



FORMULATION AND EVALUATION OF HERBAL CHURNA CONTAINING FENUGREEK EXTRACT FOR ANTI-DEPRESSANT ACTIVITY

Sonali Tukaram Chavan^{1*}, Avinash Hatkar², Swapnil Bharat Rathod³

¹Student of Bachelor of Pharmacy, Faculty of Pharmacy, Dr. Babasaheb Ambedkar Technological University, Lonere, Raigad

²Department of Pharmacology, Faculty of Pharmacology, Dr. Babasaheb Ambedkar Technological University, Lonere, Raigad

³Student of Bachelor of Pharmacy, Faculty of Pharmacy, Dr. Babasaheb Ambedkar Technological University, Lonere, Raigad

ABSTRACT

Depression is a prevalent mental health disorder with significant public health implications, often resistant to conventional drug treatments. This paper explores the formulation and pharmacology of an herbal churna aimed at addressing depression using fenugreek seeds, ashwagandha, Brahmi, turmeric, and black pepper. These ingredients are selected based on their known antidepressant and anxiolytic properties, supported by their chemical constituents. The formulation process involves gathering, cleaning, drying, grinding, and mixing the ingredients in specific quantities. Pharmacologically, the churna leverages the antidepressant effects of compounds like 4-hydroxyisoleucine, withanolides, bacosides, and curcumin. The addition of black pepper enhances bioavailability. Evaluation tests such as organoleptic assessment, loss on drying, and ash value determination ensure quality and authenticity. This herbal churna presents a promising natural approach to managing depression, offering potential benefits for mental health and overall well-being.

KEY WORDS: -Herbal Churna, Fenugreek extract, Anti-depressant.

INTRODUCTION

Depression is a common chronic recurrent syndrome often refractive to drug treatment affecting quality of life and overall productivity. In recent years clinical depression has been recognised as a major public health problem. According to the world health organisation (WHO) in its 1998 report depression (including complication of depression) affected about 12% of the world population and was supposed to be second greatest cause of premature death and disability worldwide by the year 2023⁽²⁾.

The high prevalence of suicide in depressed patients (up to 15%) along with the complications arising from mental stress and effect on the cardiovascular system have confirmed depression as contributory factor to fatal coronary disease⁽¹⁾.

Depression is a syndrome that affects a person's mood physical health and alters behaviour patients with major depression disorder have symptoms that reflects change in brain monoamine neurotransmitters specifically serotonin, nor epinephrine and dopamine. Depression is commonly accepted to be a disorder due to disturbance in neurotransmitters function, particularly serotonin (5-HT) nor epinephrine (NE) and dopamine although several classes of antidepressant are currently being used due to clinical limitations and adverse effect there is critical interest in development of efficient and safe drug for treatment of depression.

The most widely used antidepressant namely serotonin reuptake inhibitor (SSRI) increase extra cellular availability of serotonin. Depression is highly prevailing psychological ailments, characterized by sad mood, lack of interest, absence of Vigor, feeling of guilt, disturbed sleep, and appetite, decreased energy and reduce centralisation⁽³⁾.

Many plants have documents antidepressant action like hypericum perforatum and pueraria labata, Fenugreek (Trigonella foenum graecum) belonging to family Fabaceae in a renowned prehistoric plant.

Different parts of this plant are consumed in diet such as seeds and leaves Fenugreek seed have a unique rhomboid shape distinctive peppery small and acrimonious taste.

Seed are usually consumed as spice in diet as well as for the cure of various medical ailments⁽³⁾. It contains several bioactive components i.e. carpaine (alkaloids) Fenugreekine diosgenin (saponin) genettianine trigonelline flavonoids and 4- hydroxy isoleucine, arginine (amino acid).

Various activities of this plant have been reported such as hypoglycaemic, hypolipidemic and evaluate the effect of FS-ME on depression in mice.



Figure 1: Herbal Churna

Materials collection: - Herbal ingredients

1. Fenugreek seed
 2. Ashwagandha
 3. Bramhi
 4. Turmeric
 5. Black paper
1. Fenugreek seeds

Fenugreek is an annual plant in the Fabaceae (legume) family that is cultivated worldwide as a semiarid crop. Fenugreek plants are erect, loosely branched, and less than 1 metre (3 feet) tall with trifoliate light green leaves and small white flowers. The slender pods are up to 15 cm (6 inches) long, curved and beaked, and contain yellow-brown seeds that are flat rhomboids characterized by a deep furrow, less than 0.5 cm (0.2 inch) long⁽⁴⁾.



