International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 19s, 2025
https://theaspd.com/index.php

Comprehensive Pharmacological Screening Of Habenaria Intermedia D. Don Tubers For Anti-Depressant Activity

Navjit Kaur Saini[1,2], Dr. Shipra Thapar[1]
[1]Department of Pharmaceutical Sciences, CT University, Ludhiana, (Punjab) India
[2]Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial College of Pharmacy, Bela, (Punjab) India, Dr.shipra.17041@ctuniversity.in

Abstract
Although Habenaria intermedia D. Don (Vriddhi; Orchidaceae) has been used historically to alleviate depression, its traditional uses have not been thoroughly studied. Therefore, it was intended to use the forced swim test to examine the antidepressant properties of different extracts and fractions of Habenaria intermedia tubers. Habenaria intermedia tubers that had been correctly identified were extracted in a methodical and thorough manner utilizing solvents in ascending order of polarity, namely n-hexane, chloroform, methanol, and water. Only crude extracts rich in phytoconstituents were tested for antidepressant effects in mice at dosages of 100, 200, or 400 mg/kg, p.o. A statistical comparison was made between the effectiveness of Habenaria intermedia tubers and imipramine, a common antidepressant medication (15 mg/kg, i.p.). At 400 mg/kg, the methanol extract was statistically equal to the standard medication and the only one of the two extracts to show maximum antidepressant efficacy when compared to the control. n-hexane, ethyl acetate, and 1-butanol were the solvents used to partition the bioactive methanol extract in ascending order of polarity. Additionally, mice were given dosages of 25 or 50 mg/kg, p.o., of all fractions to test their antidepressant properties. At a dose of 50 mg/kg, only the ethyl acetate fraction (EAF) demonstrated significant antidepressant effect comparable to the standard medication. Current research has confirmed traditional statements about the antidepressant properties of Habenaria intermedia tubers. According to phytochemical research, phenolic and flavonoid chemicals found in Habenaria intermedia tubers may be useful in the treatment of depression disorders.
Keywords: *Antidepressant, Forced swim test, Habenaria intermedia, Orchidaceae, Vriddhi etc.*

INTRODUCTION

The world's number one factor contributing to mental health-related disability is depression, a serious global mental health issue. When depression first appears it often happens in mid- to late-adolescence (between the ages of 14 and 25), with a median 12-month prevalence of 4-5%. Major depressive disorder (MDD) has a negative impact on relationships, career, education, and is potentially linked to premature death, including suicide, obesity, and cardiac illness. When physical health issues are comorbid with depression in persons over the age of 18, the functional impacts of depression can be more severe, complicating treatment options (Ross et al., 2023; Deshmukh et al., 2023). Tricyclic and tetracyclic antidepressants, monoaminoxidase inhibitors, and selective serotonin reuptake inhibitors are among the medications used to treat depression. Common adverse effects of these antidepressant medications include headaches, mydriasis, constipation, dry mouth, transient exhaustion, and restlessness. Many allopathic medicines have been made to reduce depression. But these medicines have many side effects (Richa et al., 2017). Plants or herbs are a natural way to relieve depression. So, our study suggests that there is a natural way to cure depression without any side effects with the plant Habenaria intermedia D. Don commonly known as Vriddhi.

The orchid family member Habenaria intermedia D. Don, is also known as Vriddhi. And distributed at height of 2000–3300 m in the Himalayan Mountain countries, Pakistan, Bhutan, and Nepal. It can be found in India's temperate Himalayas, which include Kashmir, Himachal Pradesh, Uttarakhand, and Sikkim. Out of the 600 different kinds of Habenaria will (Orchidaceae) that exist worldwide, 100 species have been discovered in India (Kumar et al., 2023). Researchers have discovered that H. intermedia contain flavonoids, tannins, steroids, and coumarin glycosides (Kokate et al., 1991). Due to the presence of phenolic compounds including hydroxyl benzoic acid and gallic acid, the species looks to be a substantial source of antioxidants, consequently offering a strong anti-free radical action (Rawat et al., 2014).

