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ORIGINAL RESEARCH

Hypnotic effect of the essential oil from the leaves of *Myrtus communis* on mice

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Muluken Walle Birhanie[1]
Bizuayehu Walle[1]
Kidist Rebba[2]

[1]Department of Physiology, [2]Department of Nursing, College of Medicine and Health Sciences, Bahir Dar University, Bahir Dar, Ethiopia

Background: *Myrtus communis* has been suggested as a sleep aid in unconventional medicine. Moreover, previous studies have also indicated its sedative- and hypnotic-like activity. In this study, the hypnotic effect of *M. communis* was investigated.

Methods: Essential oil (EO) of *M. communis* (600, 800, and 1,000 mg/kg) was given orally to Swiss albino mice of both sex, and the hypnotic effect was evaluated. In addition, the EO of *M. communis* (500, 600, 800, and 1,000 mg/kg) was administered orally to Swiss albino mice of both sex 60 minutes prior to pentobarbital injection (50 mg/kg). Latency to sleep and sleep duration were recorded. The effect of the EO on motor coordination and muscle relaxation was evaluated using chimney and traction tests, 60 and 90 minutes after administration of the respective doses of the EO, respectively.

Results: There was no induction of hypnosis as the presence of the righting reflex was intact. However the EO prolonged pentobarbital-induced sleeping time and there was also 50% negative response on the chimney and traction test in a dose dependent manner.

Conclusion: The EO of *M. communis* did not produce a hypnotic effect, but it potentiated a hypnotic effect with significant central nervous system depressant activity.

Keywords: *Myrtus communis*, hypnotic, motor coordination, muscle relaxation, essential oil

Background

Hypnotics initiate sleep, which are effective for patients who are unable to achieve or maintain sleep. However, drugs of this kind are reserved for short-term use due to the fact that their long-term use will lead to physical and physiological dependence.[1] Although a multitude of pharmaceutical agents are available for the treatment of mental disorders, patients eventually cannot tolerate the side effects, do not respond adequately, or lose their response.

In comparison, many therapeutic herbs have far fewer side effects. They can provide an alternative treatment or be used to enhance the effect of prescription medications. The World Health Organization estimates that four billion people, which is 80% of the world's population, presently use herbal medicines for some aspect of primary health care in conjunction with conventional medicines.[2]

In the present study, the hypnotic effect of essential oil (EO) from the leaves of a plant known as myrtle (*Myrtus communis*) was studied. In Ethiopia, it is commonly called "addus" in Amharic.[3–7] It is grown in gardens of Addis Ababa. The plant is much more widespread than the herbarium collections indicate. This species is used for flavoring butter and a perfume is extracted that women mix with butter to make a fragrant ointment for their hair.[4–6]

Correspondence: Muluken Walle Birhanie
Department of Physiology, College of Medicine and Health Sciences, Bahir Dar University, P.O. box 16928, Bahir Dar, Ethiopia
Email ninnawal@yahoo.com

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The most important constituents of myrtle oil (up to 0.8% in the leaves) are myrtenol, myrtenol acetate, limonene (23%), linalool (20%), pinene (14%), and cineol (11%); *p*-cymene, geraniol, nerol, phenylpropanoid, and methyleugenol are the other constituents. There is considerable variability in the composition of oil from different locations.[8–11]

As a medicinal plant myrtle is better known for its antihyperglycemic,[12] antiseptic, and anti-inflammatory activities.[13]

In Ethiopia, it is used for the treatment of dandruff, tinea capitis which is called "buha ras", and also as an antipyretic substance and a sedative.[14] The leaves and fruits of the plant are also known to have vulnerary, cough-suppressing, sedative, and digestant effects.[4] Different parts of the plant find various uses in food and cosmetic industries.[15]

Traditionally, the EO from the leaves of *M. communis* is known to have antiseptic, bactericidal, anti-inflammatory, antihyperglycemic, antioxidant, antidepressant, and anti-Alzheimer- and anti-Parkinson-like effects.[13,14,16–18] It is known to improve libido and is effective against digestive problems, bronchial congestion, dry coughs, anxiety, and epilepsy.