Figure 2: fenugreek

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida (Dicot)
Order	Fabales.
Family	Fabaceae.
Scientific name	<i>Trigonella foenum-graecum</i>
Common name	methi seeds, methi dana.
Synonyms	<i>Origanum thymus</i> Kuntze <i>Thymus collinus</i> Salisb.

Table.no.1



2. **Ashwagandha**

Ashwagandha, also known as Indian Ginseng, is a medicinal herb with a wide range of health benefits. It is commonly used in Ayurvedic medicine for its tonic properties, reducing stress, improving athletic performance, enhancing cognitive function, and potentially benefiting conditions like **anxiety**, infertility, and diabetes. Research indicates it may increase testosterone levels, reduce inflammation, and aid heart health⁽⁶⁾.



Figure 3: Ashwagandha

Kingdom	Plantae
Phylum	Angiosperm
Class	Eudicots
Order	Solanales
Family	Solanaceae
Scientific name	<i>Withania somnifera</i>
Common name	Winter cherry
Synonyms	Indian ginseng.

Table.no.2

3. **Bramhi**

Brahmi, also known as *Bacopa monnieri*, is a perennial herb native to India and other parts of Asia. It is a popular ingredient in Ayurvedic medicine and is revered for its numerous health benefits. Brahmi is believed to enhance memory, cognitive function, and mental clarity. It is also used to treat various health conditions such as cold, chest congestion, and bronchitis by clearing out mucus from the air passages, which helps ease breathing⁽⁵⁾.



Figure 4: Bramhi

Kingdom	Plantae
Phylum	Anthophyta
Class	Dicotyledonae
Order	Scrophulariales
Family	Scrophulariaceae
Scientific name	<i>Bacopa monnieri</i>
Common name	Water hyssop
Synonyms	Herb of grace

Table.no.3



4. **Turmeric**

Turmeric (*Curcuma longa*) is a perennial, rhizomatous, herbaceous plant that belongs to the ginger family Zingiberaceae. It is native to the Indian subcontinent and Southeast Asia. They have a rough, segmented skin and a dull orange interior. The main rhizome is pointed or tapered at the end and measures 2.5-7 cm (1-3 inches) in length and 2.5 cm (1 inch) in width, with smaller tubers branching off⁽⁸⁾.



Figure 5: Turmaric

Kingdom	Plantae
Phylum	Magnoliophyta
Class	Liliopsida
Order	Zingiberales
Family	Zingiberaceae
Scientific name	<i>Curcuma longa</i>
Common name	Haldi
Synonyms	Indian saffron

Table no.4

5. **Black pepper**

Black pepper, scientifically known as *Piper Nigrum*, is a perennial vine native to Malabar, India, belonging to the Piperaceae family. It produces small, round berries known as peppercorns, with black peppercorns being the most common type. The plant grows best in warm, humid climates with well-draining soils, pH of 5.5-6.5, and temperatures between 10-40°C. Black pepper vines require 3-4 meters of spacing, partial to full sunlight, and 125-200 cm of annual rainfall. The plant's alkaloid Piperine gives it its pungent taste, and it is widely used as a spice globally⁽⁷⁾.



Figure 6: Black Papper



Kingdom	Plantae
Phylum	Monolipophyta
Class	Magnoliopsida
Order	Piperales
Family	Piperaceae
Scientific name	<i>Piper nigrum</i>
Common name	Pepper corns, king of spices
Synonyms	Black gold

Table no.5

Chemical constituent and their uses

Sr.no	Ingredient	Chemical constituent	Uses
1	Fenugreek seeds ⁽⁹⁾	Trigonelline, 4-hydroxyisoleucine, orientin	Anti-depressant
2	Ashwagandha ⁽¹⁰⁾	Withanolides A-Y, withaferin A, withasomniferin A	Reduce stress and anxiety
3	Bramhi ⁽¹¹⁾	Bacoside A, bacoside B, triterpenoid saponin glycosides	Improve neurotransmission
4	Turmeric ⁽¹²⁾	Demethoxycurcumin, α -turmerone, β -turmerone	Antioxidant
5	Black pepper ⁽¹³⁾	Piperine	Enhancer to turmeric