Recent studies suggests that flavonoids can also influence the metabolism of brain amines in humans neuronal and neuroendocrine cell lines and the antioxidant capacity of flavonoids also may play a positive role in specific brain pathologies (Osuchowski et al.,2004). However, no sufficient studies have been carried out to explore the role of Habenaria intermedia as an antidepressant modality, to best of our

knowledge. Flavonoids might be responsible for the antidepressant activity. The edible tubers are palatable and emollient; they have aphrodisiac, depurative, anthelmintic, and tonic properties. Leprosy, skin conditions, and asthma can all be helped by tubers (Kirtikar and Basu et al., 1918; Jagetia and Baliga et al., 2004). This plant is a key component of Chyavanprasha, a popular polyherbal rejuvenator (Pandey et al., 2005). Due to its rejuvenating effects, it has been utilized traditionally in various herbal treatments (Jagetia and Baliga et al., 2004). From its rejuvenated effects we can say that it can be used for depression also.

MATERIALS AND METHODS

Plant Material, Solvents, Chemicals, Reagents and Instruments

In July 2024 saw the acquisition of Habenaria intermedia tubers from Hans Raj and Sons, 6549-A, Khari Baoli, New Delhi, India. According to Reference No. NIP-H-3012, dated 05-08-2024, Dr. Alok Goyal, Scientist In-Charge, Natural Products Field Laboratory, National Institute of Pharmaceutical Education and Research, Mohali, Punjab, India, recognized the botanical authority of plant material available with Navjit Kaur Saini.

To prepare the plant extracts and fractions, LR-grade solvents (E Merck, Delhi, India) such as n-hexane, chloroform, methanol, ethyl acetate, and 1-butanol were utilized. The current research effort was conducted using the following instruments: a water bath (Perfit, Ambala, Haryana, India), a digital weighing balance, a hot air oven, a rotary vacuum evaporator, and a Soxhlet apparatus.

Preparation of various extracts and fractions of Habenaria intermedia tubers

In a grinder, the Habenaria intermedia tubers were ground into a powder. Ten kilograms of dried powdered plant material were added to a thimble composed of fine filter paper. Next, the plant was thoroughly extracted using n-hexane in a Soxhlet apparatus until a little amount of droplets that were collected from a siphoning tube on a watch glass evaporated without leaving any residue behind. To obtain chloroform extract, the marc was dried, packed in a thimble, and thoroughly extracted using a Soxhlet device. To obtain methanol extract, the same process was used once the chloroform extraction was finished. The plant material was boiled for two hours on a hot plate with distilled water to prepare the water extract. Using a rotating vacuum evaporator, the solvents from the crude extracts were recovered at lowered pressure.

Fig 1: - Soxhlet extraction apparatus used in the preparation of plant extracts for phytochemical analysis.

A round-bottom flask containing 250 g of methanol extract was filled with 500 ml of distilled water. The material was triturated with a glass rod for thirty minutes to create the extract suspension in distilled water. It was then divided using 50 milliliters of n-hexane by boiling it to 50 degrees Celsius for 30 minutes while stirring constantly. After cooling the contents, the upper layer, known as the n-hexane layer, was separated. New 500 milliliters of n-hexane were added to the extract. The process of partitioning using n-hexane was repeated until a few milliliters of ethyl acetate layer evaporated without leaving any appreciable residue on the watch glass. To ultimately produce n-hexane fraction (HF), all of the separated n-hexane layers were combined and condensed under low pressure. To obtain the ethyl acetate fraction (EAF), the remaining bioactive extract underwent a similar process. A similar process was used to obtain 1-butanol fraction (BF) from the residual bioactive extract. The residual bioactive extract (RBE) was also concentrated via sequential partitioning with n-hexane, ethyl acetate, and 1-butanol. The existence of

distinct groups of phytoconstituents was checked in all plant extracts and fractions of the bioactive extract (Farnsworth, 1966).

Antidepressant Activity

Animals

Swiss Albino mice of either sex, weighing between 20 and 30 grams were employed to assess the antidepressant efficacy. The animals were acquired from the All India Institute of Medical Sciences in New Delhi. Prior to conducting any animal investigations, permission was obtained from Institutional Animal Ethics Committee (IAEC) of ASBASJSM College of Pharmacy, Bela (Ropar) Punjab with approval number ASCB/IAEC/19/24/19.

