Pharmacognostical & Phytochemical Potential of *Matricaria chamomilla* Linn Flowers

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ABSTRACT

Globally distributed, Matricaria chamomilla L (M. chamomilla, Asteraceae) is a well-known medicinal plant. In traditional medicine, it is commonly used to treat a wide range of illnesses, such as infections, liver disorders, gastrointestinal, respiratory, and neuropsychiatric conditions. Additionally, it has antibacterial, antiemetic, sedative, and antispasmodic properties. This study looked into the pharmacognostical characteristics, phytochemical makeup, and anxiolytic potential of M. chamomilla flower extracts made from alcohol. The various pharmacognostical factors were assessed using modified versions of established methods. The well-established test technique that is documented in the literature was used to determine the qualitative analysis of different phytochemical elements. The hole board, open field test, and elevated plus maze models were used to assess the anxiolytic activity. The standard anxiolytic medication diazepam (1 mg/kg) and the extract (250, 500, and 750 mg/kg) were tested for efficacy. Alcoholic extract was subjected to phytochemical examination, which identified the presence of proteins, carbohydrates, flavonoids, and glycosides. Animals given extract had exploratory behavior that was comparable to benzodiazepines in every test. The elevated plus maze apparatus's number of entries and duration of time spent in the open arm were both considerably increased by the extract, according to the findings. The extract significantly increased the frequency of square crosses, assisted rearings, and rearings in the open field test—all indicators of exploratory activity. All things considered, these findings point to the possibility that the alcoholic extract of M. chamomilla flowers has anxiolytic properties and support the traditional belief.

Keywords: Anxiolytic, *Matricaria chamomilla* L, Elevated plus maze, Open field test, Hole board models.
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Introduction

The prevalence of anxiety disorder, which has a lifetime prevalence of 28.8% and an incidence of 18.1%, is becoming more well acknowledged as a chronic condition that usually manifests during adolescence [1]. Significant handicap, both educational and occupational, is linked to the disease and negatively affects quality of life [2]. Psychotropic medications are one type of pharmacotherapeutic method used to treat anxiety disorders; however, their use is constrained by their dietary restrictions, adverse effect profiles, and drug interactions [3]. When benzodiazepines are used frequently, tolerance, anterograde amnesia, addiction, psychomotor impairment, disorientation, aggression, excitation, and physical dependence all worsen [4]. These are a few of the elements that piqued the curiosity of numerous researchers who are analyzing novel compounds derived from plants in the hopes of discovering alternative anxiolytic medications

with less undesirable side effects. Many different kinds of herbal medicines have been used as anxiolytics in different parts of the world. For example, the roots of the kava plant from the topical pacific region, the saponin-containing fraction of the leaves of Albizialebbeck from India, and Citrus aurantium from Brazil-Indians, Afro-Brazilians, and Caboclos are all known to have anxiolytic effects [6]. The primary barrier to the integration of herbal medicine into clinical practice is the deficiency of adequate scientific and clinical evidence, as well as a deeper comprehension of the safety and effectiveness of herbal products. The Asteraceae family includes the well-known medicinal herb chamomilla, also known as chamomile. This annual plant can thrive in any type of soil and can withstand cold temperatures. M. Native to northern and western Asia, as well as southern and eastern Europe, is chamomilla [7]. It is now extensively available everywhere in the world [8]. M. In many nations, chamomilla has been traditionally used to treat a variety of illnesses, such as respiratory, neuropsychiatric, hepatic, and gastrointestinal issues as well as the common cold. This herb is also commonly used to treat illnesses of the skin, eyes, and mouth as well as to reduce pain and infections [9]. The content of phytochemicals in M. More than 120 components of chamomilla essential oil (EO) and extracts have been reported. Terpenoids were, on the whole, the most significant chemical category in M. chamomilla EO, the most significant constituents being β-farnesene, chamazulene, bisabolol and its oxides A and B, and bisabolone oxide A. Phenolic substances such as flavonoids, coumarins, and phenolic acids dominated chamomilla extracts. Furthermore, research has been done on the makeup of amino acids [10]. Its impact on CNS activity, however, has not yet been investigated. As a result, we conducted the investigation to assess M's potential for reducing anxiety. chamomilla by utilizing various animal prototypes.