Table no.6

Procedure of formulation

- Gather and Prepare Ingredients:** Acquire high-quality fenugreek seeds, ashwagandha root powder, Brahmi leaf powder, turmeric powder, and black pepper powder.
- Cleaning and Drying:** Ensure all ingredients are clean and free from impurities. If the seeds are not pre-powdered, dry roast the fenugreek seeds lightly in a pan until aromatic. Allow them to cool before grinding.
- Grinding:** Use a grinder or mortar and pestle to finely grind the fenugreek seeds into a powder.
- Mixing:** Combine all the powdered ingredients together - fenugreek seed powder, ashwagandha root powder, brahmi leaf powder, turmeric powder, and black pepper powder. Mix thoroughly until the powders are evenly distributed.
- Storage:** Store the churna in an airtight container, away from moisture, heat, and sunlight. Use a dark-colored glass container, if possible, to protect the Churna from light exposure.

Formula: -

Sr.no.	Ingredient	Quantity(gm)	Uses
1	Fenugreek seeds	50	Anti-depressant
2	Ashwagandha	25	Reduce stress and anxiety
3	Bramhi	25	Improve neurotransmission
4	Turmeric	10	Antioxidant
5	Black pepper	5	Enhancer to turmeric

Table no.7

Pharmacology of Used Herbs (Crude Drug): -

Several of the herbs in this formulation have shown antidepressant-like effects in various studies.

Fenugreek (*Trigonella foenum-graecum*) contains compounds like 4-HI that have exhibited anti-anxiety and antidepressant-like effects, potentially by increasing serotonin turnover in the brain.⁽¹⁶⁾

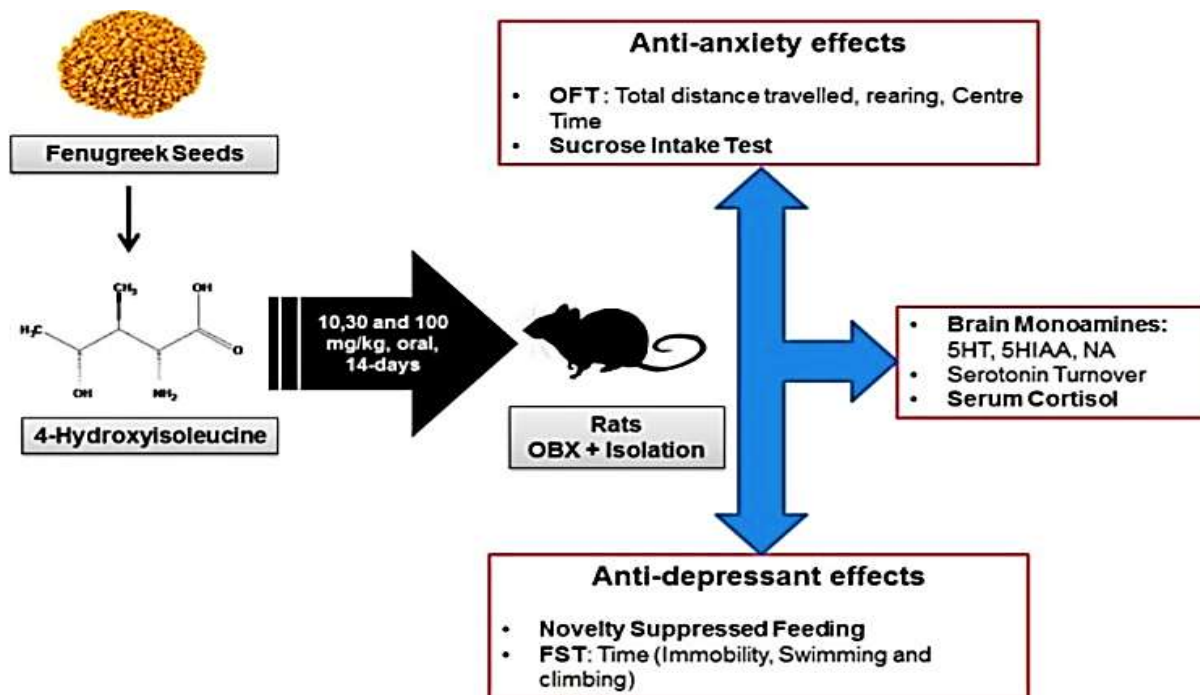


Figure 7: MOA

Ashwagandha (*Withania somnifera*) is an adaptogenic herb that has been used traditionally to treat psychiatric disorders and has shown antidepressant properties in preclinical studies.⁽¹⁷⁾

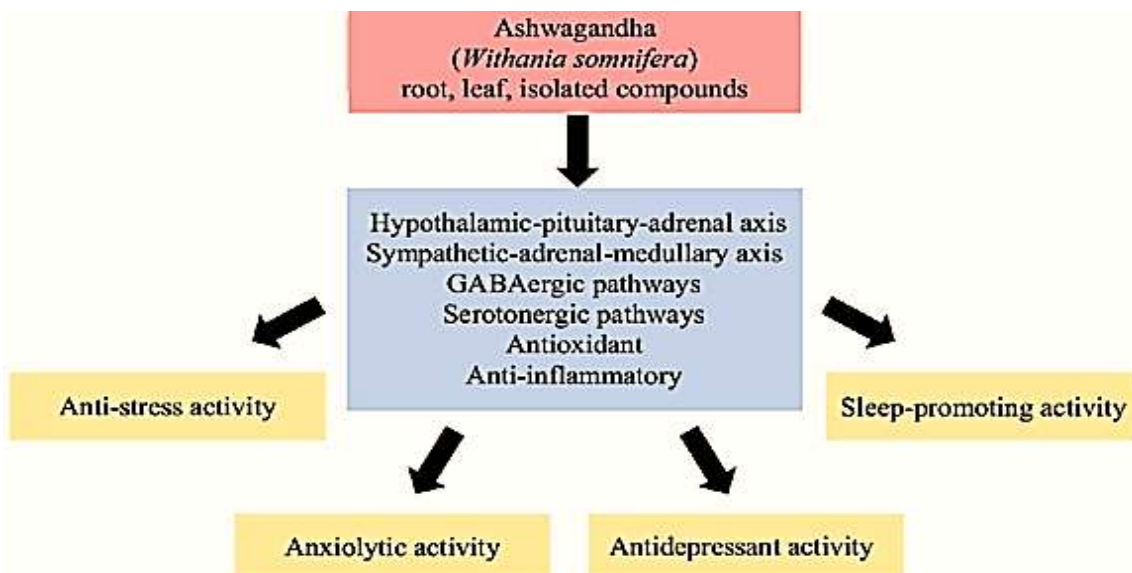


Figure 8: MOA

Brahmi (*Bacopa monnieri*) has demonstrated antidepressant activity in animal models of depression, likely through mechanisms like modulating neurotransmitters and reducing oxidative stress.⁽¹⁹⁾

