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REVIEW

Pharmacognosy, Phytochemistry and Pharmacological Properties of *Achillea millefolium* L.: A Review

Sofi Imtiyaz Ali, B. Gopalakrishnan and V. Venkatesalu*
Department of Botany, Annamalai University, Annamalainagar 608 002Tamil Nadu, India

***Achillea millefolium* L. (Yarrow) is an important species of Asteraceae family with common utilization in traditional medicine of several cultures from Europe to Asia for the treatment of spasmodic gastrointestinal disorders, hepatobiliary, gynecological disorders, against inflammation and for wound healing. An extensive review of literature was made on *A. millefolium* L. using ethno botanical text books, published articles in peer-reviewed journals, unpublished materials and scientific databases. The Plant List, International Plant Name Index and Kew Botanical Garden databases were used to authenticate the scientific names. Monoterpenes are the most representative metabolites constituting 90% of the essential oils in relation to the sesquiterpenes, and a wide range of chemical compounds have also been reported. Different pharmacological experiments in many *in-vitro* and *in-vivo* models have proved the potential of *A. millefolium* with antiinflammatory, antiulcer, anticancer activities etc. lending support to the rationale behind numerous of its traditional uses. Due to the noteworthy pharmacological activities, *A. millefolium* will be a better option for new drug discovery. The present review will comprehensively summarize the pharmacognosy, phytochemistry and ethnopharmacology of *A. millefolium* reported to date, with emphasis on more *in vitro*, clinical and pathological studies needed to investigate the unexploited potential of this plant. Copyright © 2017 John Wiley & Sons, Ltd.**

Keywords: Achiella millefoilum; pharmacognosy; phytoconstituents; ethnopharmacology; traditional uses; drug discovery.

INTRODUCTION

Natural products, especially those derived from plants, continue to provide new and important leads in the drug discovery process (Balunas and Kinghorn, 2005). The first step in drug discovery is to document traditionally used materials to treat an ailment. The knowledge of medicinally used important plants and practices are passed verbally from one generation to another, and because of this tradition, there is fear that indigenous knowledge about traditional medicine is slowly being lost (Bhatia *et al.,* 2014). Documentation of such knowledge may help in conservation and facilitate future research on medicinal plant safety and efficacy to validate. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Bunalema *et al.,* 2014) as well as prevent the destructive changes in the knowledge of medicinal plants during transmission between generations (Khoshbakht and Hammer, 2005).

The genus *Achillea* belongs to the family Asteraceae, comprises over 130 perennial herb species indigenous to the Northern Hemisphere from Europe to Asia and grows in temperate climates in dry or semi-dry habitats (Si *et al*., 2006). *Achillea millefolium* L. (yarrow or milfoil), the best-known and most widespread species, is listed among the most commonly used plant species in both folk and conventional medicine for over 3000 years (Radusiene and Gudaityte, 2005). It is commonly known as Yarrow in English and has different vernacular names in India like Biranjasipha, Gandana, Gandrain, Puthkanda, Bhut Kesi (Hindi), Bimjasif (Joshimath), Rajmari (Konkani), Rojmaari (Marathi), Achchilliya (Tamil), Tukhm gandana, Buiranjasif and Brinjasuf (Urdu). A diversity of pharmacological properties is ascribed to *A. millefolium*, such as spasmolytic, antiinflammatory, analgesic, haemostatic, antidiabetic, cholagogue, antitumor, antioxidant, antifungal, antiseptic and liver protective effects attributed due to the presence of several chemical constituents viz., essential oils, sesquiterpenes, phenolic compounds etc (Karamenderes and Apaydin, 2003; Stojanovic *et al.,* 2005; Cavalcanti *et al.,* 2006; Si *et al.,* 2006; Tajik *et al.,* 2008; Lazarevic *et al.,* 2010; Fierascu *et al.,* 2015).

The flowering tops containing essential oil are the most active part of the plant, used mainly for the treatment of influenza, hemorrhage, dysmenorrhea, diarrhea and as a haemostatic (Baser et al., 2002; Benedek *et al.,* 2008). Tea from *A. millefolium* is also used to treat diseases of gastrointestinal tract like dyspepsia, flatulence, abdominal pain, diarrhea, stomachache and digestive complaints. Recently, in a double-blind randomized clinical trial, it has been shown that tea prepared from powder of the flowers of *A.*

* Correspondence to: V. Venkatesalu, Department of Botany, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India.
E-mail: venkatesalu@yahoo.com

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millefolium relieved the severity of pain in primary dysmenorrheal (Jenabi and Fereidoony, 2015). Nowadays, it can be found in herbal pharmacies as tinctures and capsules containing dry flowers or aerials. The plant is also used as a component of a variety of industrial tea mixtures and an ingredient of phytoremedies (e.g. Amersan) (David *et al.,* 2010). *A. millefolium* can be used as an essential oil, infusion or alcohol extract, decoction, hydroalcoholic, methanolic and aqueous extract (Dias *et al.,* 2013).

A. millefolium is of great concern as evidenced by the ample research carried out by different researchers in the recent past. The present review is intended to pile up the widespread information on the pharmacognosy, phytochemistry and pharmacology of *A. millefolium* to explore its therapeutic potential and evaluate future research opportunities.

BOTANY

Plant occurrence

Asteraceaeous plants are distributed throughout the world and most common in the arid and semi-arid regions of subtropical and lower temperate latitudes. *Achillea* contains around 130 flowering and perennial species, found in Europe and temperate areas of Asia and America. *Achillea* is represented in Turkey with 46 taxa, of which 25 are endemic, and in Iran with 19 species, of which seven species are endemic (Mozaffarian, 2009). *A. millefolium* is native to Europe and western Asia but is also widespread in most temperate regions including North America and is represented by about 85 species mostly found in Europe, Asia and in North America (Anne *et al.*, 2006). *A. millefolium* grows at 3500MSL and is often found in grasslands and open forests. The plant commonly flowers from May through June, and active growth occurs in the spring.

Figure 1. *Achiella mellifolium* L. [Colour figure can be viewed at wileyonlinelibrary.com]

Botanical description

A. millefolium belongs to family Asteraceae which is the largest family of vascular plants. It is an erect herbaceous perennial plant that grows up to 50 cm tall, with a slender cropping rootstock throwing numerous roots and stolons with a blunt, succulent scale at each node. The leaves are 5–20 cm long, bipinnate or tripinnate, almost feathery, having varying degrees of hairiness (pubescence) and arranged spirally near the middle and bottom of the stem. The flowers are typically white, but either pink or pale purple flowers with corymbose, ovoid, flat-topped heads at the end of stems and branches, having densely arranged petals in flattened clusters. Fruits are 2-mm, shiny, oblong achenes, with broadly winged margins and no pappus (Akram, 2013) (Fig. 1).

A. millefolium—a polyploid complex

A. millefolium is one of the most diverse polyploid complexes of the Northern hemisphere in terms of morphological, genetic and ecological features (Guo *et al.,* 2005, 2008 and 2012; Ehrendorfer and Guo, 2006). This group includes *A. millefolium*, together with a set of Eurasian and North American related lineages (Guo *et al.,* 2005), some of them naturalized in temperate and cold areas of other continents but its basal diploids are limited to Eurasia (with four species in Europe and three in Asia). The complexity of the aforementioned group is the result of multiple processes of hybridization, polyploidization and evolution linked with different types of habitats. The existence of different autopolyploids and allopolyploids representing four ploidy levels (2×, 4×, 6× and 8×) is widely documented (Guo *et al.,* 2012), and analysis of genetic diversity carried out using AFLP markers revealed substantial polymorphism, significantly higher in polyploid than in diploid strains (Guo *et al.,* 2008). The polyploidy species are mostly difficult to define; their number varies depending on the authorities and may reach 17 or more.

Separation of *A. millefolium* agg. from other members of sect. *Achillea* was not possible by ITS and trnL-Fs sequences (Guo *et al.,* 2004). By contrast, the AFLP data clearly characterize the polyploidy complex as a clade, with mostly well separated 2× species, but with a rather diffuse superstructure of 4× and 6× taxa. By morphological, phytochemical, DNA analytical and ecogeographical criteria, *A. millefolium* agg. appears as the most apomorphic, polymorphic, diverse and widespread, highly polyploid (2×, 4×, 6×, 8×) but nevertheless monophyletic 'crown group' of the genus (Guo *et al.,* 2005).

Taxonomy

A. millefolium is native to Europe and includes three subspecies: subsp. *millefolium* (small white flowers), pink flowered subsp. *alpestris* (Wimm. & Grab.) and subsp. *ceretanum* Sennen. (large white flowers) confined to Spain and southern France (Applequist and Moerman, 2011). Several closely related species or microspecies belong to *A. millefolium* species complex,

with *Achillea collina* J. Becker ex Reichenb. and *Achillea pannonica* Scheele. most frequently treated as subspecies of *A. millefolium*, though usually excluded today. Ploidy level is informative in the recognition of species; for example, *A. millefolium* is hexaploid, while *A. collina* is tetraploid and *A. pannonica* are usually reported to be octoploid (Applequist and Moerman, 2011).

Additionally, the common native North American *A. millefolium* has been recognized at the species level as *A. lanulosa* Nutt. (Itself sometimes divided into multiple species) or at the subspecific level as *A. millefolium* subsp. *lanulosa* (Nutt.) Piper. As narrowly defined, this taxon is tetraploid (Ehrendorfer, 1973; Gervais, 1977), while North American populations recognized by some authorities as *A. borealis* Bong. may be tetraploid or hexaploid (Ehrendorfer1973; Ramsey, 2007). Guo *et al.* (2005) concluded from AFLP data that the North American polyploids were genetically from *A. millefolium*, having a closer or more direct relationship to diploid *Achillea asiatica* Serg. Gharibi *et al.* (2011) assessed extensive genetic differentiation among *A. millefolium* subsp. *elbursensis* and *A. millefolium* subsp. *millefolium* from the Northern regions of Iran by using ISSR and morphological markers.

HISTORY

The name of the genus *Achillea* originates from the ancient use as a wound-healing remedy by the Trojan hero Achilles, a powerful warrior in Greek mythology (Benedek *et al.,* 2007b). *A. millefolium* is one of the oldest known botanicals used by humans (*sensu lato*): it is among the six medicinal plants whose pollen was found in a *Homo neanderthalensis* grave at Shanidar, dated to 65 000 B.P. (Leroi, 1975; Solecki, 1975). Although it is impossible to know whether usage since then has been continuous, but it has certainly been persistent over a prolonged period, as it has been broadly accepted as a medicine by many recent cultures within its range. Duke has repeatedly argued (Duke and Ayensu, 1985; Duke, 1986) that plants used by unrelated groups for similar purposes are likely to be potent, given the statistical unlikelihood that multiple cultures would randomly adopt and retain the same use for an inert plant (Moerman, 2007), a plant whose use is independently adopted and retained by multiple cultures has increased likelihood of being genuinely bioactive.

The oldest surviving texts to record the use of *A. millefolium* in the European classical medical tradition are by Pliny the Elder and Dioscorides, both during the first century AD. These texts set a lower limit on the age of *A. millefolium* used by western cultures, although, as for most botanicals, folk use is an oral tradition may have long preceded the plants appearance in scholarly medicine. A necessary caveat is that, although most authorities have interpreted these references as being to *A. millefolium*, the traditional assignments of Linnean names to plants in classical texts are seldom certain: if, for example, a classical common name were later used for another species with similar appearance, range and uses, errors would result. Dioscorides described the herb achilleios, or millefolium (among other names), as being useful to stop bleeding, including from wounds and abnormal menstrual bleeding and reduce inflammation; a decoction could be used as a douche for menstrual bleeding and be drunk for dysentery (Osbaldeston and Wood, 2000). Pliny's natural history indicated that a plant probably identifiable as yarrow was known by names including achilleos, sideritis and millefolia. Some people also used the former two names for several other species, some quite different in description, with all being considered valuable for wounds (Jones, 1956).