Materials and methods

Plant materials

The flowers of *M. chamomilla*were collected from Bhopal Region, Madhya Pradesh, India. The identification and authentication of plant was done by Dr. Saba Naaz, Botanist, from the Department of Botany, Saifia College of Science and Bhopal. A voucher specimen number **252/Saif./Sci./Clg/Bpl** was kept in Department of Botany, Saifia College of Science, Bhopal for future reference. Fresh flowers of plants were used for pharmacognostical studies. Flowers of *M. chamomilla*were dried under shade and powdered to 60# separately and stored in airtight containers and used for phytochemical and pharmacological studies.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Macroscopical characterization

The macroscopical description of *M. chamomilla*flowers include size, shape, nature of outer and inner surfaces, types of fracture, and organoleptic characters like color, odour, taste etc. were studied [11].

Proximate analysis

In order to prevent batch-to-batch variations in dried crude medication quality, proximate analysis aids in the establishment of specific standards. Their research also provides insight into the kind of phytoconstituents that are present. Using techniques outlined in the Indian Ayurvedic pharmacopoeia, proximate analysis of M. chamomilla flowers was conducted. The flowers were subjected to a number of determinations, including total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value, water soluble extractive value, loss of moisture content, and swelling index.

Determination of ash value

Ash values of powder of *M. chamomilla* flowers were determined by the following method:

Determination of total ash

2 gm of accurately weighed powder of *M. chamomilla* flowers were incinerated in a crucible at a temperature 500-600ºC in a muffle furnace till carbon free ash was obtained. It was then cooled, weighed and percentage of ash was calculated with reference to the air-dried drug.

Determination of acid insoluble ash

In order to capture insoluble debris, the entire amount of ash that was obtained above was heated for five minutes with 25 milliliters of 2M hydrochloric acid and then filtered through ash-less filter paper. After being cleaned with hot water, the ash was burned in a muffle furnace until the filter paper was at a constant weight. The air-dried powered medication (60#) was used as a reference to compute the percentage of acid-insoluble ash.

Determination of water soluble ash

Insoluble matter was gathered on ash-less filter paper, cleaned with hot water, and ignited for 15 minutes at a temperature not to exceed 450ºC in a muffle furnace after the entire ash was boiled for 5 minutes with 25 milliliters of water. The weight of water-soluble ash was determined by dividing the weight of ash by the weight of water-insoluble materials. The air-dried powered medication was used as a reference to compute the percentage of water-soluble ash.

Determination of extractive values

Extractive values of powder *M. chamomilla* flowers were determined by the following methods.

Determination of alcohol soluble extractive value

Dried by air In a closed flask, 4g of powdered M. chamomilla flowers were macerated with 100ml of alcohol for 24 hours, with frequent shaking every 6 hours. After that, it was left to stand for eighteen hours before being quickly filtered to stop any loss during evaporation. Empty a porcelain dish, evaporate 25 ml of the filtrate, and dry it at 105 oC until its weight remains constant. With reference to the medication that had been air-dried, the percentage of alcohol-soluble extractive was computed.

Determination of water soluble extractive value

A 4 gm powdered material of M. chamomilla flowers that had been air-dried was taken and immersed in 100ml of water in a closed flask for 1 hour while being shaken constantly. After a gentle 1-hour boil in a water bath, it was cooled, weighed, and the weight was adjusted. A porcelain dish containing 25 ml of filtrate was dried at 105 oC until it reached a consistent weight by evaporation. The air-dried powered medication (60#) was used as a reference to compute the percentage of water soluble extractive.

Determination loss of moisture content

After precisely weighing 100g of powdered M. chamomilla flowers, they were placed in a tarred evaporating dish without any prior drying. Once the medicine has been added to the tarred evaporating plate, it will be dried at 105ºC for five hours and then weighed. Weighing was done every hour while drying until the difference between two subsequent weigh-ins looked to be no more than 0.25%. After drying for 50 minutes and cooling for 30 minutes in a desiccator, two consecutive weigh-ins revealed a variation of no more than 0.01 grams, indicating that the weight was constant.

