Exploration of anxiolytic activity of *Lagenaria siceraria* with mechanistic action via $GABA_A$ ionotropic receptors

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Abstract

Lagenariasiceraria is commonly called as bottle gourd.It is a rich source of bioactive constituents. In the present study, anxiolytic potential along with receptor antagonist i.e. flumazenil was evaluated in albino mice. The animals were administered with different doses of plant extract and different behavioral paradigms such as hole board, light and dark, stair case and elevated plus maze were employed. The results obtained from the current study showed significant anti-anxiety effects however flumazenil i.e. receptor antagonist failed to block the anxiolytic effect of extract.

Keywords

Lagenaria siceraria, flumazenil, albino mice.

1. INTRODUCTION

Anxiety is defined as the state of extreme uncertainty and fear that results in future threat (O'Donovan *et al*. 2013). It is the most common prevailing disease globally and has become an important area of research in psychopharmacology. Despite of commercially available anxiolytic agents such as benzodiazepine and barbiturates, the adverse effects such as dependance, sedation are associated with these drugs leading to treatment discontinuation in patients (Mandrioli & Mercolini *et al*., 2015). Consequently, there has been immense need to explore novel anxiolytic agents that can overcome such complications (Ballinger *et al*. 1990).

Now a days active research is underway to investigate safe pharmacologically active herbal medicines. Previously, numerous traditional plants such as Hibiscus rosa sinensis, Raphanus sativus, Moringa oleifera etc. have been reported with remarkable therapeutic application in the treatment of anxiety (Faustino *et.al*., 2010).

Lagenaria siceraria (Molina) Standley, commonly known as bottle-gourd belongs to the family *Cucurbitaceae*. The plant is widely grown throughout the sub-continent includingPakistan and India. It is a mounting herb, with bottle or dumb-bell shaped fruits.

The aerial parts are traditionally consumed as a vegetable (Fig-1).

Lagenaria siceraria fruit contain rich amount of ascorbic acid, fibers, proteins, vitamin B complex, saponins, flavone-Cethan-glycoside, polyphenolics and fucosterols (Duke, 1999, Sonja, 2000, Baranawska *et al.* 1994, Shah *et al*., 2010). It also contain aglycones, curcunitacins and 22-deoxycucurbitacin. The seeds and fruit of the plant contain lagenin, which possess ribosome inactivating activity, antitumour, anti HIV, immunoprotective and antiproliferative properties (Wang *et al*., 2000). Apart from lagenin, the plant also contain higher percentage of choline, which is involved in brain functioning (Rahman *et al*., 2003).

Traditionally, it has been used for the treatment of jaundice, ulcer, diabetes, piles, hypertension, insanity, colitis, hypertension, skin diseases and cardiotonic (Kirtikar *et al*., 2003). Moreover, it has also been reported for its hypolipidemic, hypoglycemic,hepato-protective, antioxidant, cardio protective, immunomodulatory effects, hyperthyroidism, hyperglycemia and lipid peroxidation, analgesic and anti-Inflammatory, diuretic, cytotoxic activity (Shirwaikar *et al*., 1996; Ghule *et al*., 2006; Deshpande *et al*., 2007; Deshpande *et al*., 2008; Shah, Seth 2008, Kubde *et al*. 2010).

Previously, the plant has also been reported for antide pressant, anxiolytic and sedative activities (Muhammad *et al*. 2013), however the aim of the present study was to evaluate the anxiolytic activity of *Lagenaria siceraria* seeds in mice along with possible anxiolytic mechanism via GABA ionotropic receptor antagonistic activity that has not been reported yet.

2. MATERIALS AND METHOD

2.1. Collection of Plant Material

The seeds of *Lagenaria siceraria* werepurchasedfrom the local market of Karachi in 2016. The taxonomic identification was conducted by Botanist at Faculty of Pharmacy, Hamdard University Karachi.

2.2.Preparation of Plant Material

The air-dried seeds (10 g) of *Lagenaria siceraria* were powdered and then macerated in ethanol (98 % w/v) for 15 days. The crude extract was filtered and evaporated under reduced pressure to give a viscous dark mass (2.145 g). The concentrated extract of 14.3% w/w yield was obtained. This extract was stored at 4°C in an airtight container.