The animals were maintained in the quarantine area until their health conditions were monitored, at which they were moved to the housing area. The animals were acclimated to its home conditions at the ASBASJSM College of Pharmacy's Central Animal House Facility for seven days. Animals were kept under standard laboratory conditions in polypropylene cages with bedding made of dust-free rice husk and kept at a constant temperature of (23±2°C) and humidity of (40±10%). Mice were fed a standard lab pellet diet and were allowed unlimited access to water. The experiment was carried out between 09:00 and 17:00 hr. The care of laboratory animals was carried out in accordance with the standards set by the CPCSEA, the Ministry of Forests & Environment, and the Government of India.

Vehicle and standard drugs

The vehicle for preparing different test dosages of crude extracts, fractions, and reference medication was distilled water plus 2 percent Tween 80. Imipramine (15 mg/kg, p.o.) was the typical medication used to treat depression.

Acute toxicity studies

Organization for Economic Cooperation and Development (OECD) guidelines-423 (Kumar and Kumar, 2015) were followed in the assessment of the acute oral toxicity of several extracts, including CE and ME of H. intermedia tubers. For each series of experiments, a single oral dose of 2000 mg/kg (limit test) was administered to six mice.

Experimental design

Two experimental protocols were designed consisting of 14 groups of mice.

Experimental protocol I was created to evaluate the antidepressant potential of several crude extracts of H. intermedia tubers, encompassing groups 1 through 8. Group 1 was the control group and was given vehicle (0.25 ml, p.o.); Group 2 was the standard group and was given imipramine (15 mg/kg, p.o.); Groups 3,4 and 5 received doses of CE of 100, 200, and 400 mg/kg, respectively; Groups 6, 7 and 8 received doses of ME of 100, 200, and 400 mg/kg.

Experimental protocol II, The purpose of groups 9 through 14 was to evaluate the antidepressant potential of different H. intermedia tubers fractions. Group 9: This group was given a vehicle (0.25 ml, p.o.); Group 10: This group was given imipramine (15 mg/kg, p.o.); Groups 11 and 12: This group was given a dose of 25 and 50 mg/kg of EAF; Groups 13 and 14: This group was given a dose of 25 and 50 mg/kg of BF.

Forced swim test

Water was poured into a plexiglass cylinder of 40 cm in height by 18 cm in diameter to a depth of 15 cm (Richa et al., 2017). The temperature of water was maintained at 25 ± 2°C throughout the experiment. Mice were made to swim in the cylinder for six minutes following the administration of a vehicle, test drug, or standard drug for 45 minutes. The entire amount of time the animal remained immobile that is, floating in the water with its nose above the surface was recorded during this test period.

(a) (b)

Fig 2: Animal exposed to force swim test (a) immobile phase (b) mobile phase

Statistical analyses

The findings were presented in the form of mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare the test medications with the standard drug and control, and Student Newman Keul's test was then administered (Scheffer, 1980).

RESULTS AND DISCUSSION

The percentage yields (% w/w) of various extracts prepared by Soxhlet technology (Kumar and Kumar, 2017) of H. intermedia tubers named HE, CE, ME and WE were found to be 0.60, 0.51, 3.52 and 2.68 % w/w respectively.

Because of its possible pharmacological properties, which include antineoplastic, antimicrobial, antioxidant, anti-inflammatory, analgesic, anti-diabetic, anti-hypertensive, and antidiarrheal effects, medicinal plants have been utilized to treat a wide range of illnesses. A medicinal plant's therapeutic value is determined by its phytoconstituents, either separately or in combination. Among the significant phytochemicals with a wide range of biological activity are alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, and terpenes. By identifying the phytochemicals in a plant, one can anticipate its pharmacological activity. Although phytochemicals are now identified using a variety of contemporary methods, traditional qualitative assays are still widely used for plants' initial phytochemical screening (Kumar and Kumar, 2016). Several types of components found in different crude extracts of H. intermedia tubers were qualitatively screened using general chemical reagents. The results of preliminary chemical testing suggested the presence of fixed oils in HE, alkaloids, coumarins in CE, flavonoids, tannins, coumarins in ME and carbohydrates, proteins in WE.

Amongst many unstandardized extracts, only CE and ME contained bioactive phytomolecules on the basis of preliminary chemical testing whereas HE and WE did not contain any bioactive phytomolecules. Therefore, only CE and ME were subjected to acute toxicity and antidepressant activity.

According to OECD-423 guidelines, an acute oral toxicity study of CE and ME of H. intermedia tubers was conducted at a dose of 2000 mg/kg/b.w/p.o. Mice did not exhibit any mortality from the crude extracts. Additionally, for 24 hours, the autonomic (urination and defecation), neurological (spontaneous activity, reactivity, touch reaction, and pain response), and behavioral (alertness, restlessness, irritability, and fearfulness) profiles of treated mice were monitored. However, during this experimental time, no adverse responses or mortality were seen in mice. The dose is considered "unclassified" on the toxicity scale in accordance with OECD-423 guidelines. Therefore, additional research with lower dosages was not carried out.

It is clear from phytochemical screening results that no major class of phytoconstituents was detected during the phytochemical screening of HE and WE. Therefore, the forced swim test was used to screen mice for antidepressant activity in CE and ME. When mice were made to swim in a restricted space, the mean amount of time they spent motionless was used to measure the antidepressant activity of the test medications. Using a normal statistical analysis procedure, the outcomes of the test medications were compared to those of the control and standard pharmaceuticals. Table 1 and Figure 3 display the length of immobility that mice displayed following acute administration of 100, 200, or 400 mg/kg, p.o. dosages of crude extracts, imipramine (15 mg/kg, p.o.), and the control (vehicle, p.o.). Only ME showed substantial antidepressant efficacy compared to the control among the two crude extracts. Although the ME (100, 200 mg/kg) considerably shortened the mice's immobility period when compared to the control, it was unable to reach a therapeutic level that was comparable to that of the conventional medication. ME reached a therapeutic level comparable to that of the conventional medication at a greater dose, 400 mg/kg. Table 1 makes it abundantly clear that, at a dose of 400 mg/kg, the ME considerably decreased the amount of time the mice spent immobile (antidepressant action), which is statistically equal to what was observed in animals given a standard medication. Conversely, CE had a slight antidepressant effect. At dosages of 100, 200, or 400 mg/kg, CE of the plant considerably decreased the amount of time that mice were immobile compared to the control group, but it was not comparable to the usual medication. ME is the most bioactive extract of H. intermedia tubers, according to these findings.

Table 1: Antidepressant activity of CE and ME of H. intermedia tubers using FST.

Treatment	Dose (mg/kg)	Mean^{n} immobility time (sec)
Control	Vehicle	285.80 ± 8.10^{a}

Imipramine	15	61.80 ± 5.80*
CE	100 200 400	191.20 ± 11.81*a 165.60 ± 6.26*a 145.00 ± 7.90*a
ME	100 200 400	103.20 ± 5.80*a 88.20 ± 6.01*a 65.40 ± 9.78*

Mean ± S.D., *P<0.05 vs. Control, aP<0.05 vs. Standard medication (Imipramine), one-way ANOVA, and Student-Newman-Keul's test are used to express the data for n=5.

