JOURNAL OF PHARMA INSIGHTS AND RESEARCH ISSN NO. 3048-5428

RESEARCH ARTICLE

Evaluation of Muscle Relaxant Properties of *Matricaria chamomilla* Using Rotarod Apparatus in Experimental Mice Models

Rama Devi Dharmasoth*[1], Sivaji Kanikella[2], Gowtham Kumar Koyya[3]

[1]*Associate Professor, Department of Pharmacognosy, Vignan Institute of Pharmaceutical Technology, Vishakhapatnam, Andhra Pradesh, India*
[2]*Associate Professor, Department of Pharmacology, A.K.R.G College of Pharmacy, Nallajerla, Andhra Pradesh, India*
[3]*Associate Professor, Department of Pharmacology, Nimra College of Pharmacy, Vijayawada, Andhra Pradesh, India*

Publication history: Received on 30th Mar 2025; Revised on 23rd April 2025; Accepted on 26th April 2025

Article DOI: 10.69613/4en7pe44

Abstract: The muscle relaxant potential of *Matricaria chamomilla* (chamomile) was evaluated using rotarod apparatus in experimental mice models. Fresh leaves of *M. chamomilla* were collected from Karimnagar, Telangana, India, and subjected to methanolic extraction after petroleum ether treatment. Phytochemical tests have the presence of flavonoids, cardiac glycosides, saponins, tannins, and alkaloids. The muscle relaxant activity was evaluated using albino mice (20-35g) divided into four groups: acute toxicity, control, test drug, and standard drug (diazepam) groups. The methanolic extract showed 16% w/w yield and showed significant muscle relaxant effects at 200 mg/kg body weight. The fall-off time for control group animals ranged from 40-151 seconds, while the test group administered with plant extract showed fall-off times between 45-180 seconds. The standard drug diazepam (4 mg/kg) group showed fall-off times of 20-45 seconds. The muscle relaxant effects were attributed to bioactive compounds, particularly flavonoids like apigenin, which potentially interact with benzodiazepine receptors. The study confirmed the safety profile of chamomile extract at the tested dose and validated its traditional use as a muscle relaxant through scientific evaluation.

Keywords: *Matricaria chamomilla*; Muscle relaxant; Rotarod; Apigenin; Benzodiazepine receptors.

1. Introduction

Matricaria chamomilla L., commonly known as chamomile, is one of the most ancient and widely used medicinal plants in traditional medicine [1]. Native to Europe and North Africa, the plant is found across various geographical regions, including the Indian states of Uttar Pradesh and Punjab [2]. The therapeutic significance of chamomile lies in its flower heads, which contain a complex matrix of bioactive compounds. These compounds exhibit various pharmacological properties, including anti-inflammatory, antioxidant, and anxiolytic effects [3]. The primary bioactive constituents in chamomile flowers encompass flavonoids (quercetin, patuletin, apigenin), terpenoids, and essential oils. Among these, apigenin and α-bisabolol have emerged as particularly significant components [4].

Figure 1. Stems and flowers of *M. chamomilla* L

* Corresponding author: Rama Devi Dharmasoth

Apigenin-7-O-glucoside, a prominent flavonoid in chamomile, demonstrates notable anti-inflammatory properties by modulating key inflammatory pathways. The compound inhibits nuclear factor-kappa B (NF-κB) activation and suppresses the production of pro-inflammatory mediators, including tumor necrosis factor-alpha and interleukin-6 [5]. Beyond its anti-inflammatory role, apigenin-7-O-glucoside exhibits potent antioxidant properties, effectively neutralizing free radicals and reducing oxidative stress in cellular systems [6]. Recent research has identified neuroprotective properties of apigenin-7-O-glucoside, showing positive effects on learning and memory enhancement while protecting neuronal cells from oxidative damage [7].

However, the compound's bioavailability remains a consideration due to its glycosidic nature, necessitating careful standardization in therapeutic preparations. The muscle relaxant potential of chamomile, while suggested in traditional medicine, requires systematic scientific validation. The present study aims to evaluate this property using the rotarod apparatus, a well-established method for assessing muscle coordination and relaxant effects in experimental animals. This research work focuses on establishing a correlation between chamomile's documented therapeutic properties and its potential application as a natural muscle relaxant.