2.3.Selection and Housing of Animals

Swiss albino mice of weighing 20 – 30 g were procured from Animal house of Hafiz Muhammad Ilyas Research lab (HMI), Hamdard University. Animals were kept under controlled environmental (25±2 ^{0}C) temperature with 12/12 hours light-dark cycle with free access to water and standard diet. Use and care of animals were carried out in accordance with the guidelines of the 'Principles of Laboratory Animal Care' (NIH publication 1985).

2.4.Drug treatment

Mice were randomly divided into four groups;each group contains seven (n=7) animals. Following are the specifications of each group: Control: Distilled water 1ml/kg, Standard: Diazepam 1 mg/kg, EELS I: Ethanolic extract of

Lagenaria siceraria seeds 200 mg/kg and EELS II:Ethanolic extract of *Lagenaria siceraria* seeds 400 mg/kg.

The dose of the extract was calculated rendering to body weight of the animals (Wang, 2000, Duke, 1992). Dilution was prepared by dissolving extract in distilled water using Tween 80 as a suspending agent. All drugs were administered via oral intubation between 8-10 am for 14 consecutive days, respectively.

2.5. Receptor Antagonist Activity Exploration.

At day 14th, flumazenil (Sigma)(0.5 mg/kg, i.p. in 2%DMSO-water mixture) was given to standard and EELS II groups, 1 h before diazepam and EELS (400 mg/kg) treatment respectively for identification of receptor antagonist mechanism.

2.6. Anxiolytic Activity:

All anxiolytic activities were performed at 1 and 14th day of treatment as described below:

2.6.1. Hole-board Test

The hole-board apparatus used was consisting of a rectangular plexiglass box (40 × 40 × 30 cm) with 4 holes equally spaced with a diameter of 3 cm on the floor. Animal from each group was placed in the center of arena to explore the apparatus for duration of 5 min. the total number of head dips and rearing behavior of mice were observed (Hossain and Uma Devi 2001).

2.6.2. Light/Dark Box Test

The apparatus consisted of two plexiglas boxes of (25×25×25 cm) joined together by a small opening (7 × 7 cm high).). The dark area was opaque with black walls without access to any light source whereas, the light area was transparent and equipped with a 40-W illuminated lamp. The light source was mounted 25 cm above the light area. For the assessment of anxiolytic activity animal of the respective group was placed individually in light area after treatment. Time spent and number of entries in light area were recorded for 5 min trial (Jain *et al*., 2003).

2.6.3. Staircase Activity

Staircase apparatus consisted of five identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. Mice was placed singly on the floor of the box with its back to the staircase. The number of steps climbed and number of rears during 5 min duration. After each step the apparatus was cleaned with ethanol swab. A step was considered when mice had all four paws on the step (Soulimani *et al*., 1991).

2.6.4. Elevated Plus Maze Test

The apparatus consisted of two open arms (35×5 cm) and two closed arms (35 × 5 × 20 cm) connected in plus figure with a central square of 5×5 cm was used. It was placed 40 cm above the floor. After treatment each animal was placed at the center of the maze, facing one of the closed arms. Observations were recorded during 5 min duration (Lister et al. 1987). The number of entries into open arms and the time spent in open arms were noted.

2.7. STATISTICAL ANALYSIS

The results were expressed as Mean ± SEM. Data was analyzed by SPSS version: 20 using one-way ANOVA followed by post hoc Scheffe test. Statistical significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

Hole Board Test

The results of hole board test are depicted in

Table 1. After the administration of ethanol seed extract of *Lagenaria siceraria* 200 and 400 mg/kg significantly decreased the number of head dips at 1st and 14th test session. However, the extract showed considerable increase in rearing behavior after day 1 of treatment in comparison with control. Whereas, no significant increase in rearing behavior was observed at day 14th of test session. These observed effects were similar to that of standard anxiolytic diazepam with significant reduction in number of head dips and augmentation in number of rearing behaviors.