Determination of swelling index

1 gram of M. chamomilla flower powder placed in a 25 milliliter glass measuring cylinder with a stopper. 25 milliliters of water were added, and the mixture was vigorously agitated every ten minutes for one hour. It was then left to remain at room temperature for three hours. Three copies of the determination were made. Measured was the volume in milliliters that the plant material, including any sticky mucilage, occupied. The swelling index of a plant material was determined by taking the mean volume value, which was correlated with 1g of plant material [12, 13].

Extraction

Plant material fattening

After allowing the blossoms of M. chamomilla to air dry in the shade, powder was created. The plant material that had been shade-dried was roughly crushed and then subjected to a petroleum ether extraction procedure using soxhlet apparatus. Up till the material was sufficiently defatted, the extraction procedure was repeated.

Extraction by soxhlation process

Defatted M. chamomilla flowers were thoroughly extracted using a variety of solvents (ethanol, ethyl acetate, and chloroform) using the soxhletion process. When the extract reached its boiling point, it evaporated. To determine the extractive yield, the dried crude concentrated extract was weighed. When it was prepared for analysis, it was placed in glass vials measuring 6 by 2 cm and kept at 4°C in the refrigerator [14].

Phytochemical screening of the extract

Various phytoconstituents, including alkaloids,carbohydrates, glycosides, phytosterols, saponins, tannins,proteins, amino acids, and flavonoids were analysedqualitatively in the *M. chamomilla*extract [15, 16].

Animals

The study used male Swiss albino mice weighing between 22 and 25 grams. This was done to protect female mice against the effects of changes in ovarian hormones during the estrous cycle. The behavioral observations were conducted throughout the same time of day in soundproof rooms in order to minimize the potentially confounding effect of spontaneous behavior's daily variations. IAEC-PBRI/IAEC/PN-19117 is the animal experiment proposal number, and Reg. No. 1824/PO/RcBi/S/15/CPCSEA is the Institutional Animal Ethical Committee registration number. The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, New Delhi, directed that all processes be carried out in compliance with IAEC. All of the animals were acquired from the Pinnacle Biomedical Research Institute's animal house in Bhopal. There, they were kept in cages with six mice per group under standard environmental conditions, which included a temperature of 25±2°C, a light and dark cycle of 12 hours, a relative humidity of 45–55 percent, and unlimited access to food and water. Food was withheld six hours prior to and throughout the trial, but not water. Every experiment was run between the hours of 0800 and 1600 during the light period.

Acute oral toxicity studies

The Organization for Economic Co-operation and Development (OECD) guideline test, ANNEX-423, was followed in performing the acute oral toxicity test. Female mice were given oral dosages of 5, 50, 300, and 2000 mg/kg of an alcoholic extract of M. chamomilla. According to a literature search using standard LD50 testing, there is typically little variation in sensitivity between the sexes; nevertheless, when variations are seen, females are typically somewhat more sensitive than males. This was the rationale underlying the toxicity experiments' selection of female mice [17]. Following dose injection, the animals were watched closely for two hours for symptoms of toxicity, including hyperactivity, grooming, convulsions, drowsiness, and hypothermia, as well as for mortality up to twenty-four hours later.

Behavioral assessment of anxiolytic activity

Treatment schedule

The elevated plus maze (EPM), open field test (OFT), and hole board models (HBM) were used to assess the anxiolytic activity. There were five groups of animals total, with six male mice in each group. Groups 1 through 5 received M. chamomilla extract (250, 500, and 750 mg/kg); group 2 received diazepam (1 mg/kg). Group 1 received vehicle (normal saline).