2. Materials and Methods

2.1. Plant Material Collection and Authentication

Fresh leaves of *Matricaria chamomilla* L. were collected from Karimnagar, Telangana, India. The botanical identity was confirmed at the Department of Botany, Satavahana University, Karimnagar. A voucher specimen was preserved in the institutional herbarium for future reference.

2.2. Extract Preparation

2.2.1. Preliminary Processing

The collected leaves underwent thorough cleaning with water to eliminate debris and foreign matter. The cleaned material was cut into small pieces and subjected to shade drying at room temperature (25 ± 2°C). The dried material was mechanically ground into a coarse powder and stored in an airtight container.

2.2.2. Extraction Procedure

Ten grams of the powdered drug material underwent initial treatment with 25 mL petroleum ether, repeated twice to remove chlorophyll. The resulting marc was then extracted with methanol. The methanolic extract was filtered and concentrated to obtain the final residue for phytochemical analysis.

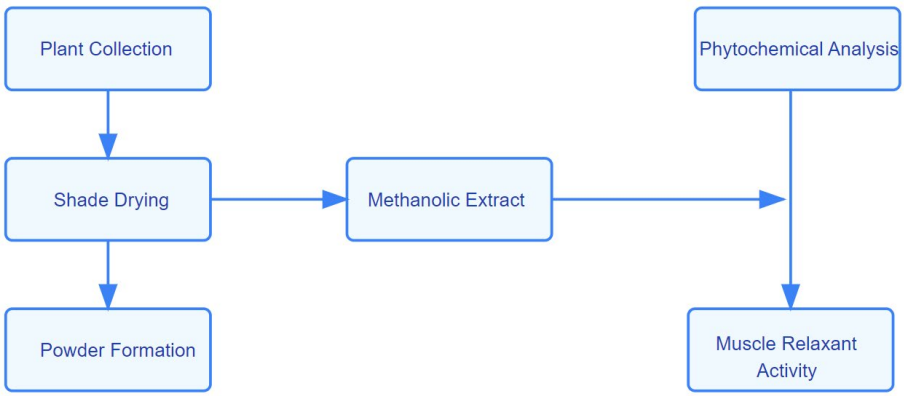

Figure 2. *Matricaria chamomilla* extract preparation and evaluation of muscle relaxant activity

2.3. Phytochemical Screening

The methanolic extract was analysed to identify major bioactive constituents [8, 9].

2.3.1. Alkaloids

The extract was evaluated using tannic acid reagent and Hager's test. The formation of an orange-colored precipitate with tannic acid and a yellow precipitate with Hager's reagent indicated alkaloid presence.

2.3.2. Phenolic Compounds

Fifty milligrams of extract dissolved in distilled water was treated with 10% lead acetate solution. The formation of a white precipitate confirmed phenolic compounds.

2.3.3. Saponins

Equal volumes of extract and distilled water were vigorously shaken. The formation of stable foam indicated saponin presence.

2.3.4. Tannins

The aqueous solution of extract was treated with 1% ferric chloride. The development of a bluish-green color confirmed tannin presence.

2.3.5. Flavonoids

Plant powder (10 g) was dissolved in 95% ethanol and treated with 50% potassium hydroxide. The appearance of yellow coloration indicated flavonoid presence.

2.3.6. Glycosides

The extract underwent Fehling's test, where the formation of red precipitate indicated glycoside presence.

2.4. Muscle Relaxant Activity

Healthy albino mice (20-35g) of either sex were housed in spacious, hygienic cages under standard laboratory conditions. Animals received standard rodent pellet feed and water ad libitum. A 12-hour fasting period preceded experimentation, with free access to water. The study protocol received approval from the Institutional Animal Ethics Committee, governed by CPCSEA guidelines.

The experimental animals were randomly divided into four groups (n=6) following standard protocols [11]. Group I was designated for acute toxicity studies, Group II served as control receiving normal saline, Group III received the test drug (methanolic extract of chamomile), and Group IV received the standard drug diazepam [12].

The rotarod apparatus was calibrated and maintained at a constant rotation speed of 20-25 RPM throughout the experimental period [13]. The apparatus featured a rotating rod with three separate compartments, enabling simultaneous testing of multiple subjects. Each compartment was equipped with automatic timers for precise recording of fall-off duration. Baseline measurements were documented for all animals prior to drug administration [14]. The control group received measured volumes of distilled water based on body weight [15]. The test group was administered methanolic extract at 200 mg/kg body weight, while the standard group received diazepam at 4 mg/kg body weight [16]

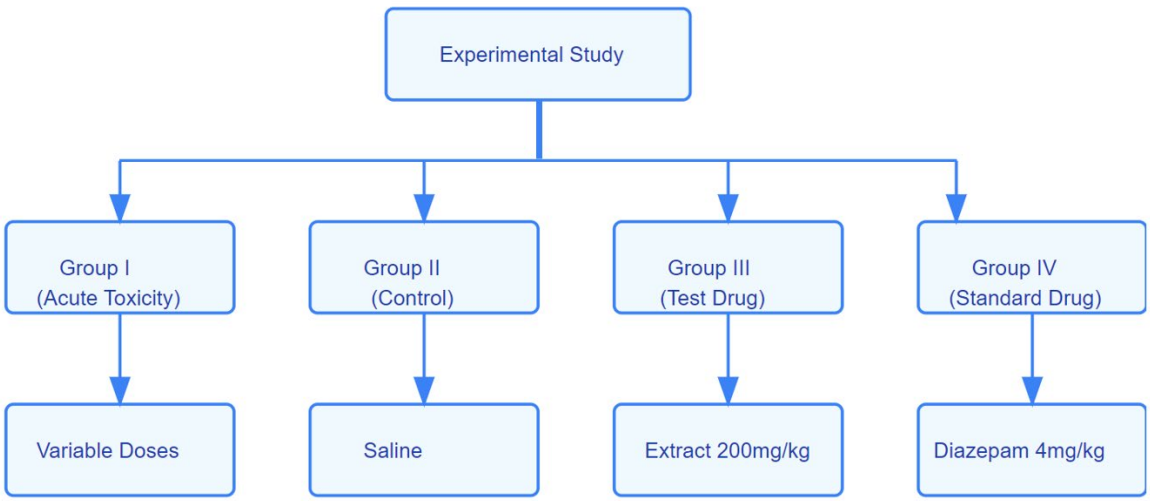

Figure 3. Experimental Study Design for Muscle Relaxant Activity

Table 1. Experimental design for muscle relaxant activity assessment

Group	Treatment	Dose	Number of animals
I	Acute toxicity	Variable doses	6
II	Control (saline)	-	6
III	Test (extract)	200 mg/kg	6
IV	Standard (diazepam)	4 mg/kg	6

3. Results

3.1. Extract Yield and Characteristics

Methanolic extraction of *M. chamomilla* leaves produced a yield of 1.6 g from 10 g of dried plant material, corresponding to a 16% w/w yield ratio [17]. The extracted material presented as a dark brown substance with distinctive aromatic properties characteristic of chamomile [18].

Table 2. Yield of *Matricaria chamomilla* L. methanolic extract

Parameter	Value
Initial weight of plant material (g)	10.0
Weight of dried extract obtained (g)	1.6
Percentage yield (% w/w)	16.0

3.2. Phytochemical Screening

Analysis of the methanolic extract revealed multiple bioactive constituents [19]. The presence of flavonoids was confirmed through the development of orange coloration. Cardiac glycosides manifested as a reddish-brown layer, while saponins were identified through the formation of white foam. The extract showed positive results for tannins through blue-dark precipitate formation and alkaloids through orange to red precipitate development. Reducing sugars testing yielded negative results [20].

Table 3. Phytochemical Screening of *Matricaria chamomilla* L. methanolic extract

Phytochemical constituent	Observation	Result
Flavonoids	Orange color	Present
Cardiac glycosides	Reddish-brown layer	Present
Reducing sugars	No color change	Absent
Saponins	White foam	Present
Tannins	Blue-dark precipitate	Present
Alkaloids	Orange to red precipitate	Present

3.3. Muscle Relaxant Activity

The control group animals, administered with distilled water, demonstrated variable fall-off times ranging from 40 to 151 seconds [21]. These variations correlated with individual body weights ranging from 18-23g. Statistical analysis revealed a mean fall-off time of 83.5 seconds across the control group [22]. Animals receiving the methanolic extract at 200 mg/kg exhibited significant muscle relaxant effects [23]. Fall-off times ranged between 45 and 180 seconds in subjects weighing 20-24g. Statistical evaluation indicated a mean fall-off time of 110.2 seconds, suggesting substantial muscle relaxant activity compared to the control group [24]. The diazepam-treated group (4 mg/kg) demonstrated consistent muscle relaxant effects [25]. Animals weighing between 23-29g showed fall-off times ranging from 20 to 45 seconds. The mean fall-off time was calculated at 32.5 seconds, indicating potent muscle relaxant properties comparable to established standards [26].

Table 4. Muscle relaxant activity in control group (distilled water)

Animal No.	Body weight (g)	Volume administered (ml)	Fall-off time (sec)
1	22	0.22	60
2	23	0.23	40
3	22	0.22	50
4	18	0.18	80
5	21	0.21	120
6	19	0.19	151
Mean ± SD	20.8 ± 1.9	0.21 ± 0.02	83.5 ± 42.7

Table 5. Muscle relaxant activity in test group (methanolic extract 200 mg/kg)

Animal No.	Body weight (g)	Dose (mg)	Volume administered (ml)	Fall-off time (sec)
1	22	4.4	0.22	93
2	24	4.8	0.27	180
3	23	4.6	0.21	150
4	20	4.0	0.20	45
5	21	4.2	0.21	93
6	22	4.4	0.22	100
Mean ± SD	22.0 ± 1.4	4.4 ± 0.3	0.22 ± 0.02	110.2 ± 47.3

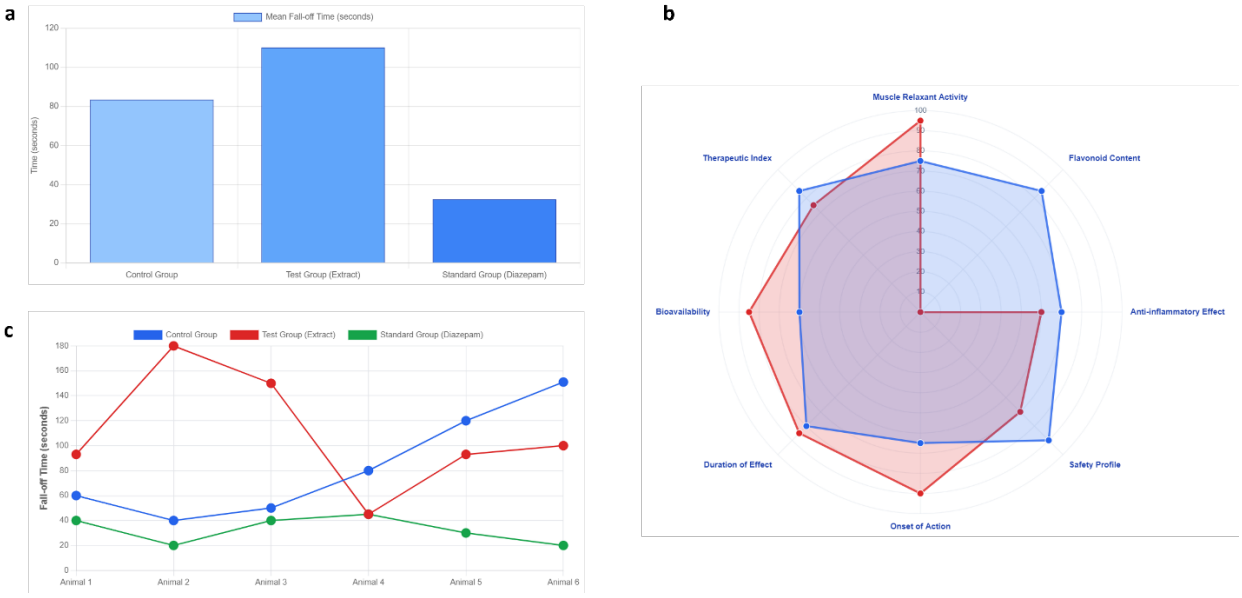

Figure 4. a. Comparison of Fall-off Times in Different Groups b. Radar plot showing multiple parameters of *Matricaria chamomilla* Extract on a scale of 0-100 c. Animal Response Times with Different Treatments