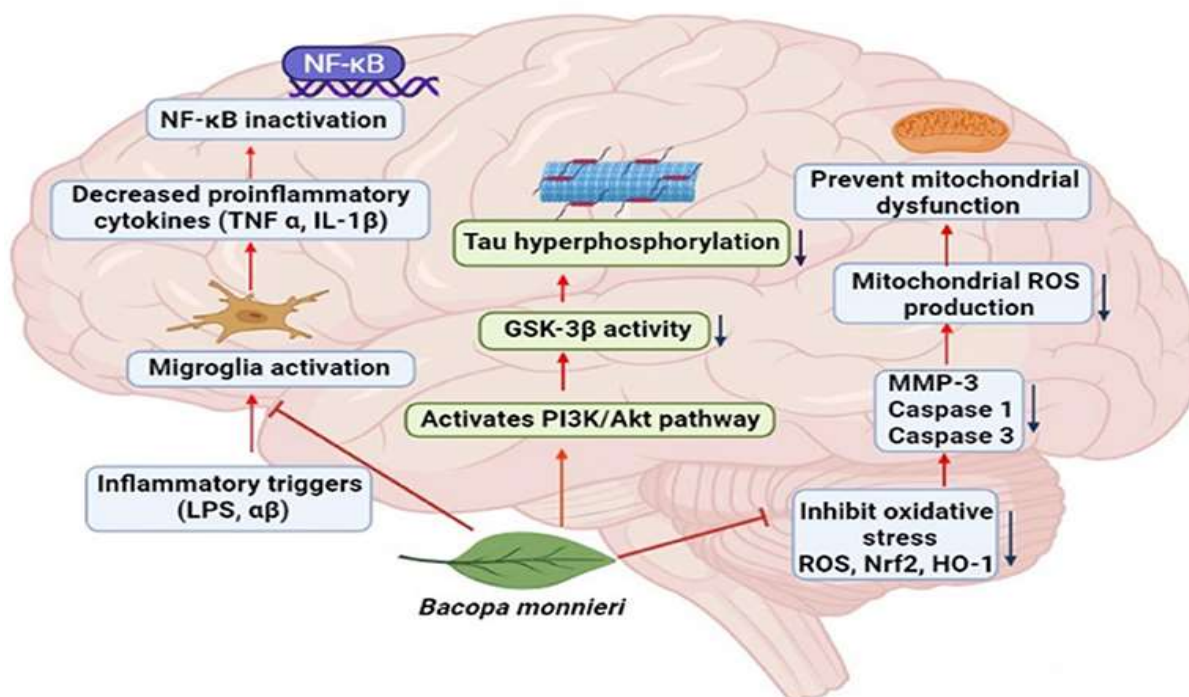


Figure 9: MOA

Turmeric (*Curcuma longa*) contains curcumin, which has demonstrated antidepressant-like effects in animal models, possibly by regulating monoaminergic systems.⁽¹⁸⁾

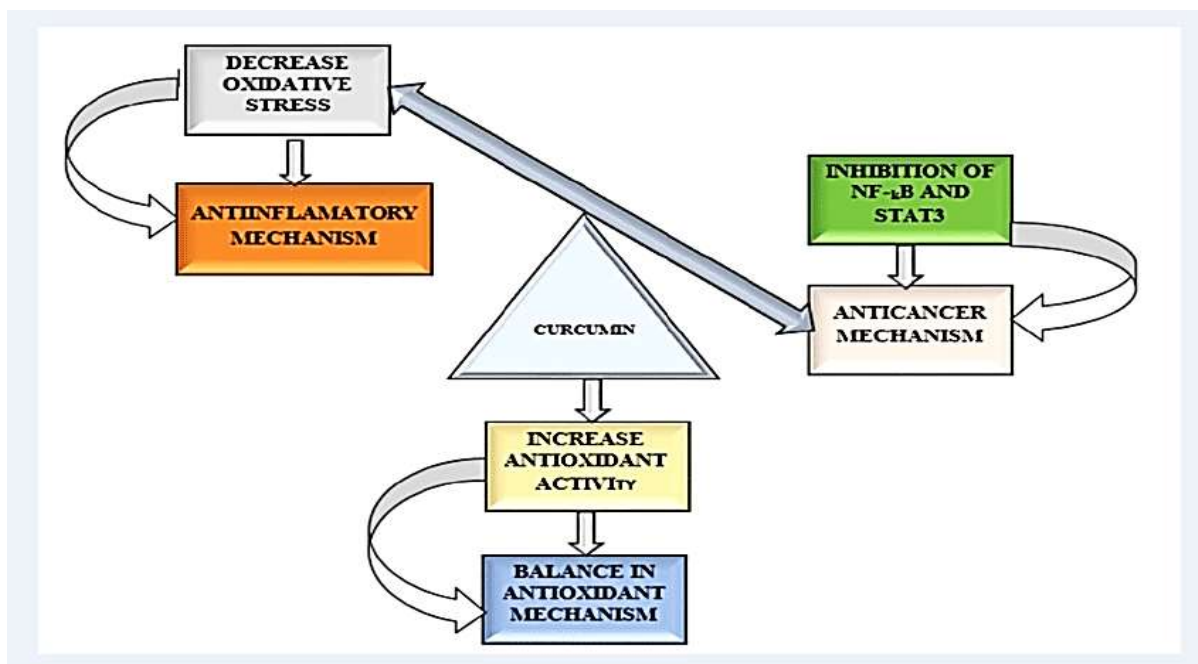


Figure 10: MOA

The addition of **black pepper** may enhance the bioavailability of some of these phytochemicals.⁽²⁰⁾

Evaluation Test For Formulation: -

1. **organoleptic properties:** The organoleptic properties of herbal churna, like other churna formulations, are important in assessing the quality and authenticity of the product. Parameters such as colour, odour, taste, and texture are evaluated as part of the standardization process.