The literary record has recently been supported by marine archeology. *A. millefolium* is among plants reported, using DNA analysis, to be present in two pressed tablets of plant material recovered in the 1980s from a collection of medical supplies in a Roman ship

Table 1. Traditional uses of *A. millefolium* in different cultures

S. no.	Culture	Treatment	Reference
1.	European	Gastrointestinal disorders, loss of appetite, menstrual problems, as a diaphoretic, skin inflammations, wounds and external bleeding	Wichtl (2004) and Willuhn (2002)
2.	Iranian	Inflammation, pain and gastrointestinal disturbances, hemorrhoids, dyspepsia, dysmenorrhea and gastritis	Miraldi *et al.* (2001)
3.	Italy	Menstrual problems, as a diuretic or for urinary problems, toothache, as a sedative and gastrointestinal disorders	Applequist and Moerman (2011)
4.	Hungary	Internal ailments as well as for burns and wounds	Applequist and Moerman (2011)
5.	Peru	Gastritis, diabetes, cholesterol and skin infections	Bussmann *et al.* (2007)
6.	Brazil	Wounds, skin problems, diarrhea and gastrointestinal problems	Pires *et al.* (2009) and Baggio *et al.* (2008)
7.	Britain and Ireland	Wounds, nosebleeds, uterine hemorrhage, high blood pressure, respiratory infections, fevers and rheumatic complaints	Allen and Hatfield (2004)
8.	China	Snakebite, wounds, hemorrhoids, varicose veins, dysmenorrhea and tuberculosis	Applequist and Moerman (2011)
9.	India	Gastric problems and fever	Sharma *et al.* (2004)

that sunk off the coast of Tuscany between 140 and 120 BC. The DNA analysis organized by Alain Touwaide and Emanuela Appetiti of the Institute for the Preservation of Medical Traditions and performed by Robert Fleischer at the Smithsonian Institution. Their investigation tentatively identified several of the tablets ingredients, all considered in writings of the time to be medicinal; in addition to yarrow, the study found DNA evidence of carrot, radish, parsley, celery, wild onion and cabbage (Applequist and Moerman, 2011).

TRADITIONAL USES

A. millefolium, a very important medicinal plant in Unani (Greco-Arab) system of medicine under the name of Biranjasif (Applequist and Moerman, 2011), has been used in traditional medicine for hundreds of years internally as herbal teas for headaches, hepato-biliary disorder, gastrointestinal complaints and as an appetite enhancing drug and externally as lotions and ointments against skin inflammations, wounds, cuts and abrasions (Cavalcanti *et al.,* 2006; Benedek *et al.,* 2007a & 2008; Nadim *et al.,* 2011) (Table 1).

A. millefolium has a long history of use as traditional herbal medicine even in veterinary medicine (Eghdami and Sadeghi, 2010). Preparations in the form of infusions, decoctions or fresh juices have been used against anorexia, stomach cramps, flatulence, gastritis, enteritis, internal and external bleeding (coughing blood, nosebleed, hemorrhoidal and menstrual bleeding, bloody urine), wounds, sores, skin rash, as well as dog and snake bites. It has been used internally, usually as a tea, and externally as a lotion, ointment or poultice (Grieve, 1971; Chandler *et al.,* 1982a).

The aerial parts of *A. millefolium,* a well-known species among other members of *Achillea*, are commonly used in European and Asian traditional medicine for the treatment of gastrointestinal disorders and hepatobiliary complaints, as well as for wound healing and skin inflammations (Jaradat, 2005; De *et al.,* 2007; Ugulu *et al.,* 2009). The ancient Europeans called it *Herba Militaris*, the military herb, an ointment made from it was used as vulnerary drug on battle wounds. *A. millefolium* flower was formerly official in the United States Pharmacopeia. In European folk, *A. millefolium* is used for gastrointestinal disorders, loss of appetite, for menstrual problems, as a diaphoretic, for skin inflammations, wounds and external bleeding (Willuhn, 2002 Wichtl, 2004).

A. millefolium is widely used in Iranian folk medicine to treat diverse diseases including inflammation, pain and gastrointestinal disturbances. However, the infusion of dried flowers is considered suitable for the treatment of hemorrhoids, dyspepsia, dysmenorrhea and gastritis (Miraldi *et al.,* 2001). In Italy, *A. millefolium* is used for a variety of conditions like menstrual problems, as a diuretic or for urinary problems, for toothache and as a sedative but primarily for gastrointestinal disorders (Passalacqua *et al.*, 2007). In Hungary, the plant is known as cickafark (kitten tail) and has been used for internal ailments as well as for burns and wounds (Applequist and Moerman, 2011).

Bussmann *et al.* (2007) reported that *A. millefolium* is used in Peru under the names of *Milenrama* and C*honchón*, for gastritis, diabetes, cholesterol and mainly for skin infections. Likewise, it is used in Brazil, under the names of *mil-folhas* and *erva de cortadura*, to treat wounds, skin problems, diarrhea and other gastrointestinal problems (Baggio *et al.,* 2008; Pires *et al*., 2009), although plant infusion or the decoction of the aerial parts of the plant is indication for calmness (Manfrini *et al.,* 2009). This latter indication is also seen in Mexico (Molina-Hernandez *et al.,* 2004). One third of the 125 records of folk use of *A. millefolium* in Britain and Ireland were for wounds, nosebleeds, uterine hemorrhage, high blood pressure, respiratory infections, fevers and rheumatic complaints (Allen and Hatfield, 2004). In China, *A. millefolium* has been used to stop bleeding, snakebite, wounds, hemorrhoids, varicose veins, dysmenorrhea and tuberculosis (Applequist and Moerman, 2011). *A. millefolium* has also been listed in the Indian Ayurvedic Pharmacopeia for fevers and wound healing. In India, the leaves and flowers are used for gastric problems and fever in Parvati valley of Himalayan region (Sharma *et al.,* 2004).

CHEMICAL CONSTITUENTS OF *A. millefolium*

Studies on the chemical constituents of *A. millefolium* could be traced back to 19th century, and many compounds have been found until now. Active ingredients reported in *A. millefolium* are summarized below, and chemical properties of major phytochemicals present in *A. mellifolium* are shown in Table 2.

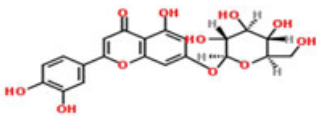
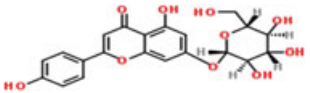
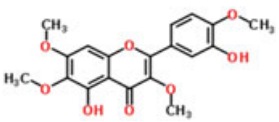
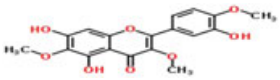
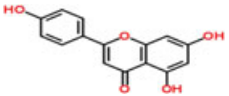
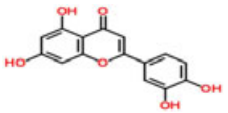
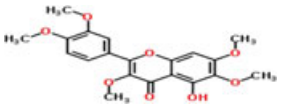
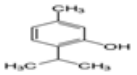
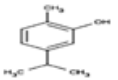
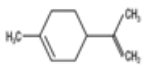
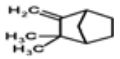
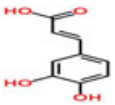
Phenols

Choline (Borrelli *et al.*, 2012), 1,3-dicaffeoylquinic acid (DCQA), 1,4-DCQA, apigenin 4-O-glucoside and luteolin 4-O-glucoside (Vitalini *et al.,* 2011), 3,4-DCQA (Benedek *et al.,* 2007c; Vitalini *et al.,* 2011), 3,5-DCQA (Innocenti *et al.,* 2007; Benedek *et al.,* 2007c; Fraisse *et al.,* 2011; Vitalini *et al.,* 2011), 1, 5-DCQA (Fraisse *et al.,* 2011), 4, 5, DCQA (Benedek *et al.,* 2007c; Fraisse *et al.,* 2011), chlorogenic acid (Tunón *et al.,* 1994; Innocenti *et al.,* 2007; Benetis *et al.,* 2008; Fraisse *et al.,* 2011; Vitalini *et al.,* 2011), luteolin-7-β-D-Oglucuronide (Benedek *et al.,* 2007c), caffeic acid (Tunón *et al.,* 1994; Wojdyło *et al.,* 2007; Yassa *et al.,* 2007; Pires *et al.,* 2009), p-coumaric acid and neochlorogenic acid (Wojdyło *et al.,* 2007), ferulic acid (Tunón *et al.,* 1994; Wojdyło *et al.,* 2007) and stachydrine, carboxylic acid, salicylic acid, pyrocatechol, adenine, mandelic acid, methyl esters of caprylic-linolenic- and undecylenic acid (Tunón *et al.,* 1994) are the phenols reported from different parts of *A. mellifolium*.

Flavonoids

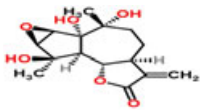
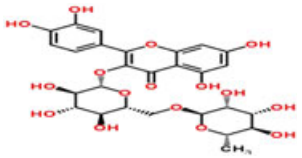
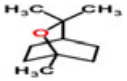
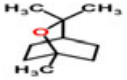
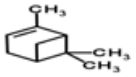
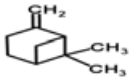
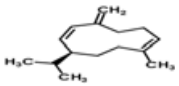
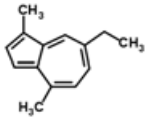
The various flavonoids reported from *A. mellifolium* include resveratrol, morin, myricetin, naringin and naringenin (Keser *et al.*, 2013), quercetin and kaempferol (Greger, 1969; Wojdyło *et al.,* 2007; Keser

Table 2. Chemical properties of major phytochemicals present in *A. millefolium*

S. no.	Compounds	Molecular formula	2DStructure	Systematic name	Average mass	Structure ID
01.	Cynaroside	C21H20O11		2-(3,4-Dihydroxyphenyl)-5-hydroxy-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	448.377 Da	ChemSpider ID-4444241 Pubchem CID-5280637
02.	Cosmosiin	C21H20O10		5-Hydroxy-2-(4-hydroxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	432.378 Da	ChemSpider ID-4444290 Pubchem CID-5280704
03.	Casticin	C19H18O8		5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,6,7-trimethoxychromen-4-one	374.341 Da	ChemSpider ID-4474632 Pubchem CID-5315263
04.	Centaureidin	C18H16O8		5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,6-dimethoxychromen-4-one	360.315 Da	ChemSpider ID-4474997 Pubchem CID-5315773
05.	Apigenin	C15H10O5		5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one	270.237 Da	ChemSpider ID-4444100 Pubchem CID-5280443
06.	Luteolin	C15H10O6		2-(3,4-Dihydroxyphenyl)-5,7-dihydroxychromen-4-one	286.236 Da	ChemSpider ID-4444102 Pubchem CID-5280445
07.	Artemetin	C20H20O8		2-(3,4-Dimethoxyphenyl)-5-hydroxy-3,6,7-trimethoxychromen-4-one	388.368 Da	ChemSpider ID-4478461 Pubchem CID-5320351
08.	Thymol	C10H14O		5-Methyl-2-propan-2-ylphenol	150.218 Da	ChemSpider ID-21105998 Pubchem CID – 6989
09.	Carvacrol	C10H14O		2-Methyl-5-propan-2-ylphenol	150.218 Da	ChemSpider ID-21105867 Pubchem CID-10364
10.	Limonene	C10H16		(4R)-1-Methyl-4-prop-1-en-2-ylcyclohexene	136.234 Da	ChemSpider ID-20939 Pubchem CID-440917
11.	Camphene	C10H16		3,3-Dimethyl-2-methylidenebicyclo[2.2.1]heptane	136.234 Da	ChemSpider ID-6364 Pubchem CID-6616
12.	Caffeic acid	C9H8O4		(2E)-3-(3,4-Dihydroxyphenyl) prop-2-enoic acid	180.157 Da	ChemSpider ID-600426 Pubchem CID-689043

(Continues)

Table 2. (Continued)