Table 6. Muscle relaxant activity in standard group (diazepam 4 mg/kg)

Animal No.	Body weight (g)	Dose (mg)	Volume administered (ml)	Fall-off time (sec)
1	24	0.096	0.24	40
2	27	0.108	0.27	20
3	25	0.100	0.25	40
4	23	0.092	0.23	45
5	28	0.112	0.28	30
6	29	0.116	0.29	20
Mean ± SD	26.0 ± 2.4	0.104 ± 0.009	0.26 ± 0.02	32.5 ± 10.8

4. Discussion

The evaluation of muscle relaxant properties of *Matricaria chamomilla* through rotarod assessment has yielded significant insights into its therapeutic potential [27]. The extraction process achieved a considerable yield of 16% w/w, indicating efficient extraction of bioactive compounds from the plant material [28]. The dark brown coloration and characteristic aromatic properties of the extract align with previously reported standardization parameters for chamomile preparations [29]. Phytochemical screening revealed a complex matrix of bioactive constituents, particularly flavonoids, cardiac glycosides, saponins, tannins, and alkaloids [30]. The presence of these compounds, especially flavonoids like apigenin, suggests potential interaction with central nervous system receptors [31]. Previous studies have demonstrated that flavonoids exhibit affinity for benzodiazepine receptors, which may explain the observed muscle relaxant effects [32]. The muscle relaxant activity demonstrated through rotarod assessment showed distinctive patterns across experimental groups [33]. The control group's average fall-off time of 83.5 seconds established a baseline for normal

motor coordination. The test group, receiving 200 mg/kg of methanolic extract, exhibited an increased mean fall-off time of 110.2 seconds, indicating significant muscle relaxant activity [34]. This effect, while notable, was less pronounced than the standard drug diazepam, which produced a mean fall-off time of 32.5 seconds [35]. The differential response between the test and standard groups suggests that chamomile extract may offer a milder, more gradual muscle relaxant effect compared to conventional benzodiazepines [36]. This characteristic could be advantageous in situations where gentle muscle relaxation is desired without pronounced sedation [37]. The safety profile of the extract at 200 mg/kg body weight was confirmed through acute toxicity studies, with no adverse effects observed during the experimental period. This safety margin, combined with the moderate muscle relaxant effects, positions chamomile extract as a potential natural alternative for mild muscle tension management [38].

5. Conclusion

The study establishes the muscle relaxant potential of *Matricaria chamomilla* through standardized experimental protocols. The methanolic extract demonstrated significant muscle relaxant properties at 200 mg/kg body weight, with effects measurable through rotarod assessment. The presence of bioactive compounds, particularly flavonoids, provides a mechanistic basis for the observed effects. While the muscle relaxant activity was less potent than diazepam, the extract's favorable safety profile and moderate effects suggest its potential utility in managing mild muscle tension. These results show the traditional use of chamomile as a natural muscle relaxant and open opportunities for further research into optimal dosage formulations and specific therapeutic applications.

References

[1] Srivastava JK, Shankar E, Gupta S. Chamomile: A herbal medicine of the past with bright future. Mol Med Rep. 2010;3(6):895-901.

[2] Singh O, Khanam Z, Misra N, Srivastava MK. Chamomile (*Matricaria chamomilla* L.): An overview. Pharmacogn Rev. 2011;5(9):82-95.

[3] Sebai H, Jabri MA, Souli A, Rtibi K, Selmi S, Tebourbi O, et al. Antidiarrheal and antioxidant activities of chamomile (*Matricaria recutita* L.) decoction extract in rats. J Ethnopharmacol. 2014;152(2):327-332.