Elevated plus maze test

The four arms that made up the EPMT device were each positioned 90 degrees from the other three arms, and they were all raised 30 cm above the ground. Two arms had tall walls measuring thirty by seven by twenty centimeters, while the other arms were joined to form a plus sign by a central section measuring seven by seven centimeters. The

walls of the enclosed arms and the floor of the maze were painted black. On the middle platform, a 40-W lamp provided lighting for the entire space. Sixty minutes before the test, the animals received treatments with diazepam, extract, and vehicle. Before any behavioral testing, the mice were allowed to spend thirty minutes getting used to the experimental laboratory's dim lighting. The experiment was conducted between 0900 and 1400 hours. On the middle platform, a single mouse was positioned with its back to an open arm. For five minutes, the frequency and length of entry into the closed and open arms were noted. When a mouse's four paws fit inside an open or closed arm, that entry was counted. The amount of time (duration) that each animal spent in the open arms (100 × open/(open + enclosed)) and the percentage of entries made in the open arms (frequency, 100 × open/total entries) were then computed. Following every trial, the apparatus was meticulously cleaned [18].

Open field test

The device was a wooden box measuring 60 by 60 by 60 centimeters. The open field arena was partitioned into 16 squares, each measuring 15 by 15 centimeters. There were four inner squares located in the center, and 12 outside squares situated along the walls. The experimental chamber was a dim, sound-attenuated space. A 40-W lamp was used to illuminate the open field arena, with the light source aimed between 75 and 100 cm above the field. Animals were treated for 60 minutes with a vehicle, 1 mg/kg of diazepam, and 250, 500, and 750 mg/kg of M. chamomilla extract. They were then placed individually in one of the corner squares, and the number of rearings, aided rearings, and crossing squares were examined for 5 minutes [19].

Hole board test

The hole board is a 40 cm by 40 cm painted white wooden board with four evenly spaced holes (1 cm in diameter and 2 cm in depth). Two thick colored lines that intersected in the middle were used to split the board into four equal sections squares, each measuring 20 cm by 20 cm. One mouse at a time was placed in each corner of the board, and the mice were then allowed to roam around and stick their heads through the holes. For each mouse, the number of head dips and sectional crossings in five minutes was noted [20].

Statistical analysis

All the results were expressed as mean ± SEM and the data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's "*t*" test. A *P* value of <0.05 was considered as the level of significance.

Results and discussion

The soxhletion extraction technique yielded crude extracts, which were then concentrated on a water bath to completely evaporate the solvents and determine the extraction yield. The yields of the M. chamomilla extracts in ethanol, ethyl acetate, and chloroform were, respectively, 4.3, 5.7, and 8.9% w/w. The physical characteristics of M. chamomilla flowers are summarized in Table 1.Early to midsummer is when the plants start to bloom, and they continue to do so until the seeds ripen in late summer, or until frost if they are deadheaded, when they produce an abundance of solitary terminal flowers. Their golden yellow blooms are 10–30 mm in diameter, with a distinct flavor, and a pleasant, aromatic scent.In order to measure various physiochemical parameters, such as total ash value, water soluble ash, acid insoluble ash, extractive soluble in alcohol, extractive soluble in water, loss on drying, foreign organic matter determination, and foaming index, M. chamomilla flowers were shade dried and ground into powder (Table 2). The phytochemical analysis of several M. chamomilla extracts revealed the types of phytochemicals found in the flowers; they are included in table 3 together with the results of the chemical tests conducted on the various phytoconstituents. It was discovered that alcoholic extracts included high concentrations of proteins, amino acids, flavonoids, and carbs. In chloroform extract, M. chamomilla was discovered to contain fixed oils and fats as well

as phytosterols. Furthermore included in the ethyl acetate extract were flavonoids, phytosterols, fixed oils, and fats and steroids. Table 3. Three mice per group were given varied doses of the alcoholic extract of M. chamomillawa orally at rates of 5, 50, 300, and 2000 mg/kg body weight, respectively. For a duration of 48 hours, the animals were studied in order to examine their overall behavior and identify any indications of discomfort or nervousness. For the anxiolytic activity, dose levels at 1/8th (250 mg/kg body weight, p.o.) and 1/2.6th (750 mg/kg body weight, p.o.) of this greatest dose were chosen, since even the mice receiving the highest dose of M. chamomilla (2000 mg/kg body weight, p.o.) did not exhibit any mortality.