In one of our previous studies, the EO from the leaves of this plant was shown to have a muscle relaxing effect and also potentiate the hypnotic effect of pentobarbital.[7]

Studies were conducted on the antihyperglycemic, antiseptic, anti-inflammatory, and sedative- and hypnotic-like effects of the EO of *M. communis*; however not on the antidepressant and hypnotic effects on the central nervous system (CNS).[7,14,19,20] Therefore, in this study, the use of EO from the leaves of *M. communis* as a hypnotic was assessed.[13,21]

Methods

Chemicals

Chemicals and solvents used included diazepam, pentobarbital, and 5% Tween 80 in distilled water (vehicle of the extract).

Plant material preparation

Fresh leaves of *M. communis* were purchased from Merkatto, a big market in Addis Ababa where fresh leaves of different plants are sold. The leaves were separated from the branches and stored in a refrigerator from 3–8˚c' until distillation. Their botanical identity was confirmed by the National Herbarium, Department of Biology, Faculty of Life Sciences, Addis Ababa University (AAU). The leaves were deposited in the herbarium with voucher number 01.

Preparation of experimental animals

Male and female Swiss albino mice weighing 16–40 g were obtained from the animal house of Tikur Anbessa Hospital, Faculty of Life Sciences, AAU, and Ethiopian Health and Nutrition Research Institute (EHNRI). They were housed in a cage at 21°C±1°C under a 12-hour light/dark cycle in the animal house of the Department of Pharmacology, School of Medicine, AAU. Tap water and standard food pellets were offered ad libitum as per Bahir Dar University animal care guidelines. Drug administration and testing of mice were performed in the Core Lab of the School of Medicine, AAU. Ethical approval was provided by Bahir Dar University Institutional Ethical Committee.

EO extraction

Hydrodistillation was done in the Drug Formulation Laboratory, EHNRI. Clevenger-type apparatus with a round bottom flask of 5 L capacity was used. Three thousand and five hundred grams of the fresh leaves of *M. communis* was hydrodistilled in the Clevenger-type apparatus.

Myrtle leaves (500 g) were hydrodistilled using 2.5 L of tap water for 3.5 hours at 30°C–50°C, after the water containing the fresh leaves started to boil. The oil was then stored in amber glass bottles in a freezer until used.[9,17] An amount of 6.7 mL of oil was obtained, and the percentage yield of oil from the fresh leaves was 0.17%.

Grouping and dosing of animals

Four experimental models were performed in this study to evaluate the hypnotic effect; evaluation of the hypnotic effect, pentobarbital-induced sleeping time (PIST; hereafter PIST test), chimney test, and traction test.

For evaluation of the hypnotic effect, the mice were divided into five experimental groups of six each. Group I served as negative control (Tween 80 [5%, v/v] in distilled water). Group II served as positive control (diazepam [3 mg/kg] suspended in the vehicle). Groups III–V served as test groups and were given the EO orally (po) at doses of 600, 800, and 1,000 mg/kg, respectively.

For the PIST test, the mice were divided into six (I–VI) experimental groups of six each. Group I served as a negative control and was given a vehicle, Tween 80 (5%, v/v) in distilled water. Group II served as a positive control and was given a standard drug, diazepam (3 mg/kg) suspended in the vehicle. Groups III–VI served as test groups and were given the EO po at doses of 500, 600, 800, and 1,000 mg/kg, respectively. Following oral administration, pentobarbital, 50 mg/kg, was administered intraperitoneally to all the groups, and the potentiating effect of EO on sleep induced by pentobarbital was recorded. This was done by recording the

latency to sleep and duration of sleep by taking loss and onset of righting reflex as a behavioral marker, respectively. Then, latency time to sleep, which was the time from administration of the standard pentobarbital, 50 mg/kg, to the loss of righting reflex, was recorded. And the time from offset to the onset of righting reflex was recorded as the duration of sleep. If there was any doubt as to the reappearance of the righting reflex, the mouse was placed gently on its back again, and the righting reflex within 1 minute was considered as the end point. Mean values of duration of anesthesia (defined as the time from loss of righting reflex up to the time of regaining of the reflex in minutes) and pentobarbital were recorded in the control and experimental groups. The percent change in the duration of anesthesia was calculated in the experimental groups compared to those of the controls. This experiment was repeated three times. All administrations were oral by gavage and the volume administered was 0.5 mL.