Table 1a-Effects of EELS on no. of head dips in Hole board Test

	No. of Head dips	
TREATMENT	**DAY 1**	**DAY 14**
Control (Distilled water 1 ml/kg)	24.5±7.7	29.16±8.18
Standard (Diazepam 1 mg/kg)	58.5±7.86**	46.33±12.46**
Standard (Diazepam 1mg/kg) + Flumazenil (0.5mg/kg)	-	11.13±10.22**
EELS I (200 mg/kg)	50±4.93**	35.83±5.23**
EELS II (400 mg/kg)	59.5±6.09**	27.83±6.79**
EELS II (400 mg/kg) + Flumazenil (0.5mg/kg)	-	37.83±5.02**

Data expressed as mean ± SEM; n= 7; *P<0.05 and ** P<0.005

Table 1b-Effects of EELS on no. of rearings in Hole board Test

TREATMENT	**No. of rearings**	
	DAY 1	**DAY 14**
Control (Distilled water 1 ml/kg)	1.83±1.03	0.666±0.89
Standard (Diazepam 1 mg/kg)	3.33±1.1**	2.0±0.81*
Standard (Diazepam 1mg/kg) + Flumazenil (0.5mg/kg)	-	0.4±0.63*
EELS I (200 mg/kg)	3±0.89**	0.5±0.54

EELS II (400 mg/kg)	4.5±1.37**	0.66±0.95
EELS II (400 mg/kg) + Flumazenil (0.5mg/kg)	-	0.54±0.91

3.1. Light and Dark Box Test

Results obtained from light dark test showed significant increase in number of entries and time spent in light area after administration of Diazepam in comparison with control group. Similar results were obtained by EELS I and II with significant escalation in number of entries and time spent in light area after day 1 and 14 day of treatment (Shown in table 2).

Table 2-Effects of EELS on mice in Light and Dark Test

	Time spent in light area (sec)		No. of entries in light area	
TREATMENT	**DAY 1**	**DAY 14**	**DAY 1**	**DAY 14**
Control	72.33 ± 6.83**	71.0 ± 6.41**	5.8 ± 0.5	5.7 ± 0.5
Standard	53.83 ± 4.79**	202.5 ± 11.96**	14.1 ± 0.5**	14.8 ± 0.6**
Standard + Flumazenil		91.72 ± 10.11**		4.5 ± 0.42**
EELS I	56.83 ± 5.38**	197 ± 11.61**	15.2 ± 0.7**	16 ± 0.7**
EELS II	68.33 ± 3.66**	182.66 ± 15.09**	12.8 ± 0.6**	16.2 ± 0.35**
EELS II + Flumazenil		191.65 ± 11.11**		18.2 ± 0.31**

Data expressed as mean ± SEM; n= 7; *P<0.05 and ** P<0.005.

3.2.Staircase Activity

Mice treated with ethanolic extract of *Lagenaria siceraria seeds* 200 and 400 mg/kg produce significant increase in number of stairs climbed with decline in rearing behavior. These observations were comparable with standard anxiolytic drug as shown in (table 3).

Table 3- Effects of EELS on mice in Stair Case Test

	No. of stairs climbed		No. of Rearing	
TREATMENT	**DAY 1**	**DAY 14**	**DAY 1**	**DAY 14**
Control	9.7± 0.6	9±0.43	8±0.4	8.4 ± 0.3
Standard	13.5± 0.48*	24.2±0.47**	6 ± 0.4*	17.1 ± 0.4*
Standard + Flumazenil	-	11.2±0.12**	-	7.3 ± 0.4*
EELS I	16.5± 0.6**	16.1±0.7**	5.6 ± 0.3**	6.3 ± 0.5**
EELS II	14± 0.6**	17± 0.4**	4.5 ± 0.6**	9.6 ± 0.6**
EELS II + Flumazenil	-	17.2± 0.6**	-	8.5 ± 0.2**

Data expressed as mean ± SEM; n= 7; *P<0.05 and ** P<0.005.