Elevated plus maze test

The lifted test was suggested as a model to assess anxiolytic movement in addition to the labyrinth test, which was suggested for particular, identifiable evidence of anxiogenic and anxiolytic drugs. Anxiogenic mixes have the opposite effect of anxiolytic mixes, which increase open arm investigation time by reducing anxiety. When compared to the control group, animals treated with three different AEMS measurements (250, 500, and 750 mg/kg) in the EPM showed a significant increase in the amount of time spent in the raised open arm and labyrinth model. Therefore, as compared to the control group, the animals treated with diazepam (1 mg/kg) showed a significant decrease in the amount of time spent at the closed arm of the elevated and labyrinth models, as well as a critical increase in the time spent at the open arm. Furthermore, diazepam (1 mg/kg)-treated mice showed a significant increase in the amount of time spent in both the labyrinth model and the open arm of raised animals. When compared to the control, the animals treated with each of the three measurements showed a significant decrease in the number of sections in both the labyrinth model and the shut arm of the lifted animal. Thus, it should come as no surprise that animals given 1 mg/kg of diazepam showed a significant decrease in the number of sections at the open arm of raised and the labyrinth model. They also showed a significant increase in the number of passages in the open arm of hoisted and the labyrinth model compared to the control group. In addition to the labyrinth model, animals treated with 1 mg/kg of diazepam naturally showed a significant reduction in the number of sections at the open arm of the helix. When compared to low dosage (250 mg/kg), animals treated with moderate and high dosage (500 and 750 mg/kg) show a more notable increase in the number of passages and time spent at open arm of raised in addition to labyrinth model.

Hole board test

The Holeboard test is a simple behavioral test used in mice to identify mixtures that have anxiolytic effects.They used an open field on base with holes for animals to poke their noses into. Opening board tests are commonly used to assess emotionality, unease, and/or push reactions in animals. They provide a simple method for gauging a creature's response to a novel environment. It has been shown that head-plunging behavior was sensitive to changes in the creature's enthusiastic condition, and it has been suggested that an increase in head-plunging behavior throughout all locomotor action (quantities of squares crossed) may reflect the outflow of anxiolytic state in animals. The frequency and duration of head-dippings have also been noted. If both eyeballs disappeared into the gap, the head plunge was scored. When compared to controls, animals in HBT (Table 5) treated with three doses of AEMC (250, 500, and 750 mg/kg) showed a significant increase in the number of head-plunge checks and a decrease in velocity (Line crossing). It should come as no surprise that animals given diazepam (1 mg/kg) likewise showed a significant increase in the quantity of head-plunge checks and line crossings. When compared to animals treated with low dosage (250 mg/kg), those treated with high measurement and moderate dose (500 and 750 mg/kg) exhibit more critical outcomes.

Open field test

Animals treated with three AEMC measures (250, 500, and 750 mg/kg) in OFT (Table 6) showed increases in each exploratory parameter. Three doses of AEMC (250, 500, and 750 mg/kg) administered to open-ended test animals showed a significant increase in ambulation when compared to the control group. Similar to this, rats given diazepam (1 mg/kg) naturally showed a significant increase in ambulation as well as a significant increase in rising when compared to controls. Thus, it should come as no surprise that animals given diazepam (1 mg/kg) showed a significant increase in rising, which was remarkable.Every animal given three AEMC measures (Table 6) showed an expand action in the focal square, with only a high dosage showing a significant difference from the control. Accordingly, it should come as no surprise that animals given 1 mg/kg of diazepam showed a significant increase in rising and a significant decrease in self-preparing movement in comparison to the control group. As a result, animals given diazepam (1 mg/kg) clearly showed a significant reduction in their ability to prepare themselves. When compared to controls, there was a slight decrease in fecal hanging in open-ended test animals given three different doses of AEMC (250, 500, and 750 mg/kg). Furthermore, diazepam (1 mg/kg)-treated rats showed a significant increase in all exploratory parameters and a somewhat smaller decrease in garbage.