Similarly, in the chimney and traction test, the mice were divided into six (I–VI) experimental groups of six each and were dosed po with the respective doses mentioned earlier, except in Group II where 1 mg/kg of diazepam was used as a reference standard.

Hypnotic activity

The mice were divided into five groups (n=6);[23,25] the control group was treated with vehicle, standard group with pentobarbital (50 mg/kg), and the remaining groups with EO (600, 800, and 1,000 mg/kg po). Each animal was observed for onset and duration of sleep.

The duration of sleep or hypnosis was measured on the basis of the loss and regain of righting reflux. The experiment was performed only between 8 am and 6 pm.

Potentiation of PIST

This was the main test used to assess the potentiating effect of substances that prolonged sleep induced by hypnotics such as pentobarbital. The body temperature of the mice was maintained at 37°C using a lamp. The time from the administration of pentobarbital to loss of righting reflex was recorded to measure the latency to sleep. Then, the mice were observed to stay on their backs on the pad, and the time of regain of the righting reflex was recorded. The time from loss to the regain of the righting reflex was considered as the duration of sleep. The experiment was performed only between 8 am and 6 pm.[23,25]

Chimney test

Male mice weighing between 14 and 31 g were used in six groups containing six mice each per dose. Pyrex glass cylinders of 30 cm length and different diameters were used depending on the size of the mice. Initially, the tube was held in a horizontal position. At the end of the tube, near the mark, a mouse was introduced with its head forward. When the mouse reaches the other end of the tube, toward which it was pushed if necessary with a rod, the tube was turned to a vertical position. Immediately, the mouse was observed to climb backward performing coordinated movements and reach the mark within 30 seconds. The time required by the mouse to climb backward to reach the top of the cylinder was recorded. Only mice which were able to perform this activity and reach the top of the vertically positioned tube within 30 seconds were used in this experiment. Each mouse was tested on the chimney 90 minutes after administration of the dose. To measure the effect of the drug on motor coordination (muscle relaxant activity), the percentage of animals that failed to climb backward was compared between test and control groups.

Traction test

Male mice with an average weight of 22–32 g were used. In a preliminary experiment, the mice were tested for their normal reactivity. The mice were exposed to a horizontal thin metallic wire suspended ~30 cm in the air, which they immediately grasped with the forepaws. The mice were released to hang on with their forelimbs. A normal mouse was able to catch the wire with their hind limbs and go to the other end within 5 seconds. Only mice that fulfilled this criterion were included in the experiment. One hour after dosing, each mouse was subjected to the traction test. All mice unable to touch the wire with their hind limbs within 5 seconds or those that fell from the wire were considered to be impaired. In addition, the mice were observed for their behavior in the cages.

Statistical analysis

For evaluation of the hypnotic and potentiating effects on PIST, results obtained from behavioral tests were expressed as mean ± standard error of mean and compared with the corresponding control group by applying analysis of variance followed by Dunnet's *t*-test.[22,23]

For the chimney test, the ED_{50} value (the dose at which 50% of the animals fail to climb backward to reach the top of the tube within 30 seconds, with 95% confidence limits) was estimated.

For the traction test, the percentage of animals losing the catching reflex was calculated. By using different doses, the ED_{50} value was calculated.[22]

The results indicated in all the experiments were analyzed using Microsoft Excel 2003 and 2007 software.

Results

Hypnotic effect of the EO

In both the experimental groups (Groups III–V) and negative control group (Group I), none of the mice were observed to show loss of righting reflex, while there was loss of righting reflex in the positive control (Group II).