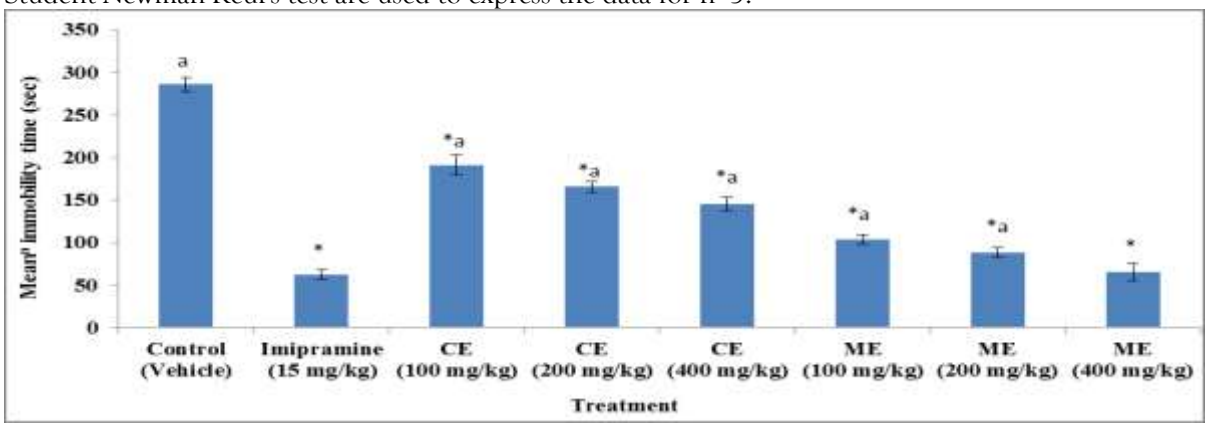

Figure 3: The duration of time that mice were immobile following treatment with a vehicle, a marketed medication, and different H. intermedia tuber extracts. ME stands for methanol extract, and CE for chloroform extract.

The methanol extract showed maximum antidepressant activity and the bioactive methanol extract was further purified by fractionation into different fractions in the order of increasing polarity such as n-hexnae fraction (HF), ethyl acetate fraction (EAF), 1-butanol fraction (BF) and remaining bioactive extract (RBE) and their percentage yields (% w/w) were found to be 1.70, 21.60, 18.23 and 54.89 (% w/w, respectively, in relation to methanol extract. The results of chemical testing suggested that HF and RBE did not show any sign of presence of phytomolecules. Only EAF contained all three types of natural compounds such as phenols, flavonoids, coumarins whereas BF contained coumarins as natural compounds.

Therefore, only EAF and BF were tested for antidepressant efficacy in mice using FST. The average duration of time the mice were immobile following the administration of EAF (25 or 50 mg/kg, p.o.), BF (25 or 50 mg/kg, p.o.), imipramine (15 mg/kg, p.o.), and the control (vehicle, p.o.) is displayed in Table 2 and Figure 4. At a dose of 50 mg/kg, only EAF demonstrated substantial antidepressant action compared to the control; this activity was statistically equal to that of the conventional medication. When compared to a normal medication, BF was unable to decrease the amount of time that mice were immobile, hence it did not reach the therapeutic level.

Table 2: Antidepressant activity of EAF and BF of H. intermedia tubers using FST.

Treatment	**Dose (mg/kg)**	**Mean^n immobility time (sec)**
Control	Vehicle	271.80 ± 5.80a
Imipramine	15	65.60 ± 7.30*
EAF	25 50	81.80 ± 5.80*a 68.40 ± 5.94*
BF	25 50	180.60 ± 7.47*a 155.00 ± 5.24*a

Mean ± S.D., *P<0.05 vs. Control, aP<0.05 vs. Standard medication (Imipramine), one-way ANOVA, and Student-Newman-Keul's test are used to express the data for n=5.