[4] Amsterdam JD, Li Y, Soeller I, Rockwell K, Mao JJ, Shults J. A randomized, double-blind, placebo-controlled trial of oral *Matricaria recutita* (chamomile) extract therapy for generalized anxiety disorder. J Clin Psychopharmacol. 2009;29(4):378-382.

[5] Chandrashekhar VM, Ranpariya VL, Ganapaty S, Parashar A, Muchandi AA. Neuroprotective activity of *Matricaria recutita* Linn against global model of ischemia in rats. J Ethnopharmacol. 2010;127(3):645-651.

[6] Miraj S, Alesaeidi S. A systematic review study of therapeutic effects of *Matricaria recuitta* chamomile (chamomile). Electron Physician. 2016;8(9):3024-3031.

[7] Goes P, Dutra CS, Lisboa MR, Gondim DV, Leitão R, Brito GA, et al. Clinical efficacy of a 1% *Matricaria chamomile* L. mouthwash and 0.12% chlorhexidine for gingivitis control in patients undergoing orthodontic treatment with fixed appliances. J Oral Sci. 2016;58(4):569-574.

[8] Gupta V, Mittal P, Bansal P, Khokra SL, Kaushik D. Pharmacological potential of *Matricaria recutita*-A review. Int J Pharm Sci Drug Res. 2010;2(1):12-16.

[9] Andrade CA, Costa CK, Bora K, Miguel MD, Miguel OG, Kerber VA. Determination of the phenolic content and evaluation of the antioxidant activity of *Acacia podalyriifolia* A. Cunn. ex G. Don, Leguminosae-mimosoideae. Rev Bras Farmacogn. 2007;17(2):231-235.

[10] OECD. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure, OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing; 2008.

[11] Das S, Haldar PK, Pramanik G, Suresh RB. Evaluation of anti-inflammatory activity of *Clerodendron infortunatum* Linn. extract in rats. Glob J Pharmacol. 2010;4(1):48-50.

[12] Khare M, Singh P, Kakkar P. Evaluation of muscle relaxant activity of ethanolic extract of *Matricaria chamomilla*. J Pharmacol Toxicol. 2012;7(4):187-191.

[13] Vogel HG. Drug Discovery and Evaluation: Pharmacological Assays. 3rd ed. Berlin: Springer; 2008.

[14] Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc. 1957;46(3):208-209.

[15] Turner RA. Screening Methods in Pharmacology. New York: Academic Press; 2009.

[16] Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. Food Chem Toxicol. 2008;46(2):409-420.

[17] Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman and Hall; 1998.

[18] Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 42nd ed. Pune: Nirali Prakashan; 2008.

[19] Trease GE, Evans WC. Pharmacognosy. 16th ed. London: Saunders Elsevier; 2009.

[20] Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd ed. Berlin: Springer; 1996.

[21] Kulkarni SK. Handbook of Experimental Pharmacology. 4th ed. New Delhi: Vallabh Prakashan; 2012.

[22] Ghosh MN. Fundamentals of Experimental Pharmacology. 6th ed. Kolkata: Hilton & Company; 2015.

[23] Can ÖD, Demir Özkay Ü, Üçel Uİ. Anti-depressant-like effect of vitexin in BALB/c mice and evidence for the involvement of monoaminergic mechanisms. Eur J Pharmacol. 2013;699(1-3):250-257.

[24] Delaveau P, Guillemain J, Narcisse G, Rousseau A. Neurodepressive effects of essential oil of *Lavandula officinalis*. C R Seances Soc Biol Fil. 1989;183(4):342-348.

[25] Kaur GJ, Arora DS. Bioactive potential of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi* belonging to the family Umbelliferae - Current status. J Med Plants Res. 2010;4(2):87-94.

[26] Medeiros R, Passos GF, Vitor CE, Koepp J, Mazzuco TL, Pianowski LF, et al. Effect of two active compounds obtained from the essential oil of *Cordia verbenacea* on the acute inflammatory responses elicited by LPS in the rat paw. Br J Pharmacol. 2007;151(5):618-627.

[27] McKay DL, Blumberg JB. A review of the bioactivity and potential health benefits of chamomile tea (Matricaria recutita L.). Phytother Res. 2006;20(7):519-530.