Effect of the EO on PIST

As revealed in Figure 1A and B, based on the recorded time of latency to sleep and sleep duration, mice in the experimental groups showed reduced latency and increased duration of sleep induced by pentobarbital in a dose-dependent manner ($P<0.01$ between the groups). The same observation was noted in the positive control, diazepam, in Group II compared with the positive control in Group I ($P<0.001$).

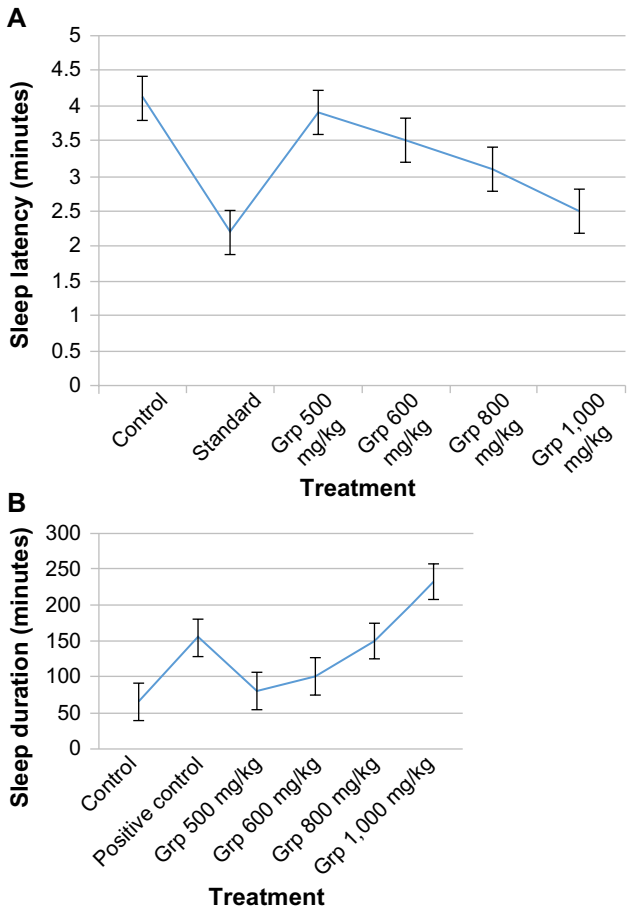

Figure 1 Effect of EO from leaves of *Myrtus communis*.

Notes: (**A**) Effect of EO from leaves of *Myrtus communis* on sleep latency in PIST test. (**B**) Effect of the EO from leaves of *M. communis* on sleep duration in PIST test. The doses of EO used were 500, 600, 800, and 1,000 mg/kg (po). The standard used was diazepam (3 mg/kg, po). n=6 in each group. Values represent mean ± SEM. $P<0.05$ between the test groups and control, and $P<0.01$ between standard and control groups (one-way ANOVA followed by Dunnet's test). Grp represents group administered with the corresponding doses (mg/kg).

Abbreviations: EO, essential oil; PIST, pentobarbital-induced sleeping time; po, orally; SEM, standard error of the mean; ANOVA, analysis of variance; Std, standard.

The effect of different doses of the EO from the leaves of *M. communis* on the latency to sleep observed during the PIST test is summarized in Figure 1A. Mice treated with 500, 600, 800, and 1,000 mg/kg of EO of *M. communis* were observed to have reduced latency to sleep induced by pentobarbital ($P<0.01$) in a dose-dependent manner (3.87, 3.52, 3.14, and 2.45 minutes, respectively) than the control (4.14 minutes), though greater than that induced by the standard (2.16 minutes).

The effect of various doses of the EO from the leaves of *M. communis* on sleep duration observed during the PIST test is summarized in Figure 1B. Mice treated with 500, 600, 800, and 1,000 mg/kg of EO of *M. communis* were observed to have increased duration of sleep induced by pentobarbital ($P<0.01$) in a dose-dependent manner (79.2, 100.2, 150.6, and 233.9 minutes, respectively) than the control (66.2 minutes) and comparable to that induced by the standard (154.5 minutes).

Effect of the EO observed during chimney test

The number of animals that showed a negative result in the chimney test following the administration of 500, 600, 800, and 1,000 mg/kg of EO increased in a dose-dependent manner (mean values of 1, 1.5, 3, and 5.5, respectively) than the control (zero) ($P<0.01$). Moreover, the inhibition of motor coordination was significant at 600, 800, and 1,000 mg/kg ($P<0.05$). The standard, diazepam, inhibited the motor coordination of mice (six) compared with the control (zero) ($P<0.001$). And the effect of diazepam 1 mg/kg was comparable with 1,000 mg/kg of the EO. Since all the mice treated with diazepam showed negative response in the chimney test, the response was taken as 100%, and the percentage of animals that could not climb backward in the Pyrex glass tube after 30 seconds following the administration of the different doses is summarized in Figure 2. Moreover, when the general locomotor activity of mice was observed within their cages after treatment with EO for the chimney test, a decrease in alertness was noted.

Effect of the EO observed during traction test

The number of animals that showed negative result in the traction test following the administration of 500, 600, 800, and 1,000 mg/kg increased in a dose-dependent manner (mean values of 1, 1.5, 3, and 5.5, respectively) than the con-

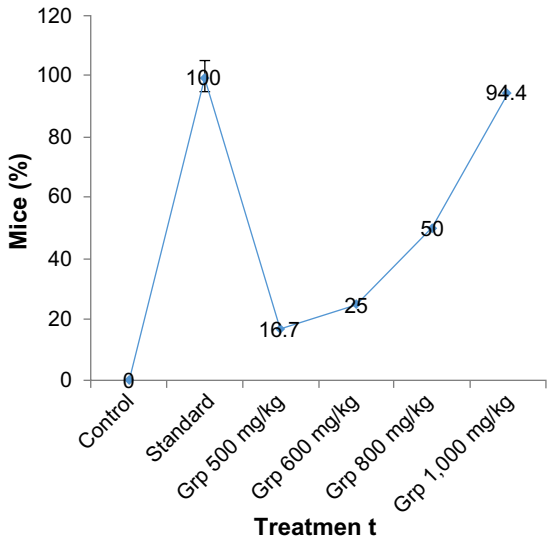

Figure 2 Effect of the EO on the negative response in chimney test.
Notes: Standard group was administered diazepam (3 mg/kg). Values represent percentage of mice showing negative effects in the tests ± SEM. n=6. $P<0.01$ between Groups III–VI and Group I, and $P<0.01$ between Group I and Group II. (Control used were administered 0.4 mL of 5% Tween 80, and standard were administered 1 mg/kg of diazepam. Values represent percentages of mice (n=6) showing negative effects in the chimney tests 1 hour after treatment with the vehicle and EO of varying doses, and 30 minutes after treatment with the standard, diazepam [1 mg/kg, po]). Grp represents group administered with the corresponding doses (mg/kg).
Abbreviations: EO, essential oil; SEM, standard error of the mean; po, orally.

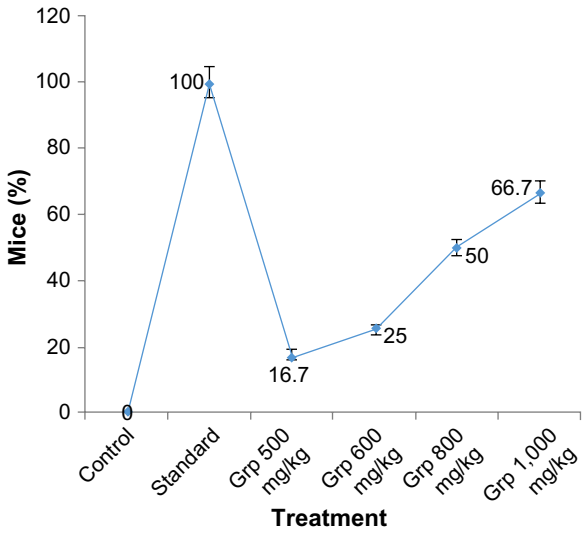

Figure 3 Effect of the EO on the negative response in traction test.
Notes: Standard group was administered diazepam (3 mg/kg). Values represent mean percentage of mice showing negative effects in the tests ± SEM. $P<0.05$ between Groups III–VI and Group I, and $P<0.01$ between Group I and Group II. (n=6. Values represent percentages of mice (n=6) showing negative effects in the traction tests 1 hour after treatment with the vehicle and EO of varying doses, and 30 minutes after treatment with diazepam. $P<0.05$ between the test groups and control, and $P<0.01$ between diazepam and control.) Grp represents group administered with the corresponding doses (mg/kg).
Abbreviations: EO, essential oil; SEM, standard error of the mean.

trol (zero) ($P<0.01$). Moreover, the negative result found in the traction test was significant at 600, 800, and 1,000 mg/kg ($P<0.05$) compared to the control. The standard, diazepam, had a negative effect on the mice during the traction test (six) than the control (zero) ($P<0.001$). Since all mice treated with diazepam showed negative response in the traction test, the response was taken as 100%, and the percentage of mice that could not place at least one hind foot on the wire within 5 seconds following the administration of the different doses is summarized in Figure 3.

Discussion

Despite the widely popular use of *M. communis* for various purposes, there is lack of scientific reports about evaluation of its pharmacological effects as a sedative and hypnotic. In this work, it was confirmed that administration of different doses of the EO of *M. communis* in mice potentiated hypnotic effects induced by pentobarbital in a dose-dependent manner; a similar finding was observed in our previous study.[7]

The interval between loss and recovery of righting reflex was used as an index of hypnotic effect.[22] However, we evaluated the hypnotic effect beyond the model indicated by Vogel, where PIST was used to assess the potentiating effect on hypnosis induced by pentobarbital. What is new about this experiment is that it confirms that the EO cannot cause hypnosis by itself (considering the loss of righting reflex as a criterion of hypnosis, as it was evidenced by pentobarbital) but potentiates the effect of hypnosis induced by pentobarbital.

The outcomes of chimney and traction tests revealed that there was significant association between the CNS depressant effects and increasing doses of the EO administered, which was evidenced by the dose-dependent decrease in locomotor activity with an ED_{50} of 800 mg/kg. In addition, there was a reduction in the time of onset and an increase in the duration of sleep induced by pentobarbital. The findings of the present study also indicated that the EO possesses sedative- and hypnotic-like effect. Similar findings were obtained in our previous studies.

The inhibition of motor coordination was possibly mediated through the $GABA_A$/benzodiazepine (BZD) and glycine inhibitory mechanisms.[24] This study proved that the EO of *M. communis* potentiates sleep induced by hypnotics like pentobarbital, but cannot induce hypnosis by itself. Hence, this research promotes the traditional use of the plant as an adjunct to other hypnotics for treatment of insomnia. However, direct extrapolation of the effective doses is not possible as the routes of administration are different. But it increases the effect of herbal medicine on which 80% of the world's population relies.[4]

The PIST test is generally used to reveal CNS depressant properties of drugs. Not only hypnotics, sedatives, and

tranquilizers but also antidepressants at high doses are known to prolong pentobarbital-induced sleep;[25] therefore, the EO could also have an antidepressant effect, which is under investigation. In this study, significant association between inhibition of motor activity and the administration of different doses of the EO was observed, indicating the CNS depressant activity of the EO. Hence, electrophysiological studies on the effect of the EO in freely moving animals with chronically implanted electrodes could also give evidence on its specific CNS depressant activity.[22]

Effect of the EO on PIST

In this study, significant potentiation of the sleep induced by pentobarbital was observed, and the duration was increased as the dose of the EO was increased from 500 to 1,000 mg/kg. Thus, the EO from the leaves of *M. communis* produced a substantial dose-related potentiation of the hypnotic effect induced by pentobarbital.