S. no.	Compounds	Molecular formula	2DStructure	Systematic name	Average mass	Structure ID
13.	Achillinin A	C15H20O6		(3aS,6R,6aR,6bR,7aS,8R,8aS) -6,6a,8-Trihydroxy-6,8-dimethyl- 3 methylenedecahydrooxireno [1,2]azuleno[4,5-b]furan-2(3H)-one	296.316 Da	ChemSpider ID-28289151 Pubchem CID-101805792
14.	Rutin	C27H30O16		2-(3,4-Dihydroxyphenyl) -5,7-dihydroxy- 4-oxo-4H-chromen-3-yl 6-O- (6-deoxy-α-L-mannopyranosyl) -β-D-glucopyranoside	610.518 Da	ChemSpider ID-4444362 Pubchem CID-5280805
15.	1,8-cineole	C10H18O2		1,3,3-Trimethyl- 2-oxabicyclo[2.2.2]octane	154.249 Da	ChemSpider ID-2656 Pubchem CID-2758
16.	Bisabolol	C15H26O		(2S)-6-Methyl-2-[(1S)- 4-methylcyclohex- 3-en-1-yl]hept-5-en-2-ol	222.366 Da	ChemSpider ID-10141 Pubchem CID-442343
17.	α-pinene	C10H16		4,6,6-Trimethylbicyclo[3.1.1]hept- 3-ene	136.234 Da	ChemSpider ID-6402 Pubchem CID-6654
18.	β-pinene	C10H16		6,6-Dimethyl-4-methylidenebicyclo [3.1.1]heptane	136.234 Da	ChemSpider ID-14198 Pubchem CID-14896
19.	Germacrene D	C15H24		(1Z,6Z)-1-Methyl-5-methylidene-8- propan-2-ylcyclodeca-1,6-diene	204.351 Da	ChemSpider ID-28288426 Pubchem CID-5373727
20.	Camazulene	C14H16		7-Ethyl-1,4-dimethylazulene	184.277 Da	ChemSpider ID-10268 Pubchem CID-10719

et al., 2013), Ten 1,10-secoguaianolides viz., millifolide A, millifolide B, millifolide C, iso-secotanapartholide, arteludooicinolide A, 3-acetyl-iso-seco-tanapartholide, 3-methoxytanapartholide, seco-tanapartholide A, seco-tanapartholide, and 5-epi-secotanapartholide A (Li *et al.,* 2012a), achillinin A (Li *et al.,* 2011), apigenin 7-O-glucoside (cosmosiin) and luteolin 7-O-glucoside (cynaroside) (Vitalini *et al.,* 2011; Benedek *et al.,* 2007c; Yassa *et al.,* 2007; Horhammer, 1961; Michaluk, 1962; Horhammer *et al.,* 1964; Oswiecimska and Miedzobrodzka, 1966; Kaloshina and Neshta, 1973; Schulz and Albroscheit, 1988), apigenin and luteolin (Csupor *et al.*, 2009; Innocenti *et al.,* 2007; Benedek *et al.,* 2007c; Wojdyło *et al.,* 2007; Guédon *et al.*, 1993), centaureidin (Csupor *et al.*, 2009), casticin (Csupor *et al.*, 2009; Haidara *et al.,* 2006; Falk *et al.,* 1975), luteolin-3,7-di-O-glucoside and vicenin-2 (Benetis *et al.,* 2008), artemetin (Csupor *et al.*, 2009; Falk *et al.,* 1975; Ivancheva *et al.,* 2002), rutin (Vitalini *et al.,* 2011; Pires *et al.,* 2009; Benedek *et al.,* 2007c; Innocenti *et al.,* 2007; Neshta *et al.,* 1972; Kaloshina and Neshta, 1973), dihydrodehydrodiconiferyl alcohol 9-O-β-D-glucopyranoside, apigenin-7-O-β-D-glucopyranoside, luteolin-7-O-β-D-glucopyranoside and luteolin-4-O-β-D-glucopyranoside (Innocenti *et al.,* 2007), 5-hydroxy 3,4′, 6,7 –tetramethoxy flavones (Gadgoli and Mishra, 2007; Falk *et al.,* 1975), isorhamnetin (Wojdyło *et al.,* 2007; Falk *et al.,* 1975; Greger, 1969) and acacetin (Greger, 1969).

Flavonoid aglycones and flavonoid glycosides—C-glycosylflavones, flavonol and flavones O-glycosides are also found in *A. mellifolium*. The flavonoid aglycones include chrysophenol-D, salvigenin, quercetagetin, centaureidin, hispidulin, cirsimarin and nepetin. The flavonoid glycosides include vitexin, vicenine, swertjponin and swertisin. The flavonol and flavones O-glycosides contain quercetin-3-O-glycoside, quercetin-3-O-rhamnoglycoside, luteolin-7-O-glycoside, diosmetin-7-O-glycoside and kaempferol-3-O-glycoside (Ivancheva *et al.,* 2002).

Sesquiterpenoids

The sesquiterpenoids identified in *A. millefolium* are sesquiterpene lactone ester A, sesquiterpene lactone ester B and sesquiterpene lactone-diol (Farooq *et al.,* 2012), seco-pseudo guaianolides viz., paulitin, isopaulitin, psilostachyin C, desacetylmatricarin and sintenin (Csupor *et al.*, 2009), achimillic acids A, B and C (Tozyo *et al.,* 1994), isoachifolidiene (Rucker *et al.,* 1992), 8-acetyl egelolide and 8-angeloyl egelolide (Ochir *et al.*, 1991), austricin (deacetylmatricarin), millefin, 8-hydroxyachillin and artelesin (Konovalov and Chelombit'ko, 1991), α-peroxyachifolid, β-peroxyisoachifolid (Hausen *et al.,* 199; Rucker *et al.,* 1991), achillifolin, dihydroparthenolide and dihydroreynosin (Ulubelen *et al.*, 1990), azulenogene sesquiterpene lactones viz., 8-acetoxy-artabsine, 8-angeloxy-artabsine and 2, 3-dihydro-desavetoxy-matricin (Verzar-Petri *et al.,* 1980), acetylbalchanolide and millefolide (Hochmannová *et al.*, 1961). The sterols identified include, β-sitosterol, stigmasterol, campesterol and cholesterol while triterpenes identified are α-amyrin, β-amyrin, taraxasterol and pseudotaraxasterol (Chandler *et al.*, 1982b).

Essential oils

Monoterpenes are the most representative metabolites constituting 90% of the essential oil of *A. millefolium* in relation to the sesquiterpenes. However, variation in the composition of essential oil may be due to various factors related to chemotype, ecotype, phenophases, altitude and variations in environmental conditions such as temperature, photoperiod, relative humidity and irradiance. Moreover, genetic background may be the factor responsible for affecting the chemistry of secondary metabolites of the plants (Zahara *et al.*, 2014).

Hydrocarbon monoterpenes

Cis-chrysanthenol (Judzentiene, 2016), α-pinene, β-pinene and β-phellandrene (Sevindik *et al.,* 2016; Kazemi, 2015; Costescu *et al.*, 2014; Falconieri *et al.,* 2011; Nadim *et al.*, 2011; Conti *et al.,* 2010; Bimbiraite *et al.,* 2008; Anne *et al.*, 2001 & 2006; Nemeth, 2005; Boskovic *et al.,* 2005; Rohloff *et al.,* 2000; Hofmann *et al.,* 1992), p-cymene (Ebadollahi *et al.,* 2016; Yousefzadeh and Zeinivand, 2013; Nadim *et al.*, 2011; Anne *et al.*, 2006; Jaimand *et al.,* 2006), α-thujane, α-terpinene and γ-terpinene (Mazandarani *et al.,* 2013), camphene and limonene (Kazemi, 2015; Nadim *et al.*, 2011; Bimbiraite *et al.,* 2008) and sabinene (Nadim *et al.*, 2011; Conti *et al.,* 2010; Boskovic *et al.,* 2005) are the hydrocarbon monoterpenes identified in *A. millefolium*.

Oxygenated monoterpenes

Oxygenated monoterpenes including camphor, borneol and bornyl acetate (Sevindik *et al.,* 2016; Ebadollahi *et al.,* 2016; Kazemi, 2015; Mazandarani *et al.,* 2013; Conti *et al.,* 2010; Rahimmalek *et al.,* 2009; Boskovic *et al.,* 2005; Candan *et al.,* 2003), piperitone (Ebadollahi *et al.,* 2016), carvacrol and carvone (Kazemi, 2015), 1, 8-cineole (Sevindik *et al.,* 2016; Yousefzadeh and Zeinivand, 2013; Nadim *et al.*, 2011; Judzentiene and Mockute, 2010; Conti *et al.,* 2010; Rahimmalek *et al.,* 2009; Anne *et al.*, 2006; Candan *et al.,* 2003; Bezic *et al.,* 2003), α-terpineol and terpinen-4-ol (Sevindik *et al.,* 2016; Nadim *et al.*, 2011; Candan *et al.,* 2003), artemisia ketone (Mazandarani *et al.,* 2013; Conti *et al.,* 2010), α-thujone, β-thujone, linalool and fenchyl acetate (Mazandarani *et al.,* 2013; Anne *et al.*, 2006), dihydrocarveol and chrysanthenyle acetate (Yousefzadeh and Zeinivand, 2013), trans-thujone and trans-crhysanthenyl acetate (Falconieri *et al.,* 2011) and myrecene (Bezic *et al.,* 2003) were isolated from *A. millefolium*.

Sesquiterpene hydrocarbons

(E)-β-caryophyllene (Sevindik *et al.,* 2016; Costescu *et al.*, 2014; Conti *et al.,* 2010; Anne *et al.*, 2006; Bezic *et al.,* 2003), β-cubebene (Costescu *et al.*, 2014), germacrene-D (Costescu *et al.*, 2014; Rahimmalek *et al.,* 2009; Bimbiraite *et al.,* 2008; Santoro *et al.*, 2007; Nemeth, 2005; Boskovic *et al.,* 2005; Anne *et al.*, 2001;

Table 3. Phytochemicals from *A. millefolium* linked to various pharmacological properties

S. no.	Phytochemical	Pharmacological effect	References
1.	Centaureidin, casticin, paulitin, isopaulitin, psilostachyin C, desacetylmatricarin and sintenin	Antiproliferative	Cuspor *et al.* (2009)
2.	3,5-DCQA (dicaffeoylquinic acid), 1,5-DCQA and 4,5-DCQA	Antioxidant	Fraisse *et al.* (2011)
3.	Neochlorogenic acid, ferulic acid, thymol, carvacrol, bornyl acetate, limonene, camphene, eucalyptol, α-pinene and β-terpineol	Antioxidant	Aneta *et al.* (2007)
4.	Terpinolene, 1,8-cineole, γ-terpinene and thujone	Antibacterial	Masumeh *et al.* (2009)
5.	Camphor and borneol	Antibacterial and antioxidant	Masumeh *et al.* (2009)
6.	Rutin	Antinociceptive and antioxidant	Pires *et al.* (2009) and Vitalini *et al.* (2011)
7.	Apigenin 7-O-glucoside and luteolin 7-O-glucoside	Antiplasmodial, antioxidant and antiinflammatory	Vitalini *et al.* (2011) and Yassa *et al.*, (2007)
8.	Quercetin	Antispasmodic	Lemmens-Gruber *et al.* (2006)
9.	Salicylic acid and pyrocatechol	Antiparasitic	Tunón *et al.* (1994)
10.	Seco-tanapartholide A (Ten 1,10-secoguaianolides)	Anticancer	Li *et al.* (2012a)
11.	Achimillic acids A, B and C,	Antitumor	Tozyo *et al.* (1994)
12.	Achillinin A	Antiproliferative	Li *et al.* (2011)
13.	Apigenin and luteolin	Antispasmodic; antioxidant, antiproliferative and estrogenic	Lemmens-Gruber *et al.* (2006); Vitalini *et al.* (2011); Aneta *et al.* (2007); Cuspor *et al.* (2009); Innocenti *et al.* (2007)
14.	Caffeic acid,	Antiparasitic and antioxidant	Tunón *et al.* (1994) and Aneta *et al.* (2007)
15.	Chlorogenic acid	Antioxidant and antiparasitic	Fraisse *et al.* (2011) and Tunón *et al.* (1994)
16.	Luteolin-7-O-beta-D-glucuronide	Choleretic	Benedek *et al.* (2005)
17.	Dihydrodehydrodiconiferyl alcohol 9-O-beta-D-glucopyranoside	Estrogenic	Innocenti *et al.* (2007)
18.	5-Hydroxy 3,4, 6,7-tetramethoxy flavone	Hepatoprotective	Gadgoli and Mishra (2007)
19.	Artemetin	Hypotensive, vasodilatory, bronchodilatory and antiproliferative	De Souza *et al.* (2011) and Cuspor *et al.* (2009)
20.	Bisabolol	Immunosuppressive	Saeidnia *et al.* (2004)

Hofmann *et al.,* 1992; Lourenco *et al.,* 1999), α-asarone and β-bisabolene (Falconieri *et al.,* 2011), bicyclogermacrene (Rahimmalek *et al.,* 2009), α-humulene and cadinene (Bimbiraite *et al.,* 2008), germacrene-B (Jaimand *et al.,* 2006), γ-muurolene (Anne *et al*., 2006) and α-elemene, trans-β-farnesene, α-cadinene, germacrene-D-4-ol (Lourenco *et al.,* 1999) are the sesquiterpene hydrocarbons reported from *A. millefolium*.

Oxygenated sesquiterpenes

Oxygenated sesquiterpenes include Umbelulone (Yousefzadeh and Zeinivand, 2013), viridiflorol, 10-epi-γ-eudesmol and selin-11-en-4α-ol (Judzentiene, 2016), bisabolol-oxides (Costescu *et al*., 2014; Nemeth, 2005; Anne *et al*., 2001), caryophyllene oxide (Sevindik *et al.,* 2016; Judzentiene and Mockute, 2010; Conti *et al.,* 2010; Boskovic *et al.,* 2005; Anne *et al*., 2006), nerolidol and eudesmol (Judzentiene and Mockute, 2010), spathulenol (Rahimmalek *et al.,* 2009), β-bisabolol (Anne *et al*., 2006) and α-bisabolol, β-eudesmol, γ-eudesmol, bisabolol oxide II and bisabolone oxide (Candan *et al.,* 2003).

Proazulenes

Chamazulene (Costescu *et al*., 2014; Mazandarani *et al.,* 2013; Nadim *et al*., 2011; Rahimmalek *et al.,* 2009; Santoro *et al.*, 2007; Nemeth, 2005; Anne *et al*., 2006; Jaimand *et al.,* 2006; Anne *et al*., 2001; Hofmann *et al.,* 1992) are the proazulenes reported from *A. millefolium*.

PHARMACOLOGICAL ACTIVITIES

Various active compounds have been reported from *A. millefolium* containing wide range of medicinal activities viz., choleretic, antimalarial, antioxidant,

antihypertensive, antimicrobial, antispasmodic, hepatoprotective, gastroprotective, etc. using different *in-vitro* and *in-vivo* methods. The different pharmacological activities of the *A. millefolium* extracts and its isolated phytoconstituents are summarized in Table 3.

Antiulcer

Hydroalcoholic extract of *A. millefolium* was evaluated on locomotor activity of ileum. The ileum contractions of Wister rats were induced by 60 mM KCl (18.83 ± 4.91%) or 1-μM acetyl choline (18.31 ± 11.12%). However, addition of 1% of the extract decreased the contraction in ileum induced by KCl (59.96 ± 11.8%) or acetyl choline (54.16 ± 12.06%) ($p > 0.05$), and this may be due to flavonoids especially quercetin and apigenin (Sedighi *et al.,* 2013).

The effect of aqueous extract obtained from *A. millefolium* flowering tops (AME) on gastric motility was evaluated on the resting tone of the isolated gastric antrum and on gastric emptying *in vivo* both in control mice and in cisplatin-treated mice. It was observed that the AME (1–30 000 μg mL^{-1}) contracted the isolated mouse and human gastric strips in a concentration-dependent manner, with an effective threshold concentration of 100 μg mL^{-1}. The contractile effect of AME was unaffected by hexamethonium (3×10^{-4} mol L^{-1}) and tetrodotoxin (3×10^{-7} mol L^{-1}) but strongly reduced by atropine (10^{-6} mol L^{-1}). Among various chemical ingredients in *A. millefolium*, choline (5.62×10^{-4} mol L^{-1} threshold concentration), but not the flavonoids rutin and apigenin, mimicked the action of AME. The prokinetic effect of *A. millefolium* extract observed *in vivo* could provide the pharmacological basis underlying its traditional use in the treatment of dyspepsia (Borrelli *et al.*, 2012).

Oral administration (30, 100 and 300 mg/kg) of the hydroalcoholic extract of *A. millefolium* aerial part inhibited ethanol-induced gastric lesions by 35, 56 and 81%, respectively. However, oral treatment of 1 and 10 mg/kg reduced the chronic gastric ulcers induced by acetic acid exposure by 43 and 65%, respectively, and promoted significant regeneration of the gastric mucosa after ulcer induction denoting increased cell proliferation. *A. millefolium* treatment (10 mg/kg p.o.) also decreased glutathione (GSH) and superoxide dismutase (SOD) activity by 53 and 37% after acetic acid-induced chronic gastric lesions. The results suggested that the antioxidant properties of the hydroalcoholic extract may contribute to its gastroprotective activity (Potrich *et al.,* 2010).

Baggio *et al.* (2008) investigated that a hot-water aqueous extract of *A. millefolium* protected rats against gastric ulcers induced by ethanol and restraint-in-cold stress, but not against indomethacin induced ulcers. The inhibitory dose 50% (ID_{50}) for the aqueous extract of *A. millefolium* was 900 mg/kg, p.o when tested against gastric ulcers induced by ethanol and or indomethacin. Antiulcer activity of *A. millefolium* may be either due to inhibition of gastric secretion or increase in protective factors (such as blood flow) in gastric mucosa.

Cavalcanti *et al.* (2006) reported that aqueous extract of *A. millefolium* efficiently heals the chronic ulcers induced by acetic acid (ED_{50} = 32 mg/kg, p.o.) in rodents. The mucosal damage was reduced up to 75% after seven days of treatment (100 mg/kg/day dose) and by 90% at 300 mg/kg/day dose. Additionally, acute administration of *A. millefolium* extract, concomitant to ulcer-inducing treatment with 70% ethanol or indomethacin, significantly reduced mucosal damage.

Hepatoprotective

Zolghadri *et al.* (2014) investigated the effect of ethanol extract of *A. millefolium* aerial parts on IL-1β and iNOS gene expression of pancreatic tissue in the Streptozotocin (STZ) induced diabetic rats. Streptozotocin (45 mg/kg body) was injected intraperitoneal (IP) for inducing diabetes in rats. Real-time PCR was used for determining quantity of pancreatic IL-1β and iNOS mRNA. Body weight, IL-1β and iNOS genes expression (about 56 and 55%, respectively) in STZ-diabetic rats was restored after administration of *A. millefolium* extract (100 mg/kg/day). This may be due to amelioration of IL-1β and iNOS gene over expression which can have a β-cell protective effect.

Antihepatotoxic activity of 5-hydroxy 3, 4′, 6, 7-tetramethoxy flavone isolated from the aqueous extract of *A. millefoliumn* aerial parts on fasted Wistar rats was assessed by estimating Serum Transaminases viz. Glutamyl Pyruvate Transaminase (GPT) and Glutamyl Oxaloacetate Transaminases (GOT), alkaline phosphatase and total bilirubin. Hepatotoxicity was induced by using carbon tetrachloride (CCl_4) and paracetamol (PCL). Pretreatment with the isolated compound, 5-hydroxy 3, 4′, 6, 7 tetramethoxy flavone, at 20 mg/kg b.w.i.p. significantly reduced GPT levels (101.28 and 141.98%), while GOT levels reduced to 99.8 and 110.12%, alkaline phosphatase (39.2 and 17.97%) and total bilirubin (55.28 and 43.64%) as compared with CCl_4 and PCL respectively, which is similar with Silymarin (50 mg/kg b. w. i. p.). The results confirmed the medicinal value of *A. millefolium* used in indigenous systems of medicines in India (Gadgoli and Mishra, 2007).

The aqueous–methanol extract of *A. millefolium* aerial parts was studied for its possible hepatoprotective effect against d-galactosamine (d-GalN) and lipopolysaccharide (LPS) -induced hepatitis in mice to rationalize some of the folklore uses. Co-administration of d-GalN (700 mg/kg) and LPS (25 μg/kg) produced 100% mortality in mice. However, pre-treatment with *A. millefolium* extract (300 mg/kg) reduced the mortality to 40%. Co-administration of d-GalN (700 mg/kg) and LPS (1 μg/kg) significantly raised the plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels compared with values in the control group ($p < 0.05$). Pre-treatment of mice with *A. millefolium* extract (150–600 mg/kg) significantly prevented the toxins induced rise in plasma ALT and AST ($p < 0.05$). These results indicate that the *A. millefolium* exhibit a hepatoprotective effect, which may be partly attributed to its observed calcium channel blocking activity (Yaeesh *et al.*, 2006).

Anticancer/Antitumor

Ten 1,10-secoguaianolides isolated from the methanolic extract of *A. millefolium* flower, were studied for their growth inhibitory activity *in vitro* against the human tumor (MCF7WT) and human prostatic cancer cell line (PC3). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used for estimating cell survival. The results showed that only Seco-tanapartholide A exhibited moderate cell growth inhibitory activity against the human cancer cell line MCF7WT (IC_{50} = 5:51 μm) (Li *et al.,* 2012a).

Cytotoxic and genotoxic effects of *A. millefolium* leaf aqueous extract on *Lactuca sativa* (lettuce) root tip meristem cells were examined. Lettuce seeds were treated for 72 h with different concentrations of *A. millefolium* aqueous extracts (5, 10, 20 and 30 mg/mL) for analyzing percentage of germination, root development and cellular behavior. The results showed that the 30 mg/mL of aqueous extract reduced the mitotic index, seed germination, root development and also induced chromosome aberrations and cellular death in the root cells of *L. sativa*. Although *A. millefolium* has a beneficial effect as a medicinal plant, serious problems and damages on cells by incorrect usage can be observed (Saulo and Viccini, 2011).

Hemmati *et al.* (2011) investigated the anticancer effect of hydroalcoholic extract of *A. millefolium* flowers on bleomycin-induced (7.5 IU/kg) lung fibrosis in Sprague Dawley rat. They were treated with different doses of *A. millefolium* extract (400, 800, 1600 mg/kg/day P.O.) for two weeks. Histopathological examination of bleomycin-treated animals showed marked alveolar thickening associated with fibroblasts, myofibroblasts proliferation and collagen production in interstitial tissue leading to pulmonary fibrosis. However, no toxicological or histopathological abnormalities were exhibited in rats after oral treatment of *A. millefolium* extract and less contraction of lung strips was also observed at 1600 mg/kg of the extract.

Anticancer potential of ethanol extract of *A. millefolium* aerial part was assayed on HFFF (normal fibroblast cell line) and six cancerous cell lines viz, AGS (human caucasian gastric adenocarcinoma), MCF7 (human breast ductal carcinoma), SW742 (human colorectal adenocarcinoma), SKLC6 (human lung carcinoma), A375 (human melanoma cancer) and PLC/?PRF/5 (human liver hepatoma). Cell lines were treated with 10 mg of evaporated ethanol extract dissolved in DMSO and ethanol (50%). The highest IC_{50} value of 66.000 μg/mL was observed against PLC/?PRF/5, followed by MCF7 (64.058 μg/mL), A537 (49.438 μg/mL), SW742 (40.279 μg/mL), HFFF (34.431 μg/mL), SKLC6 (24.106 μg/mL) and AGS cell lines (22.051 μg/mL). The findings may point at selectivity effect of extract in inducing cytotoxicity on cancerous cell lines (Ghavami *et al*., 2010).

De Santanna *et?al.* (2009) investigated the genotoxic activity of *A. millefolium* flower oil on heterozygous diploid strain of *Aspergillus nidulans* (A757//UT448 with green conidia). The genotoxic evaluation was performed at 0.13, 0.19 and 0.25 μL/mL concentrations. A significant increase in number of yellow and white mitotic recombinants, per colony of the diploid strain, was reported after oil treatment with 0.19 and 0.25 μL/mL. The genotoxicity of the oil was associated with the induction of mitotic non-disjunction or crossing-over by oil. The present results pointed to the necessity of testing the ability of *A. millefolium* essential oil to interfere with the recombinational process in mammalian cells and also suggested that the oil should be used carefully.

Casticin isolated from *A. millefolium* was studied for antitumor potential through cell cycle and apoptotic signaling pathways in two MCF-7 sub-lines MN1 and MDD2. Both cell lines were found sensitive to Casticin at concentrations above 0.25 μM, and they display a similar 50% inhibitory concentration (IC_{50}) of 2 μM. Casticin results in cell growth arrest in G2/M phase and induced apoptotic death by acting as a tubulin-binding agent (TBA). It also induced p21which in turn inhibited Cdk1 and down regulated the cyclin A expression (Haidara *et al.*, 2006).

The cytotoxic activity of *A. millefolium* leaf and flower ethanol extracts was investigated on human breast cancer (SK-Br-3, MDA-MB-435) and leukemia cell lines (U937 and K562). The inhibition of cell proliferation was measured by using MTT colorimetric assay. *A. millefolium* extract at 10 μg/mL concentration caused 50% inhibition in growth of SK-Br and K562 cells (most sensitive cells to the effect), and the proliferation of these cells was strongly decreased at 400 μg/mL (>87% inhibition). However, with increase in concentration, the proliferation activity of these cells was increased by 10 to 100 μg/mL (%inhibition range 48.8 at 10 μg/mL to 6.25 at 100 μg/mL) and suppressed (%inhibition range 6.25 and 27.6% at 400 μg/mL) (Amirghofran and Karimi, 2002).

In-vivo antitumor activity of three sesquiterpenoids, achimillic acids A, B and C, isolated from methanol extract of *A. millefolium* flower was studied against mouse P-388 leukemia cells. Test compounds were administrated as a single IP injection on the day following the tumor inoculation at a dose of 1, 2, 5, 20 and 50 mg/kg. The compounds were found to be active against mouse P-388 leukemia cells in a concentration-dependent manner (Tozyo *et al.,* 1994).

Antiinflammatory

David *et al.* (2010) examined the effects of *A. millefolium* aqueous extract on the inflammatory responses of RAW 264.7 murine macrophages cell line challenged with LPS. Cell viability was not affected at the concentration of 25–300 μg/mL of extract. Lipopolysaccharide-induced NO production was strongly suppressed in a dose-dependent manner by down regulating the expression inducible nitric oxide synthase (iNOS), an enzyme directly involved in LPS-stimulated NO synthesis. However, no significant effect was observed in PGE_2 synthesis, protein COX-2 and IL-6 levels and dose-dependent inhibition was observed in secretion of GM-CSF and IL-10. At 200 μg/mL, the production of TNF-and IL-10 was significantly enhanced. The present study clearly suggested the potential of *A. millefolium* to combat acute and chronic inflammation and may be a novel source for drug discovery.

In-vitro antiinflammatory activity of *A. millefolium* aerial part methanol and aqueous extracts inhibited the inflammation-related proteases, viz, neutrophil elastase (HNE) and matrix metalloproteinases (MMP-2 and -9). Besdes extracts, two fractions enriched in flavonoids and DCQAs, were tested in order to evaluate their contribution to the antiphlogistic activity of the plant. The extract and the flavonoid-enriched fraction inhibited HNE at IC_{50} value of 20 μg/mL, while a DCQA-enriched fraction inhibited HNE at IC_{50} value of 72 μg/mL. Dicaffeoylquinic acid fraction was more potent to inhibit MMP-2 and MMP-9 at IC_{50} values ranged from 600 to 800 μg/mL then the flavonoid fraction and the extract. The obtained results give further insights into the pharmacological activity of *A. millefolium* and confirm the traditional application as antiphlogistic drug (Benedek *et al.,* 2007c).

Three glycosylated phenolic compounds, luteolin-7-O-glucoside, apigenin-7-O-glucoside and caffeic acid glucoside were isolated from the methanol extract of *A. millefolium* aerial part, and the immunological properties of different fractions of plant extract were studied on humoral immune system of BALB/c albino female mice using microhaemagglutination test. The test compounds at 125 and 61.5 mg/kg showed a significant decrease in the anti-SRBC (sheep red blood cell) titer of mice. The immunological properties of the fractions may be attributed to glycosylated derivatives of caffeic acid (Yassa *et al.,* 2007).

Lopes *et al.* (2005) studied the effects of *A. millefolium* leaf essential oil and commercial azulene in peritoneal macrophage cell cultures from Swiss mice. Three dilutions of the essential oil (1:50, 1:100 and 1:200) and commercial azulene (1:100) were tested for H_2O_2 and TNF-α determination by using MTT assay. Higher viability level of 70% was observed in 1:100 and 1:200 dilutions. However, 66.86% viability of the cells was found in the presence of commercial azulene. The essential oil was also able to stimulate peritoneal macrophages to produce H_2O_2 and TNF-α without causing an overproduction of these compounds, but it is lower than that of commercial azulene. The results suggested that the *A. millefolium* can modulate macrophages activation.

Lopes *et al.* (2003) determined the release of nitric oxide in peritoneal macrophages cultures of Swiss mice in the presence of crude essential oil and 70% crude ethanolic extract obtained from the leaves of *A. millefolium*. Among different dilutions of the essential oil tested (1:50, 1: 100 and 1: 200), only the 1: 100 dilution produced a greater amount of nitric oxide (NO). In relation to the 70% ethanolic extract, higher NO production was observed in the more concentrated samples (6, 8 and 10 mg/mL). It was found that both the essential oil and the 70% crude ethanolic extract of *A. millefolium* are macrophage activation modulating agents at concentrations of 20, 10 and 5 mg/mL, when compared with LPS (LPS-potent stimulator of NO production).

Antiinflammatory activity of different fractions isolated from an aqueous extract of the dry flower heads of *A. millefolium* was measured by the mouse paw edema test. The most active fraction isolated (XII) reduced inflammation by 35% at a dose level of 40 mg/kg (Goldberg *et al.,* 1969).

Antiproliferative

Antiproliferative activity of methanolic extract of *A. millefolium* aerial parts (MEA) alone or combined with bleomycin was investigated on human prostate cancer (DU-145) and human nonmalignant fibroblast cell lines (HFFF2) by using MTT assay. Both the cell lines were treated with MEA at various concentrations (20, 100, 500, 1000 and 2000 μg/mL). The extract considerably improved cytotoxicity induced by bleomycin showing 60 and 49% survival rate at doses of 1000 and 2000 μg/mL, respectively. The survival rate reached 85% in bleomycin-treated cells. MEA did not exhibit any cytotoxicity on HFFF2 cells. The enhanced cell toxicity induced by bleomycin in the prostate cancer cell without any significant toxicity on normal cells may be due to cytotoxic flavonoids such as casticin and sesquiterpenoids, but the mechanisms remain to be elucidated (Shahani *et al*., 2015).

In-vitro antiproliferative activity of Achillinin A isolated from the flower of *A. millefolium* was evaluated against five human lung tumor cell lines (adenocarcinomic human alveolar basal epithelial A549, human lung adenocarcinoma RERF-LC-kj, human lung carcinoma QG-90, QG-56, PC-3) and compared with that of cisplatin. The results showed that Achillinin A exhibited potential antiproliferative activity to adenocarcinomic human alveolar basal epithelial A549, human lung adenocarcinoma RERF-LC-kj and human lung carcinoma QG-90 cells with 50% inhibitory concentration (IC_{50}) values of 5.8, 10 and 0.31 μM, respectively, and the activity was stronger than that of cisplatin (Li *et al.,* 2011).

Five flavonoids (apigenin, luteolin, centaureidin, casticin and artemetin) and five sesquiterpenoids (paulitin, isopaulitin, psilostachyin C, desacetylmatricarin and sintenin) isolated and identified from chloform extract of aerial parts of *A. millefolium* were investigated for their antiproliferative activities on three human tumor cell lines (HeLa, MCF-7 and A431 cells) by using MTT assay. Centaureidin was the most effective constituent having high cell growth inhibitory activities on HeLa (IC_{50} value of 0.0819 μM) and MCF-7 (IC_{50} value of 0.1250 μM) cells. Casticin and paulitin were also highly effective against all three tumor cell lines (IC_{50} 1.286–4.76 μM), while apigenin, luteolin and isopaulitin proved to be moderately active (IC_{50} value of 6.95–32.88 μM). Artemetin, psilostachyin C, desacetylmatricarin and sintenin did not show antiproliferative effects against these cell lines (Csupor *et al.*, 2009).

Antispermatogenic effect

Takzare *et al.* (2011)studied the effect of ethanol extract of *A. millefolium* flowers on spermatogenesis in adult male wistar rats. A dose of 200, 400 and 800 mg/kg/day of extract were administered by IP injection or through gavage for 22 days, on every other day. At a dose of 400 mg/kg/day (IP), scattered immature cells on basal membrane in seminiferous tubules were found, and also a significant decrease in cell accumulation and vacuolization in seminiferous tubule was seen. However, a dose of 800 mg/kg (IP) caused thickeness in seminiferous tubules on basal membrane, decrease

in cell accumulation in seminiferous tubule, severe disarrangement, degenerative cells and severe decrease in sperm count. Oral administration of 800 mg/kg/day of extract showed thickness in basal membrane and the disarrangement in cells. The present results suggest that the *A. millefolium* exhibit temporary antifertile activity in adult male animals.

Oral administration of alcoholic extract of *A. millefolium* flowers caused significant decrease in fertility parameters (fertility indices, body and reproductive organs weight) in male rats. At the doses of 200 and 400 mg/kg/day for 50 days, no significant difference in body weight, sperm motility and sperm viability was observed. However, significant decrease was observed in epididymis weight, epididymal sperm reserve (ESR), daily sperm production (DSP) and testosterone concentration at 200 mg/kg of body weight. The results suggested that alcoholic extract of *A. millefolium* flowers had antifertility effect, but its mechanism is not clear, and it may be due to the presence of chemical composition of *A. millefolium* (Parandin and Ghorbani, 2010).

Montanari *et al.* (1998) studied the effect of an ethanolic extract (200 mg/kg/day, intraperitoneally, for 20 days) and a hydroalcoholic extract (300 mg/kg/day, orally, for 30 days) of *A. millefolium* flowers on the spermatogenesis of Swiss mice. *A. millefolium*, at the dose of 200 mg/kg/day for 20 days or at the dose of 300 mg/kg/day for 30 days, did not cause any significant difference in body weight gain or in testis and seminal vesicle weight. However, macroscopic alterations were observed in the reproductive organs of animals treated with 200 mg/kg/day, intraperitoneally, for 20 days, and giant multinuclear cells were found in the vacuolized seminiferous tubules of animals treated with 300 mg/kg/day of the extract. The results clearly suggested further studies with *A. millefolium* as an antifertility agent.

Antioxidant

Various compounds isolated from the essential oil of aerial part of *A. millefolium* were examined for their antioxidant activity using DPPH assay. The highest free radical scavening activity against DPPH was shown by thymol (IC_{50} 12.0 ± 0.1 μg/mL), followed by carvacrol (IC_{50} 13.43 ± 0.0 μg/mL), and the lowest activity was exhibited by bornyl acetate (IC_{50} 25 ± 0.1 μg/mL). However, similar activity was observed in α-pinene (20 ± 0.1 μg/mL), limonene (20 ± 0.3 μg/mL) and camphene (20.01 ± 0.3 μg/mL). The results suggested that the antioxidant activity of the essential oil is mainly due to the action of thymol and carvacrol (Kazemi, 2015).

Georgieva *et al.* (2015) studied the antioxidant activity (DPPH, ABTS, FRAP and CUPRAC assays) of *A. millefolium* (leaves and stems). The highest free radical scavenging activity was observed against CUPRAC (55.08 ± 0.85 to 148.99 ± 1.94 μM TE/g dw), followed by FRAP (38.16 ± 0.47 to 132.71 ± 1.86 μM TE/g dw), DPPH (24.15 ± 0.15 to 116.74 ± 0.21 μM TE/g dw) and ABTS (18.59 ± 0.22 to 125.75 ± 2.24 μM TE/g dw). However, decoction extract showed two/three times higher activity than the other extracts studied.