3.3. Elevated Plus Maze

As depicted in table 4, the administration of Diazepam significantly increased the time spent in the open arms as compared to control group. The seed extract of *Lagenaria siceraria* at 200 and 400 mg/kg illustrated significant augmentation in the time spent in the open arms. Meanwhile the number of entries in open arm was also remarkably increased by EELS I and II after 1 and 14 days of treatment.

Table 4-Effects of EELS on mice in Elevated Plus Maze Test

	No. of entries in open arm		Time spent in open arm (sec)	
TREATMENT	**DAY 1**	**DAY 14**	**DAY 1**	**DAY 14**
Control	4.16 ± 0.752	4.33 ± 0.51	16 ± 0.5	15.7 ± 0.6
Standard	16.0 ±2.09**	19.33 ± 1.86**	32 ± 0.5**	33 ±0.6**
Standard + Flumazenil	-	6.21±1.25**	-	13± 0.4**
EELS I	5.16 ±1.16**	8.5 ±1.64**	12.6 ± 0.9**	23 ±0.7**

EELS II	6 ± 1.41**	14.33 ± 2.58**	12 ± 0.9**	32.3 ± 0.4**
EELS II + Flumazenil	-	13.37 ± 3.15**	-	33.1± 0.2**

Data expressed as mean ± SEM; n= 7;*P<0.05 and ** P<0.005.

Anxiety is the most prevailing neurological disorder that affect almost 3.6% of global population (Organization 2017). Different anxiolytic drugs i.e.; benzodiazepines, Selective serotonin reuptake inhibitors (SSRI's), Serotonin-Norepinephrine reuptake inhibitors (SNRI's) and tricyclic antidepressants, have been conventionally used for the treatment of anxiety disorder. Despite of multiple treatment options, the adverse effects associated with these medications lead to treatment failure (Lader, Morton, 1991). To eliminate these complications, researchers are investigating for safer, specific and low-cost therapies. Recently, conventional medicine has been reevaluated worldwide by extensive research on therapeutic principles of plants and different plant species have been reported for their anxiolytic potential (Harvey 2008, Nanumala 2018, Bum 2011, Siddiq and Younus 2018). In the current study, the anxiolytic activity of ethanolic extract of *L. siceraria* seeds at 200 and 400 mg/kg were determined using different behavioral animal models.

In our study, there was significant decline in number of head dips in mice by EELS 200 and 400 mg/kg. Decrease in head dipping behavior of animal represents escape response of animal and reduction in neophilia (Gillian and Christopher 2008). Whereas, significant increase in rearing behavior of mice was also observed at doses 200 and 400 mg/kg at 1 and 14th day of test session. These results were comparable with reference standard diazepam (5 mg/kg). The considerable reduction in head dips and augmentation in rearing behavior indicates decrease in anxiety and anxiolytic effect of plant extract (Assad and Khan, 2017).

Anxiolytic effect of *L. siceraria* (200 and 400 mg/kg) was further investigated in light/ dark test. In light dark test, animals treated with EELS I and II showed significant increase in time spent in light area in comparison with control at day 1 to 14, which represents the exploratory behavior of mice and potential aversion to novel area, similar to Salvia elegans (Herrera-Ruiz, 2006). However, the rearing behavior of mice was considerably by EELS at dose 400 mg/kg after acute treatment, which represents the investigatory behavior of animal (Arrant, 2013). Similar results were obtained with diazepam 5 mg/kg with increase in time spent in light area and significant escalation in rearing behavior in day 1 to 14 test sessions. Results obtained from EELS II were comparable with standard drug.

In addition to this, significant increase in number of stairs climbed was observed by EELS I and II in stair case test. A significant increase in number of steps climbed reflects the locomotory activity of animal when exposed to novel environment (Emmanouil, 1990). Whereas, number of rearing was significantly reduced by *Lagenaria siceraria* at both doses at day 1 and 14th test sessions. The escalation in climibing and reduction in rearing behavior represents the anxiolytic activity of EELS, similar to *Myrtus communis* L, *Eschscholzia californica Cham and Carica papaya L* (Haliu, 2012, Rolland 2001,Kebebew, 2013). Meanwhile, diazepam (1 mg/kg) produced

considerable augmentation in number of stairs climbed and reduction in rearing behavior.