Figure 11: Organoleptic Property

- Loss of drying:** Loss on drying (LOD) is a common test method used in the pharmaceutical industry to determine the moisture content and volatile substances present in a sample, such as a churna (an Ayurvedic herbal powder formulation). The key points regarding the loss on drying test for a churna are:
The test is carried out on a well-mixed sample of the churna. If the churna is in the form of large particles, the size should be reduced by rapid crushing to a powder.
The churna sample is weighed in a glass-stoppered, shallow weighing bottle that has been previously dried. The sample is distributed evenly in the bottle, not exceeding a depth of 10 mm.
The loaded bottle is then placed in a drying oven or chamber, with the stopper removed, and dried at the temperature and for the time specified in the monograph. Typical drying conditions are 105°C for 2-4 hours.
After drying, the bottle is promptly closed, allowed to cool in a desiccator, and reweighed. The loss on drying is calculated as the percentage weight loss from the initial weight. ⁽¹⁴⁾
The acceptance criteria for the loss on drying of a churna is typically specified in the individual monograph or product specification.
In summary, the loss on drying test provides a measure of the moisture and volatile content in a churna sample, which is an important quality parameter for Ayurvedic herbal formulations.



Figure 12: Loss On Drying

3. **Ash value:** The ash value represents the inorganic residues present in herbal drugs, primarily consisting of phosphates, carbonates, and silicates. These residues are a major index for evaluating the quality and purity of herbal medicines. The ash value is determined by incinerating the drug at a temperature of 450°C or above, which converts organic and carbon matter into ash. The ash content can be further divided into different types, including total ash, water soluble ash, acid insoluble ash, and sulphated ash, each providing specific information about the drug's composition.⁽¹⁵⁾



Figure 13: Ash value

4. **Microbial study:**

The evaluation test of the antimicrobial activity of herbal churna containing fenugreek, ashwagandha, turmeric, brahmi, and black pepper can be conducted by assessing the inhibitory effects of this combination on a range of bacteria, yeasts, and fungi. Each ingredient has known antimicrobial properties.

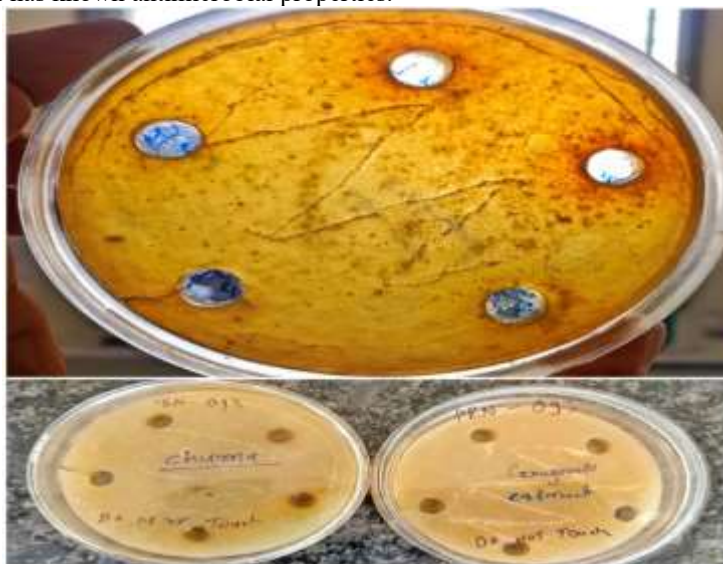


Figure 14: Microbial study

Fenugreek seeds have shown antimicrobial activity, ashwagandha has been studied for its antibacterial effects against Gram-positive cocci, turmeric is efficient against certain fungus and bacterial strains, Brahmi has antimicrobial properties, and black pepper exhibits antimicrobial activity. Combining these ingredients in churna form could potentially enhance their individual antimicrobial effects, making it a promising natural remedy against various pathogens.

