Study of sedation, pre-anesthetic and anti-anxiety effects of polar, semi-polar and non-polar fractions of yarrow (*Achillea millefolium*) extract compared with Diazepam in rats

Ali Rezaie[1*], Changhiz Ahmadizadeh[2]

1- Department of Clinical Science, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
2- Department of Microbiology, Ahar Branch, Islamic Azad University, Ahar, Iran
* Corresponding Author's Email: arezaie1@gmail.com

Abstract: The genus Achillea comprises of ~85 species, most of which are endemic to Europe and the Middle East. Turkish flora possesses 42 Achillea species and 23 of them are endemic. The aim of this study was to investigation of the sedation, pre-anesthetic and anti-anxiety effects of polar, semi-polar and non-polar fractions of yarrow (Achillea millefolium) extract compared with diazepam in rats. In the present study, 90 wistar male rats were numbered in groups consisted of 15 animals and were placed in especial cages. In order to evaluate the sedation and pre-anesthetic effects of yarrow extract compared with diazepam, 100 mg/kg of polar extract in first group, 100 mg/kg of semi-polar extract in second group, 100 mg/kg of non-polar extract in third group, 2 mg/kg diazepam in group four, 2 mg/kg amount of dimethyl sulfoxide was injected intra peritoneal in fifth group, and sixth group did not receive any drug. Elevated plus maze was used in order to evaluate anti-anxiety effects of yarrow extract. The results of dual Tokay follow up test show a significant difference between intra peritoneal injections of 100 mg/kg BW of polar extract than semi-polar and non-polar than diazepam. Intraperitoneal injection of polar fraction of yarrow extract has showed better and significant effects than semi-polar, non-polar and diazepam. In conclusion, can state that, polar-fraction of yarrow has more sufficient sedative, pre-anesthetic and anti-anxiety effects than diazepam and other under studied groups. Authors suggest that still need more studies on this plant component in order to understand the more sedative and anxiolytic effects of this plant.

[Ali Rezaie, Changhiz Ahmadizadeh. **Study of sedation, pre-anesthetic and anti-anxiety effects of polar, semi-polar and non-polar fractions of yarrow (*Achillea millefolium*) extract compared with Diazepam in rats.** *Life Sci J* 2013; 10(1):907-913] (ISSN: 1097-8135). http://www.lifesciencesite.com. 141

Keywords: Yarrow, Sedation, Anti-Anxiety, Polar, Semi-Polar, Non-Polar Fraction, Dimethylsulfoxide, Rat.

1. Introduction

The genus Achillea (Asteraceae), named after the mythological Greek warrior Achilles, who used Achillea species for healing wounded-soldiers during the Trojan War [Cheers, 1999]. The genus Achillea comprises of ~85 species, most of which are endemic to Europe and the Middle East. Turkish flora possesses 42 Achillea species and 23 of them are endemic [Duman and Achillea, 2000]. These species have some interesting properties and are used in cosmetics, fragrances and agriculture, for example, plant protection [Senatore et al., 2005]. Some Achillea species have been known to be ethnopharmacologically used in folk remedies for various purposes such as hemorrhoid and wound healing [Baytop, 1999].Herbal teas prepared from some Achillea species are very often used in folk medicine as diuretic, for abdominal pain, against diarrhea, flatulence and emmenagog, moreover for wound healing purposes [Fujita et al., 1995; Honda et al., 1996; Yesilada et al., 1993]. Achillea biebersteinii is locally named yarrow, and other species widely used as a folk remedy to treat abdominal pain, wounds and stomachache as well [Sezik et al., 2001; Baytop, 1997]. A. biebersteinii Afan. [Asteraceae, Section: Filipendulinae (D.C.) Boiss] (syn. A. micrantha) is a perennial herb, villose, stems erect, simple or branched from the base; 30–60 cm high; leaves up to 10 cm, oblong-lanceolate in outline, pinnatisect into numerous narrow segments, segments divided into minute linear-lanceolate mucronate lobes; the heads are radiate, in large dense compound corymbs; involucre 4-5 mm, oblong-ovoid; flowering period, April-May. Several biological activity studies have been performed on various Achillea species, including antibacterial, antioxidant, anti-inflammatory and antispasmodic activities [Karamenderes and Apaydın, 2003; Candan et al., 2003; Al-Hindawi et al., 1989; Skocibusic et al., 2004].