Elevated plus maze model is one of the renowned animal models employed for investigation of anxiolytic agents (Pellow S, 1985). It is well established that drugs having anxiolytic effect increases the number of entries and time spent in open arm (Helton, 1996). In the present study, diazepam (5 mg/kg) showed significant increase in the number of entries in open arm. Similar effect was observed by EELS I and II. On the other hand, *Lagenaria siceraria* depicted considerable augmentation in number of rearing at dose 200 and 400 mg/kg in EPM. This indicates the anxiolytic activity of EELS with increase in number of entries in open arm and rearing behavior, similar to previously published reports (Maqbool 2019, Mendonça 2009).

The role of GABA in pathophysiology of anxiety is well established and the widely used anxiolytic agent benzodiazepines, produce its effect via acting on GABA receptors (Rang, 2003). Likewise, plants have also been reported to produce anxiolytic effect through GABAergic pathway (Begum 2018, Ishola 2013). Similarly, in this experiment the administration of flumazenil (0.5 mg/kg), a GABA receptor antagonist was done to investigate that whether anxiolytic effect of EELS is produced by involvement of GABAergic pathway or not. Results demonstrated that flumazenil did not antagonize the anxiolytic like effect of EELS in different behavioral paradigms. This suggest that the anxiolytic property of *Lagenaria siceraria* might be attributed by neuronal pathway other than GABA.

Clinical studies support the involvement of serotonin in the pathophysiology of anxiety (Kahn *et al*., 1988). In the recent animal studies on different plant extracts indicated the contribution of serotonergic pathway to produce anxiolytic effect (Perveen et al 2009, Diniz *et al*., 2019).). On this basis it can be assumed that anxiolytic effect of *Lagenaria siceraria* seeds might be contributed due to involvement of serotonergic pathway. Whereas, some ergot, indole and different secondary alkaloids of plants were also reported to produce their anxiolytic effect *via* acting on dopaminergic and nor adrenergic pathway (Pertz *et al*., 1999; Both *et al*., 2006; lee *et al*., 2012). So, it can be predicted that the anxiolytic properties of *L. siceraria* seeds may be due to other than GABA mechanisms possibly by the dopaminergic and nor adrenergic pathway, as the seeds of *Lagenaria siceraria* contain large amount of lagenin, curcunitacins, aglycones and several other secondary alkaloids that might be responsible to reduce anxiety in mice (Ojiako *et al*., 2007, Sood *et al*., 2012).

Furthermore, research studies are required to emphasize the therapeutic potential of *L. siceraria* seeds in treatment of anxiety. Moreover, it is also important to evaluate the possible neurochemical pathway involved in contributing anxiolytic activity of *Lagenaria siceraria* seeds.

4. CONCLUSION

The current work explores the anxiolytic potential of ethanol extract of *Lagenaria siceraria* seeds in animal models. However, flumazenil i.e., GABA receptor antagonist failed to inhibit the anxiolytic potential of plant seeds. This indicate that there are certain other anxiolytic mechanisms that are needed to be explored by further investigations. Furthermore, identification of the contributing chemical constituents involved in producing anxiolytic effect of seeds is also essential.

ETHICAL CONCERN

This study was approved by the University Ethical Review Board of Hamdard University approved the study vide reference number ERB-16-02. Specifications mentioned in Helsinki Resolution 1964 were followed for animal handling and use.