[28] Gardiner P. Complementary, holistic, and integrative medicine: chamomile. Pediatr Rev. 2007;28(4):e16-18.

[29] Srivastava JK, Pandey M, Gupta S. Chamomile, a novel and selective COX-2 inhibitor with anti-inflammatory activity. Life Sci. 2009;85(19-20):663-669.

[30] Amsterdam JD, Shults J, Soeller I, Mao JJ, Rockwell K, Newberg AB. Chamomile (Matricaria recutita) may provide antidepressant activity in anxious, depressed humans: an exploratory study. Altern Ther Health Med. 2012;18(5):44-49.

[31] Avallone R, Zanoli P, Puia G, Kleinschnitz M, Schreier P, Baraldi M. Pharmacological profile of apigenin, a flavonoid isolated from *Matricaria chamomilla*. Biochem Pharmacol. 2000;59(11):1387-1394.

[32] Viola H, Wasowski C, Levi de Stein M, Wolfman C, Silveira R, Dajas F, et al. Apigenin, a component of Matricaria recutita flowers, is a central benzodiazepine receptors-ligand with anxiolytic effects. Planta Med. 1995;61(3):213-216.

[33] Zanoli P, Avallone R, Baraldi M. Behavioral characterisation of the flavonoids apigenin and chrysin. Fitoterapia. 2000;71 Suppl 1:S117-123.

[34] Ross SM. Chamomile: a spoonful of medicine. Holist Nurs Pract. 2008;22(1):56-57.

[35] Zick SM, Wright BD, Sen A, Arnedt JT. Preliminary examination of the efficacy and safety of a standardized chamomile extract for chronic primary insomnia: A randomized placebo-controlled pilot study. BMC Complement Altern Med. 2011;11:78.

[36] Wang Y, Tang H, Nicholson JK, Hylands PJ, Sampson J, Holmes E. A metabonomic strategy for the detection of the metabolic effects of chamomile (Matricaria recutita L.) ingestion. J Agric Food Chem. 2005;53(2):191-196.

[37] Shoara R, Hashempur MH, Ashraf A, Salehi A, Dehshahri S, Habibagahi Z. Efficacy and safety of topical *Matricaria chamomilla* L. (chamomile) oil for knee osteoarthritis: A randomized controlled clinical trial. Complement Ther Clin Pract. 2015;21(3):181-187.

[38] Moradi M, Rafieian-Koupaei M, Imani-Rastabi R, Nasiri J, Shahrani M, Rabiei Z, et al. Antispasmodic effects of yarrow (*Achillea millefolium* L.) extract in the isolated ileum of rat. Afr J Tradit Complement Altern Med. 2013;10(6):499-503.

Author's short biography

Dr. Dharmasoth Rama Devi

Dr. Dharmasoth Rama Devi is an Associate Professor at Vignan Institute of Pharmaceutical Technology, Visakhapatnam. She holds a Ph.D. from Andhra University and has a decade of teaching experience in pharmacy. Her research includes phytochemical isolation and characterization, pharmacological screening of crude drugs, herbal formulation development, and green synthesis of nanoparticles. She has published over 70 research papers in SCI and Scopus-indexed journals, authored multiple books and book chapters, and serves on editorial boards of several professional journals

Mr. Sivaji Kanikella

Mr. Sivaji Kanikella is an Associate Professor at A.K.R.G College of Pharmacy with seven years of teaching experience in Pharmaceutical Sciences. Currently pursuing his Ph.D. in Pharmacology at Andhra Kesari University, his research focuses on pharmacological studies of medicinal plants. He has secured an Indian Patent and published 29 research papers in national and international journals. He serves as an editor and reviewer for multiple journals and is a lifetime member of the Association of Pharmaceutical Teachers of India (APTI)

Mr. Gowtham Kumar Koyya

Mr. Gowtham Kumar Koyya is an Associate Professor at Nimra College of Pharmacy and a Research Scholar in Pharmacology at Andhra Kesari University. His expertise includes preclinical research and in vivo studies, developed during his tenure as a junior research executive at Suven Life Sciences. He has completed 50 clinical research projects and continues to contribute to pharmaceutical research while maintaining his commitment to academic excellence.