Ethanol and aqueous extracts of *A. millefolium* leaves, flowers and seeds were studied for their antioxidant activity by using ferric thiocyanate and H_2O_2 radical scavenging assays. At 100 μg, both the extracts exhibited scavenging activity (17.75 to 40.63%) on H_2O_2. However, α-tocopherol and BHA (control) exhibited 44.58% and 39.26% H_2O_2 scavenging activity. The highest H_2O_2 scavenging activity was observed in *A. millefolium* aqueous seed extract (17.75%) and the lowest (40.57%) in ethanol flower extract (Keser *et al.,* 2013).

The antioxidant activity of the methanol extract of *A. millefolium* aerial part and its compounds were evaluated by using DPPH, total antioxidant capacity (TAC), copper reducing power and TBARS assays. Methanol extract showed significant activity against all the assays. However, among compounds, rutin (IC_{50} 1.50 ± 0.11 mEq uric acid for DPPH and 0.35 ± 0.07 mEq uric acid for TAC at 1 μM), luteolin 7-O-glucoside (IC_{50} 1.10 ± 0.09 mEq uric for DPPH and 0.11 ± 0.03 mEq uric acid for TAC at 1 μM) and chlorogenic acid (IC_{50} 1.58 ± 0.11 mEq uric for DPPH and 0.30 ± 0.05 mEq uric acid for TAC at 1 μM) showed the highest activity. While luteolin-7-O-glucoside and apigenin-7-O-glucoside showed the maximum inhibition of TBARS formation (Vitalini *et al.,* 2011).

Fraisse *et al.* (2011) assessed the contribution of five main caffeoyl derivatives to the antioxidant activity of the *A. millefolium* determined by DPPH assay. Total antioxidant capacity of *A. millefolium* aerial parts was 8.29%, and main caffeoyl derivatives showed 61.80% of the total with chlorogenic acid (10.01%), 3,5-DCQA (33.17%), 1,5-DCQA (13.63%) and 4,5-DCQA (4.99%). The main caffeoyl derivatives among polyphenols can be considered as the major antioxidant compounds of aerial parts of *A. millefolium.*

Wojdyło *et al.* (2007) isolated five phenolic compounds (caffeic acid, neochlorogenic acid, ferulic acid, luteolin and apigenin) from *A. millefolium* and studied their antioxidant activity by using ABTS, DPPH and FRAP assays. The highest activity was observed against DPPH (200 ± 3.33 μM trolox/100 g dw), followed by FRAP (191 ± 4.51 μM trolox/100 g dw) and ABTS (11.2 ± 0.77 μM trolox/100 g dw).

In-vitro antioxidant activity of borneol, camphor, eucalyptol, α-pinene and β-terpineol isolated from essential oil of *A. millefolium* aerial parts strongly reduced the diphenylpicrylhydrazyl radical (DPPH) (IC_{50} = 1.56 μg/mL) and exhibited hydroxyl radical scavenging effect in the Fe^{3+-}EDTA–H_2O_2 deoxyribose system (IC_{50} = 2.7 μg/mL). It also inhibited the nonenzymatic lipid peroxidation of rat liver homogenate (IC_{50} = 13.5 μg/mL). Observations confirmed that *A. millefolium* essential oil possessed strong antioxidative activity (Candan *et al.,* 2003). The results suggested that *A. millefolium* may be used as an easily accessible source of natural antioxidants and also as a possible food supplement or in pharmaceutical industry.

Antimicrobial

Antifungal activity of the essential oil from aerial parts of *A. millefolium* was evaluated against *Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Candida*

guillermondii, *Candida parapsilosis*, *Cryptococcus neoformans*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Trichophyton verrucosum*, *Microsporum canis*, *Microsporum gypseum*, *Epidermophyton floccosum*, *Aspergillus niger*, *Aspergillus fumigates and Aspergillus flavus*. The oil showed the highest activity against the tested strains, with MIC values ranging from 0.32–1.25 μL mL^{-1} (Falconieri *et al.,* 2011).

Antibacterial activity of essential oil from aerial parts of *A. millefolium* was studied against *Shigella dysenteria*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis* and *Kelebsiella pneumoniae*. The highest antibacterial activity was observed against *S. epidermidis* and *S. aureus* (33.6 ± 0.5 mm; MIC 12.6 μg/mL and 31.4 ± 0.8 mm; MIC 15.4 μg/mL) (Mazandarani *et al*., 2009).

Different extracts (hexane, petroleum ether and methanol) of *A. millefolium* aerial parts was tested against *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Salmonella enteritidis*, *A. niger* and *C. albicans*. The mean zones of inhibition ranged between 15 and 17 mm, and the highest zone was observed in *P. aeruginosa* (Stojanovic *et al.,* 2005). Methanol extract of *A. millefolium* aerial parts was active against *Helicobacter pylori* at a MIC of 50 μg/mL (Mahady *et al*., 2005).

The methanolic extracts (both water-soluble and water-insoluble fractions) and the essential oil of *A. millefolium* aerial parts were screened for antimicrobial activity against *S. aureus*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *B. cereus*, *Acinetobacter lwoffii*, *Enterobacter aerogenes*, *E. coli*, *K. pneumoniae*, *Proteus mirabilis*, *P. aeruginosa*, *Clostridium perfringens*, *Mycobacterium smegmatis*, *C. albicans* and *C. krusei* by agar well-diffusion method. The significant activity was shown by water-insoluble fractions against *Candida perfringens* and the yeasts as compared with water-soluble fractions which exhibited slight or no activity. Essential oil possessed strong activity against the tested strains as compared with the extracts. The MIC value ranged from 4.5 mg/mL (*w*/*v*) to 72.00 mg/mL (*w*/*v*) with the lowest MIC value against *S. pneumoniae, C. perfringens* and *C. albicans* at 4.5 mg/mL (*w*/*v*). The highest zone of inhibition was recorded against *C. albicans* and *C. krusei* (21 and 16 mm) (Candan *et al.,* 2003).

Ethanol extract of aerial parts of *A. millefolium* was screened for antimicrobial activity against *E. coli, B. cereus, P. aeruginosa, S. enteritidis* and *C. albicans.* The highest MIC value of 62.50 mg/mL was observed against *B. cereus* and *S. enteritidis*. However, no activity was observed in other three tested strains (Kokoska *et al.,* 2002). These studies confirmed the ethnopharmacological use of *A. millefolium* and place them among the most promising indigenous drug to treat microbial infections.

Antiparasitic

Ebadollahi and Ashouri (2011) determined the fumigant toxicity of essential oil from *A. millefolium* against adults of *Plodia interpunctella*. The 100% mortality rate was achieved at 50, 65 and 80-μL concentrations. The LC_{50} value was 34.80 μL/L after 24 h of fumigation, and it decreased with increase of exposure time.

Methanolic extract and compounds isolated from methanolic extract of *A. millefolium* were screened for antiplasmodial activity in CQ-sensitive (D10) and CQ-resistant (W2) strains of *Plasmodium falciparum.* The methanol extract did not induce 50% mortality in the D10 strain but showed a measurable activity against the CQ-resistant W2 strain, with an IC_{50} value of 44.6 ± 8.8 μg/mL. However, among the isolated compounds, apigenin 7-O-glucoside (IC_{50} 10.1 ± 1.3 in D10 and 6.1 ± 3.8 μg/mL in W2) and luteolin 7-O-glucoside (IC_{50} 26.2 ± 13.5 in D10 and 26.8 ± 3.6 μg/mL in W2) were the most active against both strains of *P. falciparum* (Vitalini *et al.*, 2011).

Essential oil extracted from the leaves and flowers of *A. millefolium* were tested for their *in-vitro* antileishmanial activity against *Leishmania amazonensis* and murine macrophages (J774G8 cell line). The IC_{50} value of *L. amazonensis* promastigotes was 7.8 μg/mL whereas the survival of amastigotes of this pathogen within peritoneal murine macrophages was halved by treatment with the oil at 6.5 μg/mL. The mean cytotoxic value of the oil measured against adherent (uninfected) J774G8 macrophages was 72.0 μg/mL (i.e. 9.2 and 11.0 times higher than the IC_{50} against the promastigotes and intracellular amastigotes). Scanning electron microscopy revealed that the oil caused morphological changes in the treated parasites, including alterations in their shape and size. In transmission electron microscopy, promastigotes treated with the oil (at IC_{50} of 7.8 μg/mL) showed ultrastructural alterations (Santos *et al.,* 2010).

A study was conducted to investigate the efficacy of 11 flavonoids to inhibit the growth of the intraerythrocytic malarial parasite viz., chloroquine-sensitive (3D7) and chloroquine-resistant (7G8) strains. All flavonoids showed activity against 7G8 strain, and only eight exhibited activity against 3D7 strain, and luteolin was the most effectual in preventing the parasitic growth (Lehane and Saliba, 2008).

Nilforoushzadeh *et al.* (2008) evaluated the efficacy of *A. millefolium*, propolis hydroalcoholic extract and systemic glucantime against *Cutaneous leishmaniasis* in Balb/c mice. Mean of ulcer size reduction observed in glucantime was 22.57%, *A. millefolium* (43.29%) and propolis groups (43.77%). However, *A. millefolium* and propolis hydroalcoholic extract were more effective than glucantime.

Essential oil from *A. millefolium* was investigated for their antitrypanocidal activity against epimastigotes and trypomastigotes forms of *Trypanosoma cruzi*. Results showed a dose-dependent growth inhibition with IC_{50} of 145.5 and 228 g/mL after 24 h, respectively (Santoro *et al.,* 2007).

Antimalarial and antibabesial activities (*Babesia gibsoniis* is a tick-transmitted canine protozoan parasite that destroys red blood cells) of 24 aqueous extracts of traditionally used plants for the treatment of malaria in Java were investigated. *A. millefolium* was one of the six species found to exhibit strong inhibitory activity (over 80% inhibition at 1 mg/mL) (Murnigsih *et al.*, 2005).

A. millefolium flower, leaf and stem ethanolic extracts showed repellant activity against *Aedes aegypti*. The nitrogen-containing compound stachydrine, the carboxylic acids, caffeic acid, chlorogenic acid, salicylic acid and the phenolic compound pyrocatechol, were most active among 35 compounds isolated from fractions of the extracts. They showed a distance and contact-repelling activity similar to the well-known repellent *N,N*-diethyl-toluamide (DEET) (Tunón *et al.,* 1994).

A. millefolium methanol extract exhibited activity against 24-h-old larvae of *Aedes triseriatus*. The active principle was found to be N-(2-methlpropyl)-(E, E)-2, 4-decadienamide. At 5 ppm, isolated and synthesized amides resulted in 98 and 100% mortality of 24-h-old *A. triseriatus* larvae. The N-(2-methylpropyl)-amides of decanoic and (E)-2-decenoic acids showed the same order of antilarval activity as N-(2-methylpropyl)-(E, E)-2, 4-decadienamide, but N-(2-methylpropyl) sorbamide was inactive (Lalonde *et al.,* 1980).

Antispasmodic

Moradi *et al.* (2013) studied the effect of hydroalcoholic extract of *A. millefolium* aerial parts on contraction and relaxation of isolated ileum in rat. The inhibitory effect of the extract on contractions induced by KCl and acetylcholine was not significantly affected neither by propranolol (1 μM) nor by Nω-Nitro-L-arginine methylester hydrochloride (100 μM). However, *A. millefolium* extract reduced KCl and acetylcholine-induced contraction of ileum, which may be due to the blockade of voltage-dependent calcium channels and can be used for eliminating intestinal spasms.

Babaei *et al.* (2007) studied the effect of *A. millefolium* hydro-alcoholic extract on the contractile responses of the isolated guinea-pig ileum. It was found that the contractile response was repressed by the extract in a dose-dependent manner (EC_{50} = 1.5 mg/mL). The results confirmed that *in-vitro* effect of *A. millefolium* extract in inhibiting the electrical induced contractions of the guinea-pig ileum.

Several flavonoid agycones viz, quercetin, luteolin and apigenin showed potent antispasmodic activities on isolated terminal guinea pig ilea *in vivo*. The aglycones quercetin, luteolin and apigenin exhibited the highest antispasmodic activities with IC_{50} values of 7.8, 9.8 and 12.5 μmol/L, respectively. However, rutin and the flavonoid metabolites, homoprotocatechuic acid and homovanillic acid showed no significant effects on contractility of the terminal ilea (Lemmens-Gruber *et al.,* 2006).

Immunosuppressive

Yassa *et al.* (2007) studied the immunosuppressive activity of methanol extract and some fractions of *A. millefolium* aerial parts on humoral immunity in BALB/c mice by microhaemagglutination test. Only two fractions at 125 and 61.5 mg/kg showed significant decrease in the anti-SRBC titer of mice. The immunological properties of *A. millefolium* may be due to the presence of glycosylated derivatives of caffeic acid isolated from the active fraction of *A. millefolium*.

Saeidnia *et al.* (2004) studied the effects of *A. millefolium* essential oil on humoral immune responses in BALB/c mice. The essential oil decreased the anti-SRBC antibody titer in mice and accounts for the different immunological effects of this plant. However, a sesquiterpene bisabolol was the main compound isolated from *A. millefolium* essential oil.

Cardiovascular

Dall'Acqua *et al.* (2011) investigated the effect of methanol extract of *A. millefolium* aerial parts extract *in vitro* on the growth of primary rat vascular smooth muscle cells (VSMC) under different conditions of cell seeding density (2000 or 8000 SMC/well) and incubation time (24 or 48 h) using the MTS assay. *A. millefolium* extract was found to enhance primary rat VSMC by partly acting through estrogen receptors and impairing NF-κB signaling in human umbilical vein endothelial cells at a concentration below 60 μg/mL by about 30–40%.

Niazmand and Saberi (2010) investigated the inotropic and chronotropic effects of aqueous–ethanol extract of *A. millefolium* on 24 wistar rat's isolated heart. The extract was infused to the heart at three different concentrations (0.01, 0.0125, 0.02 mg/mL) for 30 s. The extract showed negative inotropic and chronotropic effects on the heart during the infusion. However, the negative choronotropic of *A. millefolium* was stronger than its negative inotropic effect. This supports some of the traditional uses of *A. millefolium* and may induce novel potential actions in the cardiovascular system.

Analgesic effect

Noureddini and Rasta (2008) studied the analgesic effects of aqueous extract of *A. millefolium* flowers in the rat's formalin test. Aqueous extract of *A. millefolium* (5, 27, 40, 80, 160 and 320 mg/kg, p.o.) was injected 30 min before formalin injection. Antinociception during 0–5 min (first phase) and 15–60 min (second phase) after formalin injection was recorded. The highest antinociceptive was observed at a dose of 160 mg/kg, and larger dose (320 mg/kg) did not further effect in the formalin test. The results of the present study justified the traditional use of the *A. millefolium* for treating pain.

Role in appetite

Nematy *et al.* (2017) studied the orexigenic effect of hydro-alcoholic extract of *A. millefolium* on 30 male wistar rats by measuring plasma ghrelin level. A dose of 50, 100 or 150 mg/kg of *A. millefolium* extract was given to rats for 7 days via gavage. The extract at the concentrations of 50 and 100 mg/kg showed significant increase in food intake by rats during 24 h, while at the 150 mg/kg no significant effect was observed. The reason for decrease in appetite after administration of 150 mg/kg of extract is not clear. However, it may be due to some side effects of *A. millefolium* at high doses.

This study indicated that *A. millefolium* had positive dose-related effects on appetite in rats.

Estrogenic

Innocenti *et al.* (2007) reported the *in-vitro* estrogenic activity of *A. millefolium* aerial parts in an assay using recombinant MCF-7 cells. Active constituents (dihydrodehydrodiconiferyl alcohol 9-O-beta-D-glucopyranoside, apigenin and luteolin) isolated from aerial part of *A. millefolium* are considered as estrogenic agents. Apigenin activated estrogen receptor α (ERα) at a minimum concentration of 1.50×10^{-2} g/L, while luteolin was inactive. However, apigenin and luteolin activated ERβ at minimum concentrations of 3.70×10^{-3} g/L and 2.20×10^{-3} g/L.

Choleretic activity

The efficiency of 20% methanol extract fraction enriched in 3,4-DCCA, 3,5-DCCA and 4,5-DCCA and luteolin-7-O-β-D-glucuronide of *A. millefolium* aerial parts was investigated for their choleretic potential in isolated perfused rat liver. The fraction caused a dose-dependent increase in bile flow of 23.1% (±6.9), 44.1% (±17.2) and 47% (±12.2), compared with the internal standard cynarin, which showed an increase of 5.1% (±2.0), 15.9% (±3.6) and 21.6% (±8.9) at the same concentrations (10, 20 and 40 mg/L). The combined effect of DCCAs and luteolin-7-O-β-D glucuronide stimulated bile flow more effectively than the single compound cynarin. Due to their polar structure, these compounds are quantitatively extracted into teas and tinctures and are active choleretic principles in the traditional applications of *A. millefolium* (Benedek *et al.*, 2006).

Anxiolytic activity

Hydroalcoholic extract of aerial parts of *A. millefolium* was evaluated for their potential anxiolytic-like effect in mice subjected to the elevated plus-maze, marble-burying and open-field tests. Flumazenil (1.0 mg/kg) and picrotoxin (1.0 mg/kg) were administered intraperitoneally 30 min before the administration of the hydroalcoholic extract of *A. millefolium* (300 mg/kg). *A. millefolium* exerted anxiolytic-like effects in the elevated plus-maze and marble-burying test after acute and chronic (25 days) administration at doses that did not alter locomotor activity. The effects of *A. millefolium* in the elevated plus-maze were not altered by picrotoxin pretreatment but were partially blocked by flumazenil. *A. millefolium* did not induce any changes in [(3)H]-flunitrazepam binding to the benzodiazepine (BDZ) site on the GABA(A) receptor indicating that the anxiolytic-like effects were likely not mediated by GABA(A)/BDZ neurotransmission and may be a promising candidate for future development as a new anxiolytic drug (Baretta *et al.,* 2012).

Antinociceptive

The hydroalcohol extract of *A. millefolium* was evaluated by the hot plate, writhing, formalin and intestinal transit tests to confirm their folk use as analgesic, antiinflammatory and antispasmodic agents. Abdominal contortions were significantly inhibited by 65 and 23% at 500 and 1000 mg/kg of the extract due to the presence of flavonoid glycoside rutin as a principal constituent. A high content of caffeic acid derivatives was also found in *A. millefolium*. None of the extracts produced differences in the intestinal transit in mice, nor in the response time in the hot plate or in the immediate or late responses in the formalin test (Pires *et al.,* 2009).

Hypotensive, vasodilatory and bronchodilatory activities

The oral administration of aqueous ethanol extract of *A. millefolium* aerial parts (100–300 mg/kg), dichloromethane fractions (10–30 mg/kg) significantly reduced the mean arterial pressure (MAP) of normotensive rats, but not ethylacetate fraction (10 mg/kg) and butanolic fraction (50 mg/kg). The dichloromethane fractions were found to contain high amounts of artemetin and when administered by either oral (1.5 mg/kg) or intravenous (0.15–1.5 mg/kg) routes in rats caused dose-dependent reduce in MAP, up to 11.47 ± 1.5 mmHg (1.5 mg/kg, i.v.). Intravenous injection of artemetin (0.75 mg/kg) significantly reduced the hypertensive response to angiotensin-I, while increasing the average length of bradykinin-induced hypotension. Artemetin (1.5 mg/kg, p.o.) was also able to reduce plasma (about 37%) and vascular (up to 63%) angiotensin-converting enzyme activity *in vitro*, compared with control group (De Souza *et al.,* 2011).

The aqueous–methanol extract of *A. millefolium* aerial parts caused a dose-dependent (1–100 mg/kg) decrease in arterial blood pressure of rats under anesthesia. In spontaneously beating guinea pig atrial tissues, the extract exerted negative inotropic and chronotropic effects. In isolated rabbit aortic rings, the extract (0.3–10 mg/mL) relaxed phenylephrine (1 mM) and high K^+ (80 mM) induced contractions. In guinea pig tracheal strips, the extract suppressed carbachol (1 mM) and K^+ induced contractions. These results indicated that *A. millefolium* exhibits hypotensive, cardiovascular inhibitory and bronchodilatory effects, thus explaining its medicinal use in hyperactive cardiovascular and airway disorders, such as hypertension and asthma (Khan and Gilani, 2011).

Skin-rejuvenating activity

Pain *et al.* (2011) evaluated the effect of *A. millefolium* extract on the expression pattern of various epidermal differentiation markers in normal human skin biopsies using quantitative image analysis and second to evaluate its capacity to rejuvenate the appearance of skin surface *in vivo*. Study showed that *A. millefolium* extract improved the expression profile of various epidermal differentiation markers (cytokeratin 10, transglutaminase-1 and filaggrin) in cultured skin

biopsies as well as increased epidermal thickness. *In vivo*, a 2-month treatment with 2% *A. millefolium* extract significantly improved the appearance of wrinkles and pores compared with placebo.

Cyclophosphamide toxicity amelioration activity

Jalali *et al.* (2012) assessed whether *A. millefolium* inflorescences aqueous extract with antioxidant and antiinflammatory activities could serve as a protective agent against reproductive toxicity during cyclophosphamide treatment induced in male Wistar rats. *A. millefolium* aqueous extract was given at a dose of 1.2 g/kg/day orally 4 h after cyclophosphamide administration (at dose of 5 mg/kg/day for 28 days by oral gavages). Cyclophosphamide-treated rats showed significant decrease in the body, testes and epididymides weights as well as many histological alterations. Stereological parameters, spermatogenic activities and testicular antioxidant capacity along with epididymal sperm count and serum testosterone concentration were also significantly decreased by cyclophosphamide. Co-treatment with *A. millefolium* extract caused a partial recovery in above-mentioned parameters.

Anticonflict behavior activity

Molina-Hernandez *et al.* (2004) found that anticonflict-like behavior actions of aqueous extract of *A. millefolium* flowers in female Wistar rats may vary according to the oestrous cycle phase. During late proestrus, conflict behavior was reduced at doses of 8.0, 10.0 or 12.0 mg/kg. Conversely, during diestrus, only the dose of 12.0 mg/kg reduced conflict behavior. During late proestrus, control rats displayed reduced conflict behavior compared with diestrus. Diazepam (2.0 mg/kg; IP) reduced conflict behavior both during late proestrus or diestrus. It was found that the anticonflict-like actions of *A. millefolium* may vary according to the estrous cycle phase.

Anthelmintic activity

Tariq *et al.* (2008) evaluated the anthelmintic efficacy of aqueous and ethanolic extracts of *A. millifolium* against the gastrointestinal nematodes of sheep. The worm motility inhibition assay was used for *in-vitro* studies, and fecal egg count reduction assay was used for *in-vivo* studies. *In-vitro* studies revealed significant anthelmintic effects of *A. millefolium* extracts on live *Haemonchus contortus* worms as evident from their paralysis and death at 8 h post exposure. Both the extracts resulted in a mean worm motility inhibition of 94.44 and 88.88%. The mean mortality index (MMI) of both extracts was 0.95 MMI and 0.9 MMI and LC_{50} was 0.05 mg/mL for aqueous extract and 0.11 mg/mL for ethanol extract. The *in-vivo* anthelmintic activity of aqueous and ethanol extracts of *A. millefolium* demonstrated the highest (88.40%) nematode egg count reduction in sheep treated with aqueous extract at 2 g/kg body weight on day 15 after treatment and 76.53% reduction in fecal egg count for the ethanol extract at the same concentration. *A. millefolium* possesses significant anthelmintic activity and could be a potential alternative for treating cases of helminth infections in ruminants.

Table 4. Clinical trials of *A. millefolium*

S. no.	Study type	Number of participants	Disease	Main outcome	Main conclusion	Adverse effect	Reference
1.	Double-blind clinical trial	140	Episiotomy	Reduce perineal pain level, redness, edema and ecchymosis of episiotomy wound	Episiotomy wound healing improver in primiparous women	—	Hajhashemi *et al.* (2016)
2.	Double-blind randomized clinical trail	91	Primary dysmenorrheal	Significant decrease in primary dysmenorrheal pain	Pharmacologic alternative for primary dysmenorrheal pain	—	Jenabi and Fereidoony (2015)
3.	Randomized controlled trial	56	Oral mucositis	Significantly reduced severity of oral mucositis	Chemotherapy-induced oral mucositis drug	—	Miranzadeh *et al.* (2015)
4.	Randomized controlled trial	31	Chronic kidney disease	Decrease in plasma nitrite and nitrate concentrations	Higher doses or longer duration of plant administration may make these changes more significant	Skin rashes	Vahid *et al.* (2012)
5.	Double blind, placebo-controlled randomized trial	49	Atopic dermatitis	Same effect as that of treatment with placebo	Clinical use is not recommended	—	Shapira *et al.* (2005)
6.	Randomized double-blind clinical trial	36	Liver cirrhosis	Significant decrease in Child–Pugh score and ascites	Hepatoprotective effect in cirrhotic patients	—	Huseini *et al.* (2005)

CLINICAL DATA

Herbal remedies continue to be an accepted complementary medical option throughout the world. However, the majority of adverse effects come into the light subsequently including herb–drug interactions, through spontaneous case reports, case series and post marketing surveillance studies from herbal remedies due to the presence of pharmacologically active molecules. Assessing the safety and efficacy of herbal medicines remain problematic, with inadequate or inconsistent methods being used. The clinical evidences on herbal medicine depend on the totality of the available clinical data (randomized controlled trials, case reports, post-marketing surveillance studies and spontaneous reporting schemes) that can be grouped in systematic reviews to provide reliable information on herbal medicines safety. Moreover, randomized controlled trial (double blind) is the most meticulous system for evaluating the efficacy of drugs (Izzo *et al.,* 2016). Different clinical trials performed on *A. millefolium* are mentioned in Table 4.

Hajhashemi *et al.* (2016) assessed the efficacy of *A. millefolium* ointments on episiotomy wound healing in 140 primiparous women by doing double-blind clinical trial. Healing process was assessed by five specifications: redness, ecchymosis, edema, discharge and wound dehiscence at 7th, 10th and 14th day after delivery, and pain level was assessed by means of visual analogue scale. A significant difference in reducing pain severity was observed between the groups ($P < 0.05$), as pain level, redness, edema and ecchymosis were less and more effective due to *A. millefolium* ointments than control groups. But discharge and dehiscence incidence showed no significant difference between groups ($P > 0.05$). The results clearly suggested the use of *A. millefolium* as episiotomy wound healing improver in primiparous women.

Jenabi and Fereidoony (2015) assessed the effectiveness of *A. millefolium* flowers on relief of primary dysmenorrheal in 91 female students (ranging between 10 and 15 years) by conducting double-blind randomized clinical trial. The subjects were randomly divided into two equal groups and were given either placebo or *A. millefolium* in tea bag form for three days in two menstruation cycles, and severity of pain was graded by using a visual analogue scale. It was observed that the mean change in pain score in the *A. millefolium* group was significantly greater than that in the placebo group at 1 month ($P = .001$) and 2 months ($P < .0001$) after treatment.

Miranzadeh *et al.* (2015) investigated the effect of *A. millefolium* distillate solution in the treatment of chemotherapy-induced oral mucositis (OM) in 56 cancer patients. The experimental group gargled 15 mL of a mixture of routine solution and distilled *A. millefolium* 4 times a day for 14 days, while the control group gargled 15 mL of routine solution. The severity of OM was assessed at three times before, 7 and 14 days after intervention. It was observed that mean severity score of OM was 2.39 ± 0.875 in both groups at start of the study that was changed to 1.07 ± 0.85 and 0.32 ± 0.54 in the intervention group in days 7 and 14 ($p < 0.001$). However, the severity of OM was increased to 2.75 ± 0.87 and 2.89 ± 0.956 in the control group, respectively, ($p < 0.001$). The results clearly suggested the use in patients with chemotherapy-induced OM.

Vahid *et al.* (2012) studied the possible effect of *A. millefolium* on plasma nitric oxide concentration in 31 chronic kidney disease patients by randomized controlled trial. Out of 31 patients, 16 patients received 1.5 g of powdered *A. millefolium* flower three days a week for two months, and 15 received placebo for the same period. Plasma nitrite and nitrate concentrations decreased (0.82 ± 0.51 μmol/L to 0.63 ± 0.42 μmol/L and 50.55 ± 17.92 μmol/L to 44.09 ± 17.49 μmole/L, respectively) after 2-month administration of *A. millefolium* without any overdose symptoms, but adverse reaction of skin rashes was observed in one female subject who was excluded from the study. However, these concentrations were slightly increased in the placebo group. Higher doses or longer duration of administration may make these changes more significant.

Ramadan *et al.* (2006) reported that when healthy human volunteers were given 500 mg of matricin (one of the proazulenic sesquiterpene lactone 'prodrugs' in yarrow) orally, micromolar levels of its antiinflammatory metabolite, chamazulene carboxylic acid, were found in their plasma. Another human study has found that use of yarrow leaf extract significantly reduces biting by *Aedes mosquitoes* (Jaenson *et al.*, 2006).

Huseini *et al.* (2005) studied the efficacy of herbal medicine Liv-52 (herbal extract of *A. millefolium*) on liver cirrhosis in 36 cirrhotic patients by randomized double-blind clinical trial. All the patients underwent clinical examination for the Child–Pugh score, ascites, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total billirubin, albumin, prothrombin time, platelet and white blood cells counts before and after 6 months of drug or placebo treatment. The results demonstrated that the hepatoprotective effect in cirrhotic patients treated with Liv-52 for 6 months had significantly better Child–Pugh score, decreased ascites, decreased serum ALT and AST, thus, suggesting the possible use of Liv-52 in the treatment of cirrhotic patients.

Shapira *et al.* (2005) tested the efficacy of tri-herbal combination (*Sibirian ginseng*, *A. millefolium* and *Lamium album*) on atopic dermatitis in a randomized placebo-controlled trial in 49 patients (22 were treated with the study medication and 22 with placebo). It was observed that treatment with tri-herbal combination for atopic dermatitis does not differ from treatment with placebo. Therefore, the study does not support clinical use of tri-herbal combination.

SAFETY AND TOXICOLOGY

Health hazards associated with long-term exposure to *A. millefolium* extracts are not well established. Despite the fact that, Food and Drug Administration has classified the plant as non-poisonous and has approved its utilization in alcoholic drinks (Guédon *et al.,* 1993), some toxic effects had been reported after its use by humans and in animal experiments.

A. millefolium essential oil exhibited genotoxicity in a heterozygous diploid strain of *A. nidulans*, named

A757//UT448 with green conidia at concentrations of 0.13, 0.19 and 0.25 μL/mL. A statistically significant increasing number of yellow and white mitotic recombinants per colony of the diploid strain were reported after oil treatment with 0.19 and 0.25 μL/mL concentrations. The genotoxicity of the oil was associated with the induction of mitotic non disjunction or crossing over. Therefore, the results pointing to the necessity of testing the ability of *A. millefolium* essential oil to interfere with the recombinational process in mammalian cells; they also suggested that the oil should be used with caution (De Santanna *et al.,* 2009).

Cavalcanti *et al.* (2006) studied biochemical and histopathological examinations in Wistar rats by giving *A. millefolium* aqueous extract up to 10 g/kg orally and up to 3 g/kg intraperitoneally, and no signs of deaths were observed. In longer-term studies, no signs of relevant toxicity were observed at doses of up to 1.2 g/kg/day by gavage for up to 90 days. However, slight changes in liver weight, blood cholesterol and glucose levels, neither correlated with dose or period of exposure nor suggestive of toxicity were observed.

Dalsenter *et al.* (2004) evaluated the toxicity of the exposure to the aqueous extract from leaves of *A. millefolium* on reproductive endpoints (reproductive organ weights, sperm and spermatid numbers as well as sperm morphology) in Wistar rats. Adult male rats were treated daily with yarrow extract (0.3, 0.6 and 1.2 g/kg/day) during 90 days by oral gavage. A significant increase in the percentage of abnormal sperm with the highest dose of *A. millefolium* extract was detected with no other important changes in the other reproductive endpoints studied in the male rats. Furthermore, a possible estrogenic/antiestrogenic activity of the extract screened after a 3-day treatment of immature female rats which did not show any uterotrophic effects. The results clearly showed that no long-term reproductive toxicological risk would occur with the doses of *A. millefolium* commonly consumed by humans.

Teixeira *et al.* (2003) determined the effects of the infusions of *A. millefolium* on chromosomes and the cell cycle. *Allium cepa* L. root-tip cells (*A. millefolium*—3.5 and 35.0 mg/mL) and Wistar rat bone marrow cells (*A. millefolium*—3.5 and 35.0 mg/100 g body weight) were used as *in-vivo* plant and animal test systems, respectively. While as, human peripheral blood lymphocytes (*A. millefolium*—0.35 and 3.5 mg/mL culture medium) were used as *in-vitro* test system. No statistically significant alterations were found, as compared with untreated controls, in either the cell cycle or the number of chromosome alterations, after treatments with *A. millefolium*, in rat cells or in cultured human lymphocytes. These results regarding the cytotoxicity and mutagenicity of *A. millefolium* provide valuable information about the safety of using them as therapeutic agents.

A. millefolium ethanolic extract given at 2.8 g/kg/day on days 1–8 or 8–15 of pregnancy in rats, which is 56 times the allegedly recommended daily human dose of 50 mg/kg (of body weight) showed neither contraceptive, abortifacient nor teratogenic activity (Boswell-Ruys *et al*., 2003).

In male rodents, *A. millefolium* has some effects on spermatogenesis, at least at extreme dosages. In mice, an ethanolic extract of yarrow, delivered intraperitoneally at 200 mg/kg/day, and a hydroalcoholic extract delivered orally at 300 mg/kg/day impaired spermatogenesis; morphological changes observed included germ cell necrosis (Montanari *et al.,* 1998).

A somatic mutation and recombination test using *Drosophila melanogaster* was performed to determine the genotoxic potential of *A. millefolium* (20 and 40%) herbal tea extract. It was found that *A. millefolium* tea was weakly genotoxic, and the effect could have been due to the presence of flavonoids (Graf *et al.,* 1994).

A. millefolium has been reported to cause allergic contact dermatitis in some people due to the presence of sensitizing compounds such as guaianolides (a subcategory of sesquiterpenoid) and especially alpha-peroxyachifolid, which is present at variable concentrations in fresh material of up to 0.6% in blossoms and 0.05% in leaf (Hausen *et al.,* 1991; Rucker *et al.,* 1991; Rucker *et al.,* 1994). The concentration may diminish in dried or processed material due to degradation of the compounds (Rucker *et al.,* 1994).

FUTURE PERSPECTIVES OF *A. millefolium*

Aromatic plants have a significant role to combat diseases from the dawn of civilization, and several researches are being performed in the area of herbal drugs for exploring newer and safer alternatives in order to combat against several diseases. The pharmacological properties of *A. millefolium* propose them as natural drug for clinical uses in different diseases and pathological conditions including inflammation, cancers, dyspepsia, bacterial, viral, parasitic, helminth infections, etc. However, further studies are required to find the exact mechanism lying behind some of their pharmacological properties viz., antifertility agent, in promotion of gastric motility and treatment of gastric ulcer, cytotoxic and genotoxic effects, cardiovascular diseases and fumigant toxicity for the management of stored pests. Moreover, many pharmacological effects of the plant have not yet been scientifically neither proven nor attributed to any plant constituents.

Regarding toxicity, *A. millefolium* seems to be almost safe in customary doses, but there are not enough clinical studies about *A. millefolium* safeties. Therefore, active constituents especially casticin; luteolin 7-O-glucoside; apigenin 7-O-glucoside; achimillic acids A, B and C; luteolin; apigenin; rutin and 1,8-cineole can be used in clinical trials for the other pharmacological properties viz., antiviral, antimicrobial, anticancer, antiulcer, hepatoprotective, immunomodulatory, neuroprotective, etc., and the efficacy of these compounds needs to be evaluated in humans. However, interaction with other drugs is the aspect of study which must be considered in clinical use of *A. millefolium*. The results of *in-vitro* and preclinical studies need to be critically evaluated and integrated into the practical applications of *A. millefolium*. This review brings together the most recent studies in the field of *A. millefolium* research; therefore, it will help to provide greater accessibility to the established experimental and clinical data and will promote further studies aimed at confirming the observed effects.

Conflict of Interest

Declared none.

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