Benzodiazepines possess sedative, hypnotic, anxiolytic, anticonvulsant, muscle relaxant, and amnesic actions [Page et al., 2002; Olkkola and Ahonen, 2008], which are useful in a variety of indications such as alcohol dependence, seizures, anxiety, panic, agitation and insomnia. Most are administered orally; however, they can also be given intravenously, intramuscularly or rectally [RPSGB, 2009]. In general, benzodiazepines are well-tolerated and are safe and effective drugs in the short term for a wide range of conditions [Perugi et al., 2007; Tesar, 1990]. Tolerance can develop to their effects and there

is also a risk of dependence, and upon discontinuation a withdrawal syndrome may occur. These factors, combined with other possible secondary effects after prolonged use such as psychomotor, cognitive, or memory impairments, limit their long-term applicability [Faught, 2004; Allgulander et al., 2003]. The effects of long-term use or misuse include the tendency to cause or worsen cognitive deficits, depression and anxiety [McIntosh et al., 2005, Ashton, 2005].

Diazepam is mainly used to treat anxiety, insomnia, and symptoms of acute alcohol withdrawal. It is also used as a premedication for inducing sedation, anxiolysis or amnesia before certain medical procedures [Bråthen et al., 2005].

Intravenous diazepam or lorazepam are first line treatments for status epilepticus [Riss et al., 2008; Walker, 2005]; However, lorazepam has advantages over diazepam, including a higher rate of terminating seizures and a more prolonged anticonvulsant effect [Prasad et al., 2005]. Diazepam is rarely used for the long-term treatment of epilepsy because tolerance to its anticonvulsant effects usually develops within six to 12 months of treatment, effectively rendering it useless for that purpose [Isojärvi and Tokola, 1998]. Diazepam is used for the emergency treatment of eclampsia, when IV magnesium sulfate and blood pressure control measures have failed [Kaplan, 2004; Duley, 2005]. Benzodiazepines do not have any pain-relieving properties of themselves and are generally recommended to be avoided in individuals with pain [Zeilhofer et al., 2009]. However, benzodiazepines such as diazepam can be used for their muscle-relaxant properties to alleviate pain caused by muscle spasms and various dystonias, including blepharospasm [Mezaki et al., 2005; Kachi, 2001]. Tolerance often develops to the muscle relaxant effects of benzodiazepines such as diazepam [Ashton, 2005]. Baclofen [Mañon-Espaillat and Mandel, 1999] or tizanidine is sometimes used as an alternative to diazepam. Tizanidine has been found to be equally effective as other antispasmodic drugs and have superior tolerability than baclofen and diazepam [Kamen et al., 2008].

The aim of this study was to investigation of the sedation, pre-anesthetic and anti-anxiety effects of polar, semi-polar and non-polar fractions of yarrow (Achillea millefolium) extract compared with diazepam in rats.

2. Materials and methods

2.1. Understudied animals

In the present study, 90 wistar male rats weighting 300±10 g and about 3 month-old were used for laboratory experiments. Animals were kept in standard condition, at 20-25°C, 70% humidity and light cycle of 12 hours lighting and 12 hours darkness. Standard plates were used in order to feeding by method of *Ad-Libitum*, 24 hours feeding. Especial dishes were used for water. The rats were numbered in groups consisted of 15 animals and were placed in especial cages.

2.2. Obtaining extract

500 g of fresh leaves of yarrow was powdered by liquid nitrogen and was dissolved in the 4 liter non-polar solvent like petroleum ether for 48 hour by soxhlet apparatus, obtained extract is non-polar fraction. Then, the remnants were dissolved in the 4 liter non-polar solvent like chloroform for 48 hour, obtained extract is semi-polar fraction. Finally, the remnant leaves were dissolved in the 4 liter high polar solvent like methanol for 48 hour, obtained extract is polar fraction. Achieved fractions de-solved by rotary evaporator and readied for use.