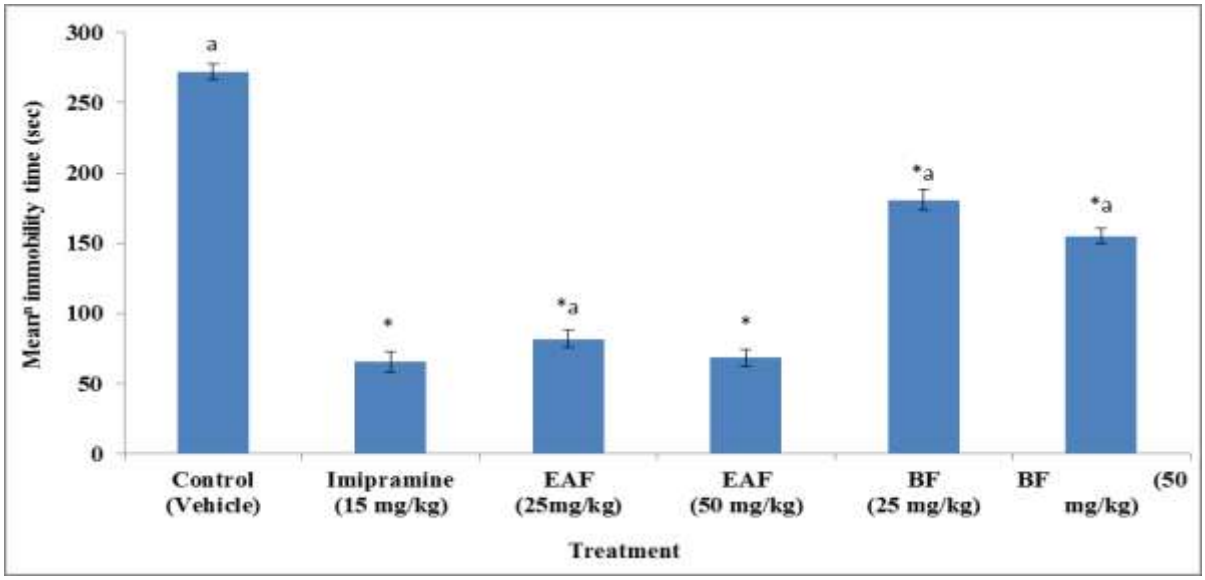

Figure 4: The duration of time that mice were immobile following treatment with a vehicle, a marketed medication, and different H. intermedia tuber fractions of bioactive extract. EAF stands for ethyl acetate fraction and BF for 1-butenol fraction.

The forced swim test, a well-researched experimental technique, was used to evaluate the antidepressant properties of Habenaria intermedia tubers. Mice are made to swim in a restricted space from which they are unable to escape during this test. Mice initially attempt to get out of the confined area while staying mobile. Mice adjust to their distinctive immobility after a few seconds. As a measure of the test drugs' antidepressant effectiveness, the length of immobility is noted (Weiss et al., 1981).

The model was selected because it works well, is inexpensive, easy to use, takes less time, doesn't require any prior training for the mice, and doesn't give the animals much distress when handled. The concept is mostly based on the findings that mice that are made to swim in a confined area from which they are unable to escape exhibit immobility as a typical trait. This conduct is indicative of a state of despair that can be lessened by a number of therapeutically effective medications for depression in humans. The reduction in motor activity, which is determined by the amount of time the animal spends immobile, is the final sign of depression in animals.

The presence of flavonoids, phenols, and coumarins in the bioactive extract and/or fraction of aerial parts of H. intermedia tubers was revealed by preliminary phytochemical tests. Our findings are consistent with the literature that has been reported to show antidepressant activity for flavonoids such as naringenin (Yi et al., 2014; 2012), icariin (Gong et al., 2016), hesperidin (Donato et al., 2014), chrysin (Filho et al., 2015); phenols such as gallic acid, protocatechuic acid, gentisic acid, salicylic acid and syringic acid, ferulic acid, ellagic acid (Kumar and Goel, 2019; Russell and Duthie, 2011; Saibabu et al., 2015; Lafay and Gil-Izquierdo, 2008) and coumarins such as scopoletin (Capra et al., 2010), psoralen (Xu et al., 2008), lacinartin (Jo et al., 2002), auraptene, umbelliferone (Jeong et al., 2006). On the basis of exhaustive scrutiny of literature survey suggested that antidepressant activity of H. intermedia tubers may be due to the flavonoids, phenols, and/or coumarins present therein.

The various mode of actions for antidepressant activity of plant based phytomolecules reported in scientific databases such as diosmin exhibit antidepressant activity by acting direct action on γ-aminobutyric acid type A ($GABA_A$) receptors (Fernandez et al., 2006); hypaconitine exhibit antidepressant activity via modulation of sensitivity to serotonin (Nesterova et al., 2010); montanine exhibit antidepressant activity via block chloride channels of $GABA_A$ receptors (Silva et al., 2006); glyco withanolides exhibit activity by acting on GABA receptors (Bhattacharya et al., 2000) and hesperidin exhibit antidepressant activity via mediated by inhibition of l-arginine-NO-cGMP pathway and elevation of BDNF levels in hippocampus (Donato et al., 2014). These literature survey of mode of actions suggested that H. intermedia tubers exhibited antidepressant profile may be due to the above-mentioned mode of actions.

CONCLUSION

Finally, it can be suggested that phenolic, flavonoidal and coumarin type of phytomolecules may be responsible for antidepressant activity of H. intermedia tubers. These types of phytomolecules will be isolated from bioactive extract / fraction using column chromatography studies in future research work.