5. **Identification test for alkaloid tannin saponin:**

The identification tests for alkaloids: Mayer's test, Dragendorff's test, Wagner's test, and Marme's test⁽²¹⁾

Identification test for saponin: Liebermann-Burchard test: Spraying the TLC plate with a reagent of ethanol and sulfuric acid. The appearance of white spots indicates the presence of saponin compounds.⁽²²⁾

Identification test for tannin: Ferric chloride test: Adding a few drops of 5% ferric chloride solution to a water extract of the plant material produces a greenish precipitate, which is a positive indication for tannins.⁽²³⁾



Figure 15: Identification Test

Observation and Result

a. organoleptic parameter:

Sr.no	Physical parameter	
1	Colour	Yellowish Brown
2	Odour	Pungent
3	Taste	Slightly Bitter
4	Texture	Smooth

b. loss of drying

W₁ – empty crucible - 30.23 gm
 W₂ – drug – 2.06 gm
 W₃ – crucible +drug -32.28 gm
 W₄ – crucible + drug after heat - 32.10 gm
Loss of drying = W₃ - W₄ = 32.28 -32.10 = 0.18 gm = 9%

c. Ash value

W₁ – empty crucible - 30.20 gm
 W₂ – drug – 2.02 gm
 W₃ – crucible +drug -32.22 gm
 W₄ – crucible + drug after heat – 31.16 gm
%ash = [(ashed wt.)-(crucible wt.)]

$$\frac{[(crucible + sample wt.)-(crucible wt.)]}{2.02} \times 100 = 95.5\%$$

- d. **microbial studies:** the solution of herbal churna shows zone of inhibition to bacteria and shows bacterial growth.
- e. **identification test:** the identification test of herbal churna is identified and results show the active ingredients present in the churna formulation.

CONCLUSION

the selected herbal ingredients, including fenugreek seeds, ashwagandha, Brahmi, turmeric, and black pepper, possess known antidepressant and anxiolytic properties, supported by their chemical constituents. The formulation process, involving gathering, cleaning, drying, grinding, and mixing of the ingredients, follows a systematic approach to ensure the quality and authenticity of the churna.



Pharmacologically, the churna leverages the antidepressant effects of compounds like 4-hydroxyisoleucine, withanolides, bacosides, and curcumin, with black pepper enhancing bioavailability.

Evaluation tests such as organoleptic assessment, loss on drying, and ash value determination confirm the quality and authenticity of the churna, meeting pharmaceutical standards.

the herbal churna presents a promising natural approach to managing depression, offering potential benefits for mental health and overall well-being. Further research and clinical studies are warranted to validate its efficacy and safety in human subjects.

Overall, this project highlights the potential of herbal remedies, particularly fenugreek-based churna, as a viable option for individuals seeking alternative treatments for depression, contributing to the growing body of evidence supporting natural therapies in mental health management.