2.3. Evaluating method as well as sedation and pre-anesthetic effects of yarrow compared with diazepam

In order to evaluate the sedation and pre-anesthetic effects of yarrow extract compared with diazepam, 100 mg/kg of polar extract in first group, 100 mg/kg of semi-polar extract in second group, 100 mg/kg of non-polar extract in third group, 2 mg/kg diazepam in group four, 2 mg/kg amount of dimethyl sulfoxide was injected intra peritoneal in fifth group, and sixth group did not receive any drug. 100 mg/kg ketamine per body weight was injected intra peritoneal in all groups 30 minutes following mentioned drugs. Induction time and sleeping time were measured immediately following administration of ketamine.

2.4. Evaluating method as well as anti-anxiety effects of yarrow compared with diazepam

Elevated plus maze was used in order to evaluate anti-anxiety effects of yarrow extract. The system consists of two arms (10×15 cm) which are open and against each other and two arms (40×10×50 cm) which are closed and against each other. They are related to each other by a central plate (10×10 cm) in a semi dark and silent. They are placed in 50 cm distance from the earth. In order to determine anti-anxiety effects of the drugs, the duration of remaining the rats on open arms is considered as non-anxiety marker and the duration of remaining the rats on closed arms is considered as anxiety marker. More duration of remaining the rats on open arms demonstrates the strong anti-anxiety effects of considered drug. Therefore, yarrow extract with dosages of 100, 200, 400 mg/kg BW and 1.2 mg/kg BW diazepam of diazepam and dimethyl sulfoxide (as placebo) were used as intra peritoneal injection. Dimethyl sulfoxide was placed in maze center 30 minutes following administration of the mentioned drugs. The time duration in which the rats remained in each of maze's arms was recorded in terms of second; time duration of their presence in maze is 5 minutes. SPSS software program was used in order to analysis statistical data as well as Tokay follow up test for

determining a significant difference among dual groups. P<0.01 has been considered as significant. Also, data were reported as mean ± SD.

3. Results

Following the injection of pre-anesthetic drugs, the injection of anesthetic inductive drugs, recording of induction time and sleeping time are considered as markers of the rate of sedation effects of a pre anesthetic drug. The results of dual Tokay follow up test show a significant difference between intra peritoneal injections of 100 mg/kg BW of polar extract than semi-polar and non-polar than diazepam (p<0.01) table 1.

Also, both of the semi-polar and non-polar fractions of extract of yarrow has showed significant difference than diazepam from aspect of induction time (P<0.01). From aspect of sleeping time, non-polar fraction had showed more sedative effect than diazepam. About semi-polar fraction, there was no significant difference in compared with diazepam from aspect of sleeping time, diagrams 1 and 2.

Table 1: group's classification and measured induction time and sleeping time

Group	Received treatment (mg/kg)	Induction time (Mean ± SD)	Sleeping time (Mean ± SD)
Group 1	100 mg/kg P.E.+ ketamine 100	83.68±2.93	4885.60±69.16
Group 2	100 mg/kg S.P.E, ketamine 100	106.60±3.57	3806.00±73.77
Group 3	100 mg/kg N.P.E, ketamine 100	114.14±2.24	3418.60±59.19
Group 4	Diazepam 2, ketamine 100	98.94±1.9	4366.60±65.72
Group 5	DMSO 2, ketamine 100	206.44±1.82	1871.40±19.67
Group 6	Without pre-anesthetic, ketamine 100	202.34±2.44	1876.00±23.02

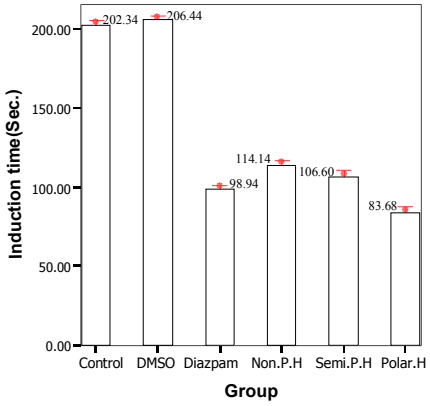

Diagram 1: mean value of data obtained from induction time in understudying groups.

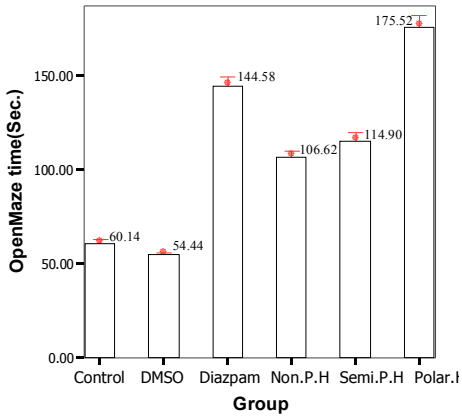

Diagram 3: mean value of data obtained from open-maze time in understudying group.

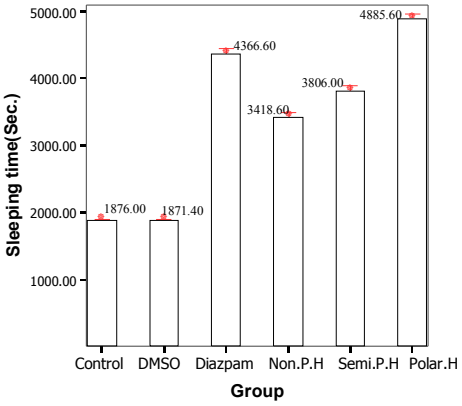

Diagram 2: mean value of data obtained from sleeping time in understudying groups.

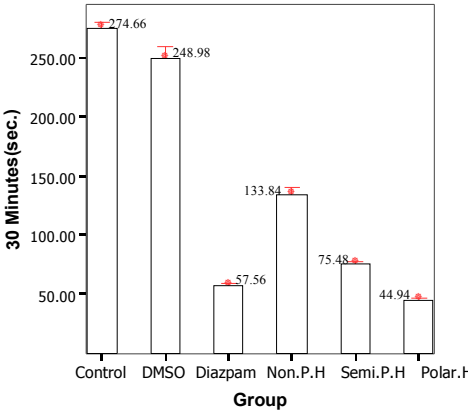

Diagram 4: mean value of data obtained from closed-maze time in understudying group

Based on dual Tokay follow up test, during the spent time in the open arm of apparatus by mice that indicating the high rate of anti-anxiety effect of drug are as follow:

Intraperitoneal injection of polar fraction of yarrow extract has showed better and significant effects than semi-polar, non-polar and diazepam ($P<0.01$), indicate that polar fraction has more anti-anxiety effect than two others, table 2.

Also, Intraperitoneal injection of all fractions of yarrow extract has showed significant difference than diazepam ($P<0.01$) (diagrams 3 and 4).

Table 2: group's classification and measure anxiety effects by focus on maze pattern

Group	Received treatment (mg/kg)	Open maze (Mean ± SD)	Close maze (Mean ± SD)
Group 1	100 mg/kg P.E.+ ketamine 100	175.52±5.27	44.94±1.69
Group 2	100 mg/kg S.P.E, ketamine 100	114.90±4.00	75.48±2.14
Group 3	100 mg/kg N.P.E, ketamine 100	106.62±2.48	133.84±5.01
Group 4	Diazepam 2, ketamine 100	144.58±4.01	57.56±1.54
Group 5	DMSO 2, ketamine 100	54.44±1.26	248.98±8.43
Group 6	Without pre-anesthetic, ketamine 100	60.14±2.25	274.66±4.02

4. Discussion and conclusion

Anxiolytic plants may interact with either glutamic acid decarboxylase (GAD) or GABA transaminase (GABA-T) and ultimately influence brain GABA levels and neurotransmission [Awad et al., 2007]. Flavonoids have recently increased in importance because they have been identified as a new type of ligand with in vivo anxiolytic properties. The flavones chrysin and apigenin, obtained from medicinal plants, have shown an anxiolytic effect in rodents exposed to behavioral tests. Apparently, these compounds modulate the γ-aminobutyric acid (GABA) ergic system to produce the biological effect [Herrera-Ruiz et al., 2008]. However, only a low content of flavonoids was found in this hydroethanolic extract. Yarrow is traditionally used as sleeping aids and probably acts via a central adenosine mechanism, which is possibly the reason for its sleep-inducing andmaintaining activity [Schiller et al., 2006]. Yarrow showed significant inhibition of GAD activity [Awad et al., 2007]. Yarrow extracts induced the response of the ionotropic (GABAA receptors) [Aoshima et al., 2006] and its fraction containing α-acids: in dose-dependently prolonged pentobarbital induced sleeping time [Zanoli et al., 2005]. Xanthohumol had been reported as modulator of the GABAA receptor response [Meissner & Haberlein, 2006].

Achillea species have been so far reported to contain diterpenes, sesquiterpenes, flavonoids, lignans, essential oil and rarely triterpenes [Ahmed et al., 2002; Barrero et al., 1990; Mockute and Judzentiene, 2003; Marchart and Kopp, 2003; Aljancic et al., 1996; Maffei et al., 1994; Oksuz et al., 1993; Kusmenoglu et al., 1995]. For instance, A. vermicularis was shown to have guaianolide- and germacrenetype sesquiterpenes as well as flavonoids, whereas A. setacea was reported to contain sesquiterpenes, essential oils and flavonoids [Marchart and Kopp, 2003; Oksuz et al., 1993; Unlu et al., 2002]. In addition to extracts, essential oils of the Achillea species were also analysed. The oil of A. pachycephala was found to contain 1,8-cineole and camphor as the major constituents, whereas 1,8-cineole and Artemisia ketone were major in A. oxyodonta. On the other hand A. biebersteinii was rich in camphor and borneol followed by 1,8-cineole. It was stated that all the oils were rich in oxygenated monoterpenes [Esmaeili et al., 2006]. Non-volatile components of A. biebersteinii afforded in addition to β-sitosterol, stigmasterol two sesquiterpene lactones, germacranolide [Badahdah and El-Orfy, 2004]. Essential oil of A. millefolium consists of a number of monoterpenes such as α-pinene, β-pinene, 1,8-cineole, camphor and borneol in addition to some sesquiterpene lactones of germacrene-derivatives [Mockute and Judzentiene, 2003]. Major component in the essential oils of both A. setacea and A. teretifolia was elucidated to be 1,8-cineole [Unlu et al., 2002] whereas α-pinene, 1,8- cineole and camphor as well as germacrene D and bisabolene as the major constituents of ten other Achillea species (A. biserrata, A. clypeotala, A. crithmifolia, A. filipendula, A. macrophylla, A. pannonica, A. pyrenaica, A. sibirica, A. taygetea and A. tenuifolia) [Maffei et al., 1994]. Various biological activity studies were also completed on Achillea species. The antimicrobial and antioxidant activities of the essential oil and the methanolic extract of A. biebersteinii were studied in vitro by Baris et al. [Baris et al., 2006]. The essential oil showed antimicrobial activity against 8 bacteria sp., 14 fungi sp. and the C. albicans, whereas the methanolic extract remained inactive.

Baretta et al., (2012) showed that Achillea 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. This behavioral profile was similar to diazepam. The effects of Achillea millefolium in the elevated plus-maze were not altered by picrotoxin pretreatment but were partially blocked by flumazenil. Furthermore, Achillea millefolium did not induce any changes in [(3)H]-flunitrazepam binding. Their results indicate that the orally administered hydroalcoholic extract of Achillea millefolium L. exerted anxiolytic-like effects that likely were not mediated by GABA(A)/BDZ neurotransmission and did not present tolerance after short-term, repeated administration, that is compatible with our research's result.

Grundmann et al., (2009) demonstrated that anxiolytic activity of kaempferol (active component of yarrow) was partially antagonized by concomitant administration of flumazenil, but not by WAY-100635. In conclusion, our study clearly demonstrates that AV extract possesses anxiolytic-like activity and that at least one of its flavonoids, kaempferol, can elicit the same kind of neuropharmacological activity.