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CONFLICT OF INTEREST

None

5. REFERENCES

1. Arrant, A.E., Schramm-Sapyta, N.L. and Kuhn, C.M. (2013). Use of the light/dark test for anxiety in adult and adolescent male rats. *Behavioural brain research,* 256, pp.119-127.
2. Assad, T. and Khan, R.A., (2017). Effect of methanol extract of Trigonella foenum-graecum L. seeds on anxiety, sedation and motor coordination. *& Metabolic brain disease*, *32*(2), pp.343-349.
3. Ballinger B.R.,(1990). New drugs. Hypnotics and anxiolytics. Br Med J. 300: 456–58.
4. Baranawska MK, Cisowaski W. (1994) High performance chromatographic determination of the flavones-Cglycosides in some species of Cucurbitaceae family. J Chromatogr A; p. 675:240.
5. Beaubrun G, Gray GE (2000). A review of herbal medicines for psychiatric disorders. PsychiatrServ, 51:11-30-4.
6. Begum, Z. and Younus, I., (2018). Hibiscus rosa sinensis mediate anxiolytic effect via modulation of ionotropic GABA-A receptors: possible mechanism of action. *Metabolic brain disease*, *33*(3), pp.823-827.
7. Biren NS, Avinash SK. (2010). Pharmacognostic studies of the *Lagenaria siceraria* (Molina) Standley. Int J PharmTech Res, 2010, 2: 121-24.
8. Both, F.L., Meneghini, L., Kerber, V.A., Henriques, A.T. and Elisabetsky, E., (2006). Role of glutamate and dopamine receptors in the psychopharmacological profile of the indole alkaloid psychollatine. *Journal of natural products*, *69*(3), pp.342-345.
9. Bum, E.N., Soudi, S., Ayissi, E.R., Dong, C., Lakoulo, N.H., Maidawa, F., Seke, P.F.E., Nanga, L.D., Taiwe, G.S., Dimo, T. and Njikam, N. (2011). The Anxiolytic activity evaluation of four medicinal plants from Cameroon. *African Journal of Traditional, Complementary and Alternative Medicines*, *8*(5S).
10. Deshpande JR, Mishra MR, Meghre VS, Wadodkar SG, Dorle AK. (2007). Antioxidant activity of *Lagenaria siceraria* (Mol) Stand.fruit. Nat Prod Rad, p. 6:127.
11. Deshpande JR, Choudhari AA, Mishra MR, Meghre VS, Wadodkar SG, Dorle AK. (2008).Beneficial effect of *Lagenaria siceraria* (Mol) Stand. Fruit epicarp in animal models. Ind J ExpBiol; p. 46:234-242.
12. Diniz, T.C., de Oliveira Júnior, R.G., Medeiros, M.A. M.B., e Silva, M.G., de Andrade Teles, R.B., dos Passos Menezes, P., de Sousa, B.M.H., Frank, L.A., de Souza Araújo, A.A., Serafini, M.R. and Guterres.., S.S., (2019). Anticonvulsant, sedative, anxiolytic and antidepressant activities of the essential oil of Annona vepretorum in mice: Involvement of GABAergic and serotonergic systems. *Biomedicine & phrmacotherapy 111*, pp.1074-1087.
13. Duke JA. (1999). Handbook of phytochemical and constituents of GRASS herbs and economic plants. Florida: CRC Press, 1999; p. 98.
14. Duke JA. (1992). Handbook of biologically active of phytochemicals and their activities. Boca Raton, FL: CRC Press, 1-8.
15. Durcan MJ, Lister RJ. (1988). Time course of ethanol effects on locomotor activity, exlporation and anxiety in mice. Psycholpharmacol; 96:67-72.
16. Emmanouil, D.E. and Quock, R.M., (1990). Effects of benzodiazepine agonist, inverse agonist and antagonist drugs in the mouse staircase test. *Psychopharmacology, 102*(1), pp.95-97.
17. Faustino, T.T., ALMEIDA, R.D. and Andreatini, R., (2010). Plantasmedicinais no tratamento do transtorno de ansiedadegeneralizada: umarevisão dos estudosclinicoscontrolados. *Rev Bras Psiquiatr*, *32*(4), pp.429-36.
18. Ghule BV, Ghante MH, Upganlawar AB, Yeole PG. (2006). Analgesic and anti-inflammatory activities of *L. siceraria* (Mol) stand. fruit juice extract in rats and mice. Pharmacognosy Magazine, p.; 2: 232.

19. Ghule BV, Ghante MH, Saoji AN, Yeole PG.2006. Hypolipidemic and antