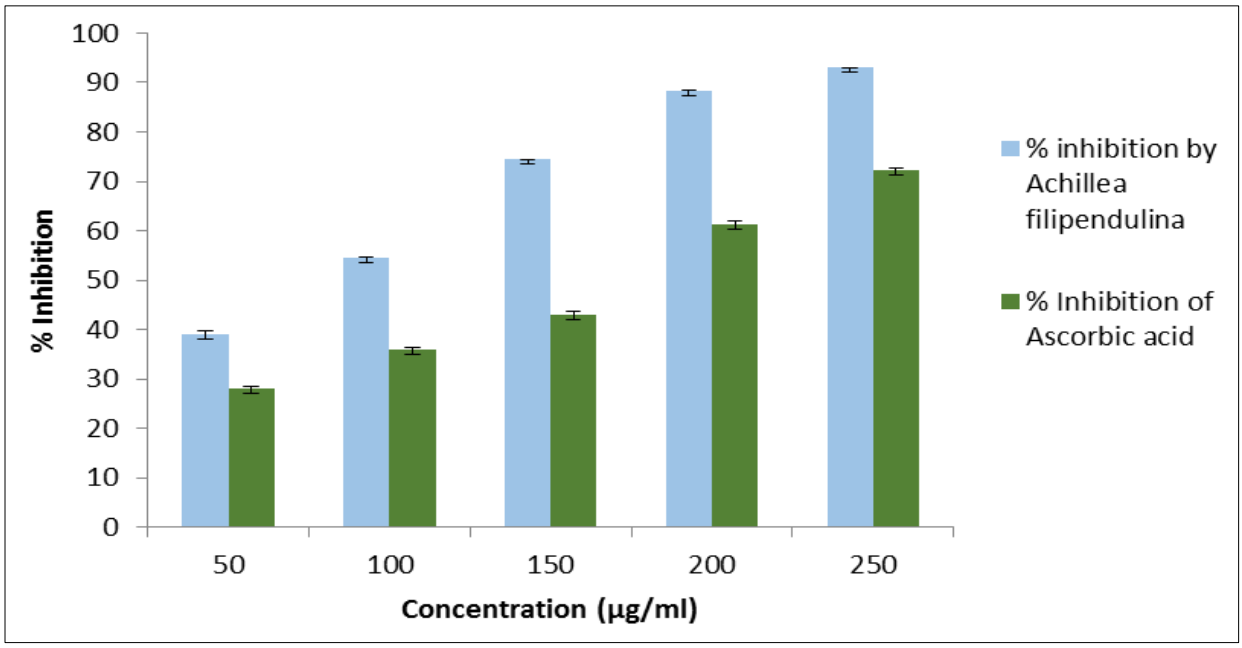
DPPH free radical scavenging activity

The ability of methanolic leaves extract to scavenge DPPH free radical was calculated as % inhibition and was compared with ascorbic acid used as standard. It was observed that at 250 µg/ml of concentration, the percentage inhibition of plant extract was found to be higher i.e. 92.98% when compared to ascorbic acid (72.24%) which is statistically significant at same concentration. The IC_{50} value was found to be 62.23±1.250 for *A. filipendulina* and for ascorbic acid it was 158.31±3.12. Percentage of inhibition was increased with increase in plant extract concentration which was presented in the Table 3 and Fig. 2.

Table 3: % Inhibition of DPPH by methanolic leaves extract of *A. filipendulina* and ascorbic acid

Concentration (µg/ml)	% Inhibition of *A. filipendulina*	IC_{50} (µg/ml) of *A. filipendulina*	% Inhibition by Ascorbic acid	IC_{50} (µg/ml) of Ascorbic acid
50	48.15±0.45	62.23±1.250	28.12±0.840	158.31±3.12
100	54.56±0.84		35.95±0.934	
150	74.44±0.24		42.94±1.360	
200	88.33±0.33		61.32±0.967	
250	92.98±0.41		72.24±0.657	

Values represent mean ± SEM of three replicates



Bar represent standard errors at 5% level

Fig 2: Percentage inhibition of DPPH by methanolic leaves extract of *A. filipendulina*

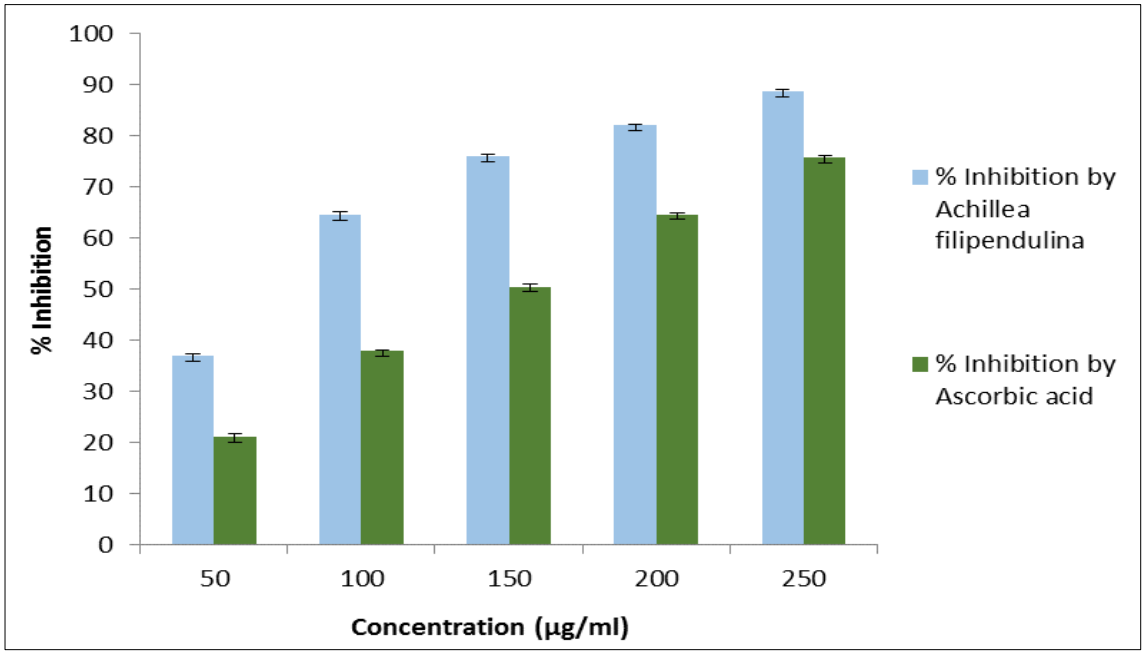
Hydrogen Peroxide radical scavenging activity

The % inhibition of hydrogen peroxide scavenging activity of leaves extract of *A. filipendulina* showed an increase from 36.9% to 88.68% as the concentration of plant extract was increased from 50 to 250 (µg/ml). The percentage inhibition of H_2O_2 by plant extract was 13.47% higher than control i.e. ascorbic acid at highest concentration (250µg/ml) and it was found to be statistically significant (Table 4 and Fig.3). The IC_{50} value was found to be 69.07±2.97 for *A. filipendulina* and 149.96±2.87 and for ascorbic acid.

Table 4: % of Hydrogen Peroxide inhibition of methanolic leaves extract of *A. filipendulina* and ascorbic acid

Concentration (µg/ml)	% Inhibition of *A. filipendulina*	IC_{50} (µg/ml) of *A. filipendulina*	% Inhibition by Ascorbic acid	IC_{50} (µg/ml) of Ascorbic acid
50	36.90±0.91	69.07±2.97	21.13±0.74	149.96±2.87
100	64.44±0.11		38.00±1.24	
150	75.99±1.15		50.45±0.84	
200	82.02±0.29		64.68±0.31	
250	88.68±0.55		75.79±0.54	

Values represent mean ± SEM of three values



Bar represents standard errors at 5% level

Fig 3: Percentage of hydrogen peroxide inhibition by methanolic leaves extract of *A. filipendulina*

Total Phenolic Content

The total phenolic content (TPC) of extract expressed in terms of gram gallic acid equivalent (GAE)/g of dry weight, calculated using the standard curve equation: y = 0.833x + 48.462

R^2 = 0.992. The values are based on the chemical reducing power which is in relation to an equivalent reducing the capacity of Gallic acid. TPC showed a sharp increase from 91.12 to 261.77 mg/ml as concentration of plant extract varied from 50µg/ml to 250µg/ml which is observed to be statistically significant and comparatively higher than the standard (Gallic acid) presented in Table 5 and Fig. 4.

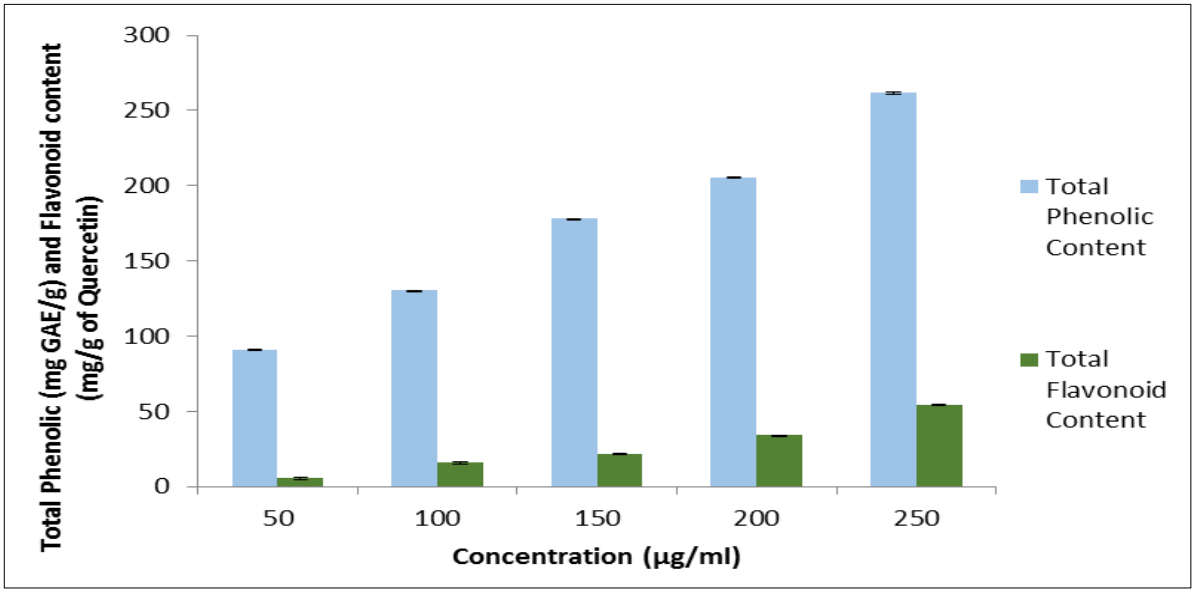
Total Flavonoid content

Total flavonoid content is expressed in terms of mg of quercetin/g of extract. The TFC at different concentrations 50, 100, 150, 200 and 250(µg/ml) varied from (5.62 to 54.51mg/g) it is again higher than standard (Quercetin) (Table 5 also expressed in Fig. 4).

Table 5: Total Phenolic and Flavonoid content of methanolic leaves extract of *A. filipendulina*

Concentration (µg/ml)	Total Phenolic Content (mg Gallic acid extract/g)	Total flavonoid Content (mg of Quercetin/g of extract)
50	91.12±0.38*	5.62±0.60*
100	130.43±0.23*	16.12±0.15*
150	178.12±0.12*	22.00±0.47*
200	205.65±0.45*	34.12±0.34*
250	261.77±0.54*	54.51±0.12*

*Each value is the average of three analysis ±SEM



Bar represent standard errors at 5% level

Fig 4: Total phenolic content represented as mg equivalent of gallic acid and Total flavonoid content represented as mg equivalent of quercetin of methanolic leaves extract of *A. filipendulina*

Discussion

The results of qualitative phytochemical analysis clearly indicate that methanolic leaves extract of *Achillea filipendulina* contains a broad spectrum of bioactive compounds which need to be screened quantitatively which was done by GC-MS. The GCMS analysis revealed the presence of 31 compounds from the leaves of *Achillea filipendulina*. From GCMS analysis compound identified *viz*., 3,4-Hexanediol, 3,4-bis-(4hydroxyphenyl) have antioxidant, anti-cancer, anti-inflammatory properties, Undecane is an alkane compound have antimicrobial agents, transducer for immunosensor and used as carcinogens (Gibka *et al.*, 2009; Krishnamoorthy and Subramaniam, 2014) [7, 15]. Decalin-8a-ol-7-one,4a,8-dimethyl-2-[2-(t-butyldimethylsilyloxy)-1-methyleneethyl]- have aromatic properties, psi. psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy- having antioxidant, anti-asthamatic, anti-cancer, gingivitis, immune enhancement and used for arthrosclerosis prevention. The other compounds Calcitrol used in the treatment of hypocalcaemia, treat osteoporosis, used for prevention of corticosteroid, 1-Heptatriacotanol have anti-hypercholeaterolemic properties (Baskaran *et al.*, 2015) [2]. Cis-Vaccenic acid shows anti-carcinogenic properties and inhibition of telomerase enzyme. Ethanol, 2-(9, 12 octadecadienyloxy), (Z, Z) is an alcoholic compound having antimicrobial activity (Elaiyaraja and Chandramohan, 2016) [5]. 1-Monolinoleoylglycerol trimethylsilyl ether is ether and steroid have antimicrobial, antioxidant, anti-inflammatory, antiarthritic, antiasthma and Diuretic properties, Rhodopsin is an carotenoid and having antioxidant properties (Sheela and Uthayakumari 2013) [26]. Another compound Ethyl iso-allocholate is a steroid having antioxidant, anti-inflammatory, anti-arthritic, anti-asthma, diuretic and antifungal for plant and human pathogenic fungi (Abubacker *et al.*, 2015) [1]. 6,9,12-Octadecatrienoic acid methyl ester (*gamma*-Linolenic acid) is a fatty acid and have anti-tumor, anti-oxidant, anti-inflammatory, antidiabetic, antiobesity and also used for treatment of eczema. Trilinolein is a fatty acid having antioxidant, anti-ischemic, antiarrhythmic activity. 6, 9, 12, 15-Docosatetraenoic acid, methyl ester is an unsaturated fatty acid ester compound have Cardio protective, hypocholesterolemic properties. Dasycarpidan-1methanol, acetate (ester) is an ester reported to have anti-inflammatory, anti-bacterial, anti-fungal, anti-diabetic and for cancer treatment (Rubaye *et al.*, 2017) [22]. When subjected to antioxidant test, the ability of methanolic leaves extract of *A. filipendulina* to scavenge DPPH free radical was found to be highest which is statistically significant over the control. IC_{50} value was found to be 62.23±1.250 for methanolic leaves extract and for standard it was 158.31±3.12. Hasini, N. (2015) [11] reported 55% inhibition at 50µg/ml of essential oil of *Achillea filipendulina*. Fateh *et al.*, 2015 [6] reported 75% inhibition by methanolic extract of *A millefolium.* Kazemi (2015) [14] observed 20.06±0.0 IC_{50} value of essential oil of *A. millefolium.* Higher the IC_{50} value lower the inhibition, so from the results we can conclude that plant extract have lower IC_{50} value which means have higher inhibition percentage as compare to standard (ascorbic acid). Hydrogen peroxide probably reacts with Fe^{2+} and Cu^{2+} ions to form hydroxyl radical which may be the origin of many of its toxic effects (Miller *et al.*, 1993) [19]. So, the elimination of H_2O_2 is very necessary for antioxidant defence in cell. H_2O_2 scavenging activity of *A. filipendulina* was found to be 88.68% at 250µg/ml which is highest as compared to standard ascorbic acid i.e. 75.21% at 250µg/ml. The IC_{50} value of leaves extract for *A. filipendulina* was found to be 69.07±2.97 µg/ml and for ascorbic acid it was 149.96±2.87 µg/ml. This could attribute to phenols which could donate electrons, subsequently neutralizing its effect. It showed that extract had an effective hydrogen peroxide scavenging activity and may protect the living system from oxidative stress (Saha and Verma, 2014) [24]. Phenolic content in leaves extract of *A. filipendulina* was found to be significantly higher than control. Phenolic compounds are potent free radical terminator (Shahidi and Wanasundara, 1992) [25] which is known to have antioxidant activity and is believed to be due to their redox properties which neutralize free radicals by decomposing peroxides. Flavonoids are effective mainly via scavenging of superoxide anion radical (hydrogen peroxide, hydroxyl radical, and singlet oxygen) (Wickens AP 2001) [31]. Total flavonoid content of *A. filipendulina* was found to be again higher than control. Kurian *et al.*, 2017 [16] reported that with an increase in the total phenolic content, the antioxidant capacity of the extracts also increases. There is no previous report of antioxidant activity of the methanolic leaves extract and therapeutic properties of the identified compounds of *A. filipendulina*. Therefore, the present experimental study was conducted to find out the possible antioxidant role of *A. filipendulina*. Present work revealed that this species of *Achillea* is a reliable source of bioactive compounds like fatty acid esters, alcohols, hydrocarbons, alkanes, amines and terpenes that justify the traditional usage of this species by the local healers for various ailments. Results showed that the level of phenolic compounds in the methanolic extracts of the *A. filipendulina* was considerable and these are important constituents of this plant. So, *A. filipendulina* could be a potential source of natural antioxidant in slowing and inhibiting the initiation of aging and oxidative stress-related degenerative diseases and some of its pharmacological effects could be attributed to the presence of these valuable bioactive components.

Conclusion

The preliminary phytochemical and GCMS analysis of *A. filipendulina* (L.) leaves revealed that plant contains many bioactive chemicals like flavonoids, alkaloids, terpenoids, triterpenoids saponins, phenolic compounds, sterols, alcohols, amines, fatty acid, esters, carotenoids, ethanols. Since the plant contains high quantities of these bioactive potential compounds, it is reliable to possess large number of pharmacological values like antioxidants, antidiabetic, antifungal, antibacterial, anti-inflammatory, cancer-preventive, lubricant, nematicide, hypercholesterolemic, immunosuppressive and hepatoprotective activities and could be employed for the treatment of different ailments. Further *in vitro* total antioxidant analysis revealed that methanolic plant extract have higher content of phenols and flavonoids, and likewise higher antioxidant activity. The results suggested possible use of *A. filipendulina* for the development of highly potent, safe and novel antioxidant drug. Further *A. filipendulina* (L.) is worthy for exploring the medicinal potential for used as a natural drugs developments**.**

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Conflicts of Interest: Nil

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