Acknowledgement

Authors duly acknowledge Department of Pharmacognosy and Head of the Department Pharmacology Dr. Ajay Singh Kushwah and Associate Professor Dr. Rahul Kumar Sharma of ASBASJSM College of Pharmacy, Bela (Ropar) Punjab, India for providing necessary instrumentation facilities for completion of research work and valuable assistance in animal handling during the course of this research.

REFERENCES

1. Bhattacharya SK, Bhattacharya A, Sairam K and Ghosal S, 2000. Anxiolytic-antidepressant activity of Withania somnifera glycowithanolides: an experimental study. Phytomedicine, 7(6), 463-469.
2. Capra, J.C., Cunha, M.P., Machado, D.G., Zomkowski, A.D., Mendes, B.G., Santos, A.R.S., Pizzolatti, M.G., Rodrigues, A.L.S., 2010. Antidepressant-like effect of scopoletin, a coumarin isolated from Polygala sabulosa (Polygalaceae) in mice: evidence for the involvement of monoaminergic systems. Eur. J. Pharmacol. 643, 232–238.
3. Deshmukh, N. N., Vyas, J. V., Paithankar, V. V., & Wankhade, A. M. (2023). A review on medicinal herbs with potential anti-depressant activities. Research Journal of Pharmacognosy and Phytochemistry, 15(3), 230-234.
4. Donato, F., de Gomes, M.G., Goes, A.T.R. et al., "Hesperidin exerts antidepressant-like efects in acute and chronic treat-ments in mice: Possible role of l-arginine-NO-cGMP pathway and BDNFlevels," Brain Research Bulletin,vol.104,pp.19–26, 2014.
5. Farnsworth, N. R. (1966). Biological and phytochemical screening of plants. Journal of Pharmaceutical Sciences, 55, 225-276.
6. Fernandez SP, Wasowski C, Loscalzo LM, Granger RE, Johnston GAR, Paladini AC and Marder M, 2006. Central nervous system depressant action of flavonoid glycosides. European Journal of Pharmacology, 539, 168–176.
7. Filho, C.B., Jesse, C.R., Donato, F., et al., "Chronic unpredictable mild stress decreases BDNF and NGF levels and Na+,K+-ATPase activity in the hippocampus and prefrontal cortex of mice: antidepressant efect of chrysin," Neuroscience,vol.289, pp. 367–380, 2015.
8. Gong, M.J., Han, B., Wang, S.M., Liang, S.W., Zou, Z.J. Icariin reverses corticosterone-induced depression-like behav-ior, decrease in hippocampal brain-derived neurotrophic factor (BDNF) andmetabolic network disturbances revealed by NMR-based metabonomics in rats," Journal of Pharmaceutical and Biomedical Analysis,vol.123,pp.63–73,2016.
9. Jagetia, G. C., & Baliga, M. S. (2004). The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants in vitro: A preliminary study. Journal of Medicinal Food, 7(3), 343-348.
10. Jeong, S.H., Han, X.H., Hong, S.S., Hwang, J.S., Hwang, J.H., Lee, D., Lee, M.K., Ro, J.S., Hwang, B.Y., 2006. Monoamine oxidase inhibitory coumarins from the aerial parts of Dictamnus albus. Arch Pharm. Res. (Seoul) 29, 1119–1124.
11. Jo, Y.S., Huong, D.T.L., Bae, K.H., Lee, M.K., Kim, Y.H., 2002. Monoamine oxidase inhibitory coumarin from Zanthoxylum schinifolium. Planta Med. 68, 84–85.
12. Kirtikar, K. R., & Basu, B. D. (1918). Indian medicinal plants (Vol. 2). Publisher not identified Basu, Bhuwaneśwari Asrama.
13. Kokate, C. K., Khandelwal, K. R., Pawar, A. P., & Gohalz, S. B. (1991). Practical Pharmacognosy. Vallabh Prakashan.
14. Kumar, C., Chandan, G., Kushwaha, M., Kumar, A., Kaur, S., Kumar, A., & Verma, M. K. (2023). Discovery of Anti-NRLP3 Inflammasome, Immunomodulatory Phytochemicals from the Extract of Habenaria intermedia D. Don: An Unexplored Plant Species. ACS Omega, 8(34), 31112-31122.
15. Kumar, D., Kumar, S. A complete monographic study on Abies pindrow Royle aerial parts. Indian Journal of Pharmaceutical Sciences, 2017, 79(6), 1001-7.
16. Kumar, D., Kumar, S. Pharmacognostic standardization of Calotropis gigantea (l.) Dryand roots. Indian Drugs, 2016, 53(10), 16-22.
17. Kumar, D., Kumar, S. Screening of antianxiety activity of Abies pindrow Royle aerial parts. Indian Journal of Pharmaceutical Education and Research, 2015, 49(1), 66-70.
18. Kumar, N.; Goel, N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnol. Rep. 2019, 24, e00370.
19. Lafay, S.; Gil-Izquierdo, A. Bioavailability of phenolic acids. Phytochemy 2008, 7, 301–311.
20. Nesterova YV, Povetieva TN, Suslov NI, Semenov AA and Pushkarskey SV, 2010. Antidepressant activity of diterpene alkaloids of Aconitum baicalense Turcz. Bulletin of Experimental Biology and Medicine, 151(4), 425-428.
21. Osuchowski, M., Johnson, V., He, Q., & Sharma, R. (2004). Alterations in regional brain neurotransmitters by silymarin, a natural antioxidant flavonoid mixture, in BALB/c mice. Pharmaceutical Biology, 42(4-5), 384-389.
22. Pandey, D. (2005). Sarangadharasanhita. Chaukhamba Amara Bharati Prakashan.
23. Rawat, S., Andola, H., Giri, L., Dhyani, P., Jugran, A., Bhatt, I. D., & Rawal, R. S. (2014). Assessment of nutritional and antioxidant potential of selected vitality strengthening Himalayan medicinal plants. International Journal of Food Properties, 17(3), 703-712.
24. Richa, Kumar, D., & Kumar, S. (2017). Screening of antidepressant activity and marker-based standardization of Baptisia tinctoria (L.) R. Vent. Indian Journal of Pharmaceutical Sciences, 79(3).
25. Ross, R. E., Van Derwerker, C. J., Saladin, M. E., & Gregory, C. M. (2023). The role of exercise in the treatment of depression: Biological underpinnings and clinical outcomes. Molecular Psychiatry, 28(1), 298-328.
26. Russell, W.; Duthie, G. Session 3: Influences of food constituents on gut health: Plant secondary metabolites and gut health: The case for phenolic acids. Proc. Nutr. Soc. 2011, 70, 389–396.
27. Saibabu, V.; Fatima, Z.; Khan, L.A.; Hameed, S. Therapeutic Potential of Dietary Phenolic Acids. Adv. Pharmacol. Sci. 2015, 2015, 823539.