REFERENCE

1. Britta Haenisch, Heinz banisch, *Depression and antidepressants: Insights from knockout of dopamine, serotonin or noradrenaline reuptake transporters, Pharmacology & Therapeutics, Volume 129, Issue 3, 2011, Pages 352-368, ISSN 0163-7258, <https://doi.org/10.1016/j.pharmthera.2010.12.002>.*
2. Vaibhav Gaur, Subhash L. Bodhankar, Vishwaraman Mohan, Prasad Thakurdesai, *Antidepressant-like effect of 4-hydroxyisoleucine from Trigonella foenum graecum L. seeds in mice, Biomedicine & Aging Pathology, Volume 2, Issue 3, 2012, Pages 121-125, ISSN 2210-5220, <https://doi.org/10.1016/j.biomag.2012.07.002>.*
3. Rajput, Muhammad. (2021). *Assessment of antidepressant activity of fenugreek seeds methanol extract. Rawal Medical Journal. 46. 236-239.*
4. Britannica, T. Editors of Encyclopaedia. "fenugreek." *Encyclopedia Britannica*, April 2, 2024. <https://www.britannica.com/plant/fenugreek>.
5. Walker EA, Pellegrini MV. *Bacopa monnieri*. [Updated 2023 Mar 17]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK589635>
6. Singh N, Bhalla M, de Jager P, Gilca M. *An overview on ashwagandha: a Rasayana (rejuvenator) of Ayurveda. Afr J Tradit Complement Altern Med. 2011;8(5 Suppl):208-13. doi: 10.4314/ajtcam.v8i5S.9. Epub 2011 Jul 3. PMID: 22754076; PMCID: PMC3252722.*
7. Britannica, T. Editors of Encyclopaedia. "black pepper." *Encyclopedia Britannica*, April 10, 2024. <https://www.britannica.com/plant/black-pepper-plant>.
8. Missouri Botanical Garden - Plant Finder Entry for *Curcuma longa* <https://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?taxonid=281676>
9. Awasthi et al. (2022). *4-Hydroxyisoleucine from fenugreek (Trigonella foenum-graecum L.) displays anxiolytic and antidepressant properties by modulating brain serotonin levels and HPA axis function. Journal of Functional Foods.*
10. Andrade C, Aswath A, Chaturvedi SK, Srinivasa M, Raguram R. *A double-blind, placebo-controlled evaluation of the anxiolytic efficacy of an ethanolic extract of withania somnifera. Indian J Psychiatry. 2000;42(3):295-301.*
11. Sairam, K., Dorababu, M., Goel, R. K., & Bhattacharya, S. K. (2002). *Antidepressant activity of standardized extract of Bacopa monniera in experimental models of depression in rats. Phytomedicine, 9(3), 207-211.*
12. Kulkarni, S. K., Bhutani, M. K., & Bishnoi, M. (2008). *Antidepressant activity of curcumin: involvement of serotonin and dopamine system. Psychopharmacology, 201(3), 435-442. doi: 10.1007/s00213-008-1300-y*
13. Chaudhary, A., Singh, N., Dalvi, M., & Wele, A. (2011). *A progressive review of Sandhana kalpana (Bioavailability Enhancers). AYU (An International Quarterly Journal of Research in Ayurveda), 32(3), 308-316. <https://doi.org/10.4103/0974-8520.93912>*
14. <https://www.pharmaguideline.com/2011/08/what-is-loss-on-drying-and.html>
15. Ajazuddin, Saraf S. *Evaluation of physicochemical and phytochemical properties of Safoof-E-Sana, a Unani polyherbal formulation. Pharmacognosy Res. 2010 Sep;2(5):318-22. doi: 10.4103/0974-8490.72332. PMID: 21589760; PMCID: PMC3093045.*
16. Yadav, R., & Kumari, R. (2021). *Antimicrobial potential of fenugreek (Trigonella foenum-graecum L.): An overview. Egyptian Journal of Biological Pest Control, 31(1), 1-10. <https://doi.org/10.1186/s41938-021-00451-5>*
17. Arora, S., Dhillon, S., Rani, G., & Nagpal, A. (2004). *The in vitro antibacterial/synergistic activities of Withania somnifera extracts. Fitoterapia, 75(3-4), 385-388. <https://doi.org/10.1016/j.fitote.2004.01.008>*
18. Moghadamtousi, S. Z., Kadir, H. A., Hassandarvish, P., Tajik, H., Abubakar, S., & Zandi, K. (2014). *A review on antibacterial, antiviral, and antifungal activity of curcumin. BioMed Research International, 2014, 186864. <https://doi.org/10.1155/2014/186864>*
19. Vijayan, P., Raghu, C., Ashok, G., Dhanaraj, S. A., & Suresh, B. (2004). *Antiviral activity of medicinal plants of Nilgiris. Indian Journal of Medical Research, 120(1), 24-29.*
20. Karsha, P. V., & Lakshmi, O. B. (2010). *Antimicrobial activity of black pepper (Piper nigrum Linn.) with special reference to its mode of action on bacteria. Indian Journal of Natural Products and Resources, 1(2), 213-215. <https://www.biologydiscussion.com/plants/detection-of-alkaloids-plants/57192>*
21. Soltani M, Parivar K, Baharara J, Kerachian MA, Asili J. *Hemolytic and cytotoxic properties of saponin purified from Holothuria leucospilota sea cucumber. Rep Biochem Mol Biol. 2014 Oct;3(1):43-50. PMID: 26989736; PMCID: PMC4757088*
22. <https://en.m.wikipedia.org/wiki/Tannin>