Table 1: Morphological characteristic of *M. chamomilla* flowers

S. No	Parameters	*M. chamomilla* flower
1	Shape	Pedunculate and heterogamous
3	Size	10-30 mm in diameter
4	Odour	Pleasant fragrant aroma
5	Taste	Characteristics
6	Colour	golden yellow
7	Foreign organic matter	No adulterants have been found

Table 2: Physiochemical analysis of powder of *M. chamomilla* flowers

S. No.	Parameters	Observations
1	Total ash	6.4
2	Water soluble ash	2.91
3	Acid insoluble ash	0.91
4	Water-soluble extractive	13.8
5	Ethanol soluble extractive	9.6
6	Loss on drying (%)	29.2
7	Foreign organic matter determination	1.05
8	Foaming index	22 (ml)

Table 3: Phytochemical screening of *M. chamomilla* flowers extracts

Phytoconstituents	Chloroform extract	Ethyl acetate extract	Alcoholic extract
Carbohydrates	-	-	+
Tanninsand Phenolics	-	-	-

Aminoacidsand Proteins	-	-	+
Flavonoids	-	+	+
Saponins	-	-	-
FixedoilsandFats	+	+	+
Alkaloids	-	-	-
Glycosides	-	-	+
Phytosterols	+	+	-

Table 4: Effect of AEMC on EPM paradigmin mice

G. No.	DrugTreatment	Dose(mg/kg)	Numberofentries(mean±SEM)		Timespentinsec(mean ±SEM)	
			Openarm	Closedarm	Openarm	Closedarm
I	Control	0.05ml/10	7.16±0.40	11.33±0.90	36.17±0.90	192.0±3.41
II	Diazepam	1	12.50±0.56***	6.33±0.42***	80.83±0.98*	129.2±2.30***
III	AEMC	250	7.50±0.34	10.17±0.40	46.33± 1.25**	160.2±2.41***
IV	AEMC	500	8.50±0.34**	9.0±0.36***	62.33±1.99***	147.00±1.71***
V	AEMC	750	11.67±0.76***	7.167±0.30***	79.17±1.49***	136.2±2.482***

Valuesweremean±S.E.M.for(n=6)expressedas time(insec)of6animalsineachgroup.*P<0.05,**P<0.01,***P <0.001vs.control

Table 5: Effect of AEMC on Whole Board test in mice

G. No.	DrugTreatment	Dose(mg/kg)	No.ofheaddipping	Linecrossing
I	Control	0.05ml/10g	22.00±0.5774	64.67±1.607
II	Standard	1	52.00±1.065***	40.67±0.7149***
III	AEMC	250	31.67±1.116***	61.00±1.065
IV	AEMC	500	38.83±1.167***	53.17±1.195***
V	AEMC	750	47.33±1.054***	47.50±1.708***

Values were mean ± S.E.M. for (n=6) expressed as time (in sec) of 6 animals in each group. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control

Table 6: Effect of AEMC on various parameters in OFT

G. No.	Drug Treatment	Dose (mg/kg)	Ambulation (N)	Rearing (N)	Self Grooming (N)	Activity in Centre (N)	Fecal dropping (N)
I	Control	0.05ml/10 g	32.67±1.520	6.67±0.33	5.50± 0.43	2.17±0.31	2.16± 0.31
II	Standard	1	75.67±1.86***	15.50±0.41	2.167±0.42***	4.167±0.32	1.0± 0.26
III	AEMC	250	34.33± 1.40	7.16±0.30	4.66±0.49	2.50±0.34	1.33± 0.23
IV	AEMC	500	49.50± 1.258***	9.500±0.4282**	3.50±0.4282*	3.33±0.3333	1.50± 0.2236
V	AEMC	750	70.00±1.506*	12.50±0.42	2.50±0.61**	4.0±0.36**	1.0± 0.25

			**	8***	*		

Values were mean ± S.E.M. for (n=6) expressed as time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test. *P<0.05,**P<0.01,***P<0.001vs.control

Conclusion

Since the effects were dosage dependent, it was determined that M. chamomilla possessed anxiolytic activity, as supported by all of the models previously mentioned. The maximum effect was noted at a dose of 750 mg/kg, which was substantially higher than the vehicle-treated control group. The outcomes from the carefully inspected models of controlled experiments using lab animals have been achieved. The results exhibit statistical validity and offer a sound scientific basis for the application of the physiologically active components of M. chamomillain anxiety. These constituents may influence specific brain mediators, hence mitigating aversion fear and eliciting anxiolytic effects. Additional research is necessary to clarify the precise mechanism and bioactive substances.

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