Similarly, hypnosis-potentiating effect of EO from the plant *Zanthoxylum budrunga* and high dose of the aqueous extract of coriander seeds was studied to prove the effect of plant materials on sleep.[27,28]

Although significant potentiation of hypnotic activity induced by pentobarbital was observed, PIST test was considered to be unspecific;[25] that is, it was difficult to predict whether the potentiation was caused by pharmacokinetic or pharmacodynamic interaction between the EO and pentobarbital. But still, there were some indications that the interaction must be pharmacodynamic. The different routes of administration of the EO (po) and pentobarbital (intraperitoneally) may rule out the possibility of interaction at the level of absorption. Moreover, as acute toxicity studies showed that the EO induced metabolizing enzymes in the liver,[30] the possibility of interaction at the level of biotransformation could also be ruled out. For potentiation to occur at this level, there must be enzyme inhibition as in the case of CCl4, which prolonged the PIST in rats[22] suggesting CNS depressant activity of the EO of *M. communis*. The possible interactions at the levels of distribution and excretion, however, are still there, which might need further investigations.

It is known that BZDs have anxiolytic effect at low doses and possess hypnotic effects at higher doses. The EO from the leaves of *M. communis* at 400 mg/kg was shown to have an anxiolytic effect in mice in one of the previous studies.[7,31] The previously mentioned findings suggest that the pharmacologic profile of the EO from the leaves of *M. communis* might resemble that of other hypnotics, BZDs, or barbiturates. Barbiturates and BZDs are known to have sedative and hypnotic effects at lower and higher dose, respectively. So in this regard, the EO seems to have hypnotic activity, but in the current studies, we confirmed that the EO could not induce hypnosis (ie, there was no loss of righting reflex upon separate administration of the varying doses of the EO). Hence, electrophysiological studies are also recommended as they could help develop other hypnotic models to evaluate hypnosis outside loss of righting reflex.

In the present study, the EO of *M. communis* was administered po, which was different from its traditional route of administration, that is, inhalation, in humans. Since the difference in the route of administration may affect the pharmacokinetics of the active components, the therapeutic doses used and the extent of hypnotic-like effect of the *M. communis* EO preparations observed in this study cannot be extrapolated to humans. However, these results advise the use of EO of *M. communis* as an adjunct, but not as a hypnotic medicine, for the treatment of insomnia.

Previous studies have reported α-pinene, limonene, and linalool to be the major monoterpinoids of the EO from the leaves of *M. communis*.[32] In the literature, linalool has been shown to have sedative and anticonvulsant activity in animal studies, and anxiolytic and sedative activity in human studies. The other monoterpinoids such as limonene and mercene were also shown to possess sedative and muscle relaxant effects in mice. Therefore, these monoterpinoids may also be responsible for the observed hypnotic-like and CNS depressant effects of the EO of *M. communis*. In other studies, it has been reported that *Sapindus emarginatus* contains triterpenoids and saponins that produce CNS depressant action, and a number of scientific reports have indicated that triterpenoids produce CNS depressant effect.[33]

The mechanism by which the EO from the leaves of *M. communis* exerts its effects has to be addressed in future studies. Compounds that act through GABA-chloride ion channel complex have been shown to prolong pentobarbital-induced sleep duration and reduce the latency to sleep.[26,31,34] Therefore, an involvement of GABAergic system may be suggested, though future studies using flumazenil, a BZD receptor antagonist, are essential to address this possibility. However, the loss of righting reflex upon administering the varying doses of the EO unlike pentobarbital indicates some other mechanisms for potentiation of hypnotic effect. There could be some allosteric receptors that potentiate the effect of hypnotics when administered together with the EO.