Molina-Hernandez et al., (2004) sowed that diazepam (2.0 mg/kg; i.p.) reduced conflict behavior both during late proestrus ($p < 0.05$) or diestrus ($p < 0.05$). Doses of 8.0 mg/kg ($p < 0.05$), 10.0 mg/kg ($p < 0.05$) or 12.0 mg/kg ($p < 0.05$) of Achillea millefolium reduced conflict behavior during late proestrus. Conversely, during diestrus, only the dose of 12.0 mg/kg ($p < 0.05$) of Achillea millefolium L. reduced conflict behavior. They concluded the anticonflict-like actions of Achillea millefolium L. may vary according to the estrous cycle phase.

In conclusion, can state that, polar-fraction of yarrow has more sufficient sedative, pre-anesthetic and anti-anxiety effects than diazepam and other under studied groups. Authors suggest that still need more studies on this plant component in order to understand the more sedative and anxiolytic effects of this plant.

Acknowledgments:
This study was Adapted from a research plan which was supported financially by Islamic Azad University, Tabriz branch. So, author declare own thankful from grant staff of research deputy of Islamic Azad University, Tabriz branch.

References:

1. Ahmed AA, Mahmoud AA, Ali ET, Tzakou O, Couladis M, Mabry TJ, Gáti T, Tóth G. Two highly oxygenated eudesmanes and 10 lignans from Achillea holosericea. Phytochemistry 2002;59(8):851–856.
2. Al-Hindawi MK, Al-Deen IH, Nabi MH, Ismail MA. Anti-inflammatory activity of some Iraqi plants using intact rats. J Ethnopharmacol 1989;26(2):163-168.
3. Aljancic I, Macura S, Juranic N, Andjelkovic S, Randjelkovic N, Milosavljevic S. Diterpenes from Achillea clyopetala. Phytochemistry 1996;43:169-171.
4. Allgulander C, Bandelow B, Hollander E. WCA recommendations for the long-term treatment of generalized anxiety disorder. CNS Spectr 2003;8(1):53-61.
5. Aoshima H, Takeda K, Okita Y, Hossain SJ, Koda H, Kiso Y. Effects of beer and hop on ionotropic gammaaminobutyric acid receptors. J Agric Food Chem 2006;54:514-2519.
6. Ashton CH. The diagnosis and management of benzodiazepine dependence. Curr Opin Psychiatry 2005;18(3):249-55.
7. Awad R, Levac D, Cybulska P, Merali Z, Trudeau VL, Arnason JT. Effects of traditionally used anxiolytic botanicals on enzymes of the gamma-aminobutyric acid (GABA) system. Can J Physiol Pharmacol 2007;85:933-942.
8. Badahdah KO, El-Orfy HS. Phytochemical constituents of Achillea biebersteinii. Journal of Saudi Chemical Society 2004;8:115-120.
9. Baretta IP, Felizardo RA, Bimbato VF, dos Santos MG, Kassuya CA, Gasparotto Junior A, da Silva CR, de Oliveira SM, Ferreira J, Andreatini R. Anxiolytic-like effects of acute and chronic treatment with Achillea millefolium L. extract. J Ethnopharmacol 2012;140(1):46-54.
10. Baris O, Gulluce M, Cahün F, Zer H, Kili H, Zkan K, Kmen M, Zbek T. Biological activities of the essential oil and methanol extract of Achillea biebersteinii Afan. (Asteraceae). Turkish Journal of Biology 2006;30(2):65-73.
11. Barrero AF, Manzaneda REA, Manzaneda RRA, Arseniyadis S, Guittet E. Achilleol B: a new tricyclic triterpene skeleton from Achillea odorata L. Tetrahedron 1990;46(24):8161-8168.
12. Baytop T. A Dictionary of vernacular names of wild, publication of the Turkish Language Society. Ankara 1997;578:163-238.
13. Baytop T. Therapy with Medicinal Plants in Turkey, Past and Present, Nobel Tıp Kitapevi, Istanbul, Turkey 1999;2nd edition.
14. Bråthen G, Ben-Menachem E, Brodtkorb E, Galvin R, Garcia-Monco JC, Halasz P, Hillbom M, Leone MA, Young AB. EFNS guideline on the diagnosis and management of alcohol-related seizures: report of an EFNS task force. Eur J Neurol 2005;12(8):575-81.
15. Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Sökmen A, Akpulat HA. Antioxidant and