28. Scheffer, W.C., 1980. Statistics for the Biological Sciences. Addison-Wesley Publishing Company, Philippines, 121-141.
29. Silva AF, de Andrade JP, Bevilaqua LR, de Souza MM, Izquierdo I, Henriques AT and Zuanazzi JA, 2006. Anxiolytic-, antidepressant- and anticonvulsant-like effects of the alkaloid montanine isolated from Hippeastrum vittatum. Pharmacology, Biochemistry and Behavior, 85(1), 48-54.
30. Weiss, J.M., Goodman, P.A., Losito, G.O., Corrigan, S., Carris, J.M., and Bailey, W.H., 1981. Behavioural depression produced by uncontrollable stressor: Relationship to norepinephrine, dopamine and serotonin levels in various regions of rat brain. Brain Research. 3, 167-205.
31. Xu, Q., Pan, Y., Yi, L.-T., Li, Y.-C., Mo, S.-F., Jiang, F.-X., Qiao, C.-F., Xu, H.-X., Lu, X.-B., Kong, L.-D., 2008. Antidepressant-like effects of psoralen isolated from the seeds of Psoralea corylifolia in the mouse forced swimming test. Biol. Pharm. Bull. 31, 1109–1114.
32. Yi, L.T., Liu, B.B., Lietal, J. BDNF signaling is necessary for the antidepressant-like efect of naringenin. Progress in Neuro-Psychopharmacology & Biological Psychiatry,vol.48,pp.135–141, 2014.