In a previously published report, pentobarbital has been shown to produce hypnosis at 50 mg/kg.[22] Likewise, barbiturates and BZDs have been shown to depress the CNS in a

dose-dependent manner, producing sedation, sleep, unconsciousness, and surgical anesthesia.[31]

In previous studies, linalol, pinene, citral, and limonene were believed to be the components responsible for the hypnotic effect of EO of *Z. budrunga*.[27] The hypnosis-potentiating activity of the EO of *M. communis* might as well be due to these compounds, as these are also present in the EO of *M. communis*.[11]

In this study, the EO of *M. communis* was shown to have potential as an adjunct for the treatment of insomnia in complementary and alternative medicine. However, the difference in dosage regimen should be considered, typically for repeated or chronic intake when the population uses the infusion, as opposed to acute treatment employed in this study. Moreover, the EO has to be given as an adjunct to other hypnotic medications, as it has been shown to only potentiate the hypnotic effect of pentobarbital.

Effects of the EO in chimney and traction tests

One of the evidences that indicated the effect of the EO on the CNS is its dose-dependent muscle relaxant activity. Experimental results of this study showed that the EO produced significant skeletal muscle relaxation, leading to negative responses in chimney and traction tests.

Likewise, to measure the effect of the EO of coriander seeds on motor coordination, the percentage of animals that fell from rod was compared between test and control groups in two studies, though the equipment used was a Rota rod.[11,27]

However, muscle relaxation could not be caused by some other preferred hypnotics like zolpidem, zaleplon, and zopiclone.[1] In this regard, considering loss of righting reflex as the only criterion of hypnosis could not be justified as muscle relaxation could occur without hypnosis or vice versa. Hence, we once again propose to develop new methods that could better confirm hypnosis. However, as a scientific trend, by using the possible available and cost-effective methods, the loss of righting reflex upon administration of varying doses of the EO was confirmed.

In this study, the EO was observed to induce and prolong sleep, potentiate sleep induced by pentobarbital, and inhibit skeletal muscle contraction and motor coordination in a dose-dependent manner. These findings were similar to that of our previous studies on EO.[7]

The EO was not observed to cause loss of righting reflex by itself but was shown to potentiate the hypnotic effect of pentobarbital, which is the new finding of this research.

In this study, the potential use of the EO from the leaves of *M. communis* as an adjunct for the treatment of insomnia has been proven, as it has mild hypnotic-like effect. This plant should be promoted, as the increasing prevalence of insomnia in the general population (26%–34%) makes it one of the common disease conditions.[1,35] Especially, the EO could be used as an adjunct for treatment of insomnia associated with problem of sleep maintenance.

Previous studies also indicate that plants with hypnotic-like activity, such as *Hypericum perforatum*, have been, are observed to prolong the duration of deep sleep and treat depression or comorbid insomnia.[3]

In previous studies, electroencephalogram (EEG) recordings were used to observe the hypnotic effect of *H. perforatum* and Valerian on sleep, and those studies showed an increase in duration of deep sleep and modulation of rapid eye movement.[29] Hence, future scientific investigations using EEG recordings should be done to observe the effect of the EO on sleep stages and cortical arousal, as there could be other unknown mechanisms by which EO potentiates sleep induced by other hypnotics.

Conclusion

This study, deploying different animal models of sedatives and hypnotics, significantly supports the Ethiopian traditional medicine claim that the EO of *M. communis* can be used as an adjunct for the treatment of insomnia. The EO of *M. communis* prolongs sleep duration and reduces sleep latency in mice, but it could not induce sleep by itself. In addition, the EO depresses CNS activity as shown from the negative results in the chimney and traction tests. EO of *M. communis* may be considered an alternative adjunct for treatment of insomnia (especially when it is associated with sleep maintenance problems), as it appears to be well tolerated in mice even at higher doses.

Recommendations

Based on the present study, the following investigations are recommended to be carried out in the future:

- Further evaluations are required to elucidate the detailed mechanism of the hypnosis-potentiating action.
- Further work is required to test the potential use of EO of *M. communis* in aiding sleep maintenance in insomnia.
- Further study using the EOs of *M. communis* should be carried out to study some of the pharmacological activities reported in aromatherapy such as epilepsy and depression.

- Electrophysiological investigations such as brain EEG analysis or electromyogram analysis should be done.

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Disclosure

The authors declare that they have no competing interests in this work.

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