antimicrobial activity of the essential oils and methanol extracts of Achillea millefolium susp. millefolium Afan. (Asteraceae). J Ethnopharmacol 2003;87:215-220.
16. Cheers G. Botanica, Konemann, Koln, Germany 1999.
17. Duley L. Evidence and practice: the magnesium sulphate story. Best Pract Res Clin Obstet Gynaecol 2005;19(1):57-74.
18. Duman H, Achillea L. in Flora of Turkey and the East Aegean Islands, Guner A, Ozhatay N, Ekim T, Baser KHC, Eds., Edinburgh University Press 2000;11:158–159.
19. Esmaeili A, Nematollahi F, Rustaiyan A, Moazami N, Masoudi S, Bamasian S. Volatile constituents of Achillea pachycephala, A. oxyodonta and A. biebersteinii from Iran. Flavour and Fragrance Journal 2006;21(2):253-256.
20. Faught E. Treatment of refractory primary generalized epilepsy. Rev Neurol Dis 2004;1(1):S34-43.
21. Fujita T, Sezik E, Tabata M, Yesilada E, Honda G, Takeda Y, Tanaka T, Takaishi Y. Traditional medicine in Turkey VII. Folk medicine in middle and west Black Sea regions. Economic Botany 1995;49(4):406-422.
22. Grundmann O, Nakajima J, Kamata K, Seo S, Butterweck V. Kaempferol from the leaves of Apocynum venetum possesses anxiolytic activities in the elevated plus maze test in mice. Phytomedicine 2009;16(4):295-302.
23. Herrera-Ruiz M, Roman-Ramos R, Zamilpa A, Tortoriello J, Jimenez-Ferrer JE. Flavonoids from Tilia americana with anxiolytic activity in plus-maze test. J Ethnopharmacol 2008;118:312-317.
24. Honda G, Yesilada E, Tabata M, Sezik E, Fujita T, Takeda Y, Takaishi Y, Tanaka T. Traditional medicine in Turkey VI. Folkmedicine inWest Anatolia: Afyon, Kutahya, Denizli, Mugla, Aydin provinces. J Ethnopharmacol 1996;53(2):75-87.
25. Isojärvi JI, Tokola RA. Benzodiazepines in the treatment of epilepsy in people with intellectual disability. J Intellect Disabil Res 1998;42(1):80–92.
26. Kachi T. Medical treatment of dystonia. Rinsho Shinkeigaku 2001;41(12):1181-2.
27. Kamen L, Henney HR, Runyan JD. A practical overview of tizanidine use for spasticity secondary to multiple sclerosis, stroke, and spinal cord injury. Curr Med Res Opin 2008;24(2):425-39.
28. Kaplan PW. Neurologic aspects of eclampsia. Neurol Clin 2004;22(4):841-61.
29. Karamenderes C, Apaydın S. Antispasmodic effect of Achillea nobilis L. subsp. sipylea (O. Schwarz) Bassler on the rat isolated duodenum. Journal of Ethnopharmacology 2003;84(2-3):175-179.
30. Kusmenoglu S, Baser KHC, Ozek T, Harmandar M, Gokalp X. Constituents of the essential oil of Achillea biebersteinii Afan. Journal of Essential Oil Research 1995;7(5):527–528.
31. Maffei M, Mucciarelli M, Scannerini S. Essential oils from Achillea species of different geographic origin. Biochemical Systematics and Ecology 1994;22(7):679-687.
32. Mañon-Espaillat R, Mandel S. Diagnostic algorithms for neuromuscular diseases. Clin Podiatr Med Surg 1999;16(1):67-79.
33. Marchart E, Kopp B. Capillary electrophoretic separation and quantification of flavone-O- and C-glycosides in Achillea setacea W. et K. Journal of Chromatography B 2003;792:363-368.
34. McIntosh A, Semple D, Smyth R, Burns J, Darjee R. Depressants. Oxford Handbook of Psychiatry (1st Ed.). Oxford University Press 2005; pp:540.
35. Meissner O, Haberlein H. Influence of xanthohumol on the binding behavior of GABA (A) receptors and their lateral mobility at hippocampal neurons. Planta Med 2006;72:656-658.
36. Mezaki T, Hayashi A, Nakase H, Hasegawa K. Therapy of dystonia in Japan. Rinsho Shinkeigaku 2005;45(9):634-42.
37. Mockute D, Judzentiene A. Variability of the essential oils composition of Achillea millefolium ssp. millefolium growing wild in Lithuania. Biochemical Systematics and Ecology 2003;31(9):1033-1045.
38. Molina-Hernandez M, Tellez-Alcantara NP, Diaz MA, Perez Garcia J, Olivera Lopez JI, Jaramillo MT. Anticonflict actions of aqueous extracts of flowers of Achillea millefolium L. vary according to the estrous cycle phases in Wistar rats. Phytother Res 2004;18(11):915-20.
39. Oksuz S, Gumus S, Alpınar K. Sesquiterpenoids and flavonoids of Achillea species. Biochemical Systematics and Ecology 1991;19(5):439.
40. Olkkola KT, Ahonen J. Midazolam and other benzodiazepines. Handb Exp Pharmacol. Handbook of Experimental Pharmacology 2008;182(182):335-60.
41. Page C, Michael C, Sutter M, Walker M, Hoffman BB. Integrated Pharmacology (2nd Ed.). C.V. Mosby 2002;ISBN 978-0-7234-3221-0.
42. Perugi G, Frare F, Toni C. Diagnosis and treatment of agoraphobia with panic disorder. CNS Drugs 2007;21(9):741–64.
43. Prasad K, Al-Roomi K, Krishnan PR, Sequeira R, Prasad K. Anticonvulsant therapy for status epilepticus. Cochrane Database Syst Rev 2005;(4):CD003723.

44. Riss J, Cloyd J, Gates J, Collins S. Benzodiazepines in epilepsy: pharmacology and pharmacokinetics. Acta Neurol Scand 2008;118 (2):69-86.
45. RPSGB (Royal Pharmaceutical Society of Great Britain). British National Formulary (BNF 57). BMJ Group and RPS Publishing 2009; ISBN 978-0-85369-845-6.
46. Schiller H, Forster A, Vonhoff C, Hegger M, Biller A, Winterhoff H. Sedating effects of Humulus lupulus L. extracts. Phytomedicine 2006;13:535-541.
47. Senatore F, Napolitano F, Arnold NA, Bruno M, Herz W. Composition and antimicrobial activity of the essential oil of Achillea falcata L. (Asteraceae). Flavour and Fragrance Journal 2005;20(3):291-294.
48. Sezik E, Yesilada E, Honda G, Takaishi Y, Takeda Y, Tanaka T. Traditional medicine in Turkey X. Folk medicine in Central Anatolia. Journal of Ethnopharmacology 2001;75(2-3):95-115.
49. Skocibusic M, Bezic N, Dunkic V, Radonic A. Antibacterial activity of Achillea clavennae essential oil against respiratory tract pathogens. Fitoterapia 2004;75(7-8):733-736.
50. Tesar GE. High-potency benzodiazepines for short-term management of panic disorder: the U.S. experience. J Clin Psychiatry 1990;51(1):4–10.
51. Unlu M, Daferera D, Donmez E, Polissiou M, Tepe B, Sokmen A. Compositions and the in vitro antimicrobial activities of the essential oils of Achillea setacea and Achillea teretifolia (Compositae). Journal of Ethnopharmacology 2002;83(1-2):117-121.
52. Walker M. Status epilepticus: an evidence based guide. BMJ 2005;331(7518):673-7.
53. Yesilada E, Honda G, Sezik E, Tabata M, Goto K, Ikeshiro Y. Traditional medicine in turkey IV. Folk medicine in the Mediterranean subdivision. Journal of Ethnopharmacology 1993;39(1):31-38.
54. Zanoli P, Rivasi M, Zavatti M, Brusiani F, Baraldi M. New insight in the neuropharmacological activity of Humulus lupulus L. J Ethnopharmacol 2005;102:102-106.
55. Zeilhofer HU, Witschi R, Hösl K. Subtype-selective GABAA receptor mimetics--novel antihyperalgesic agents. J Mol Med 2009;87(5):465-9.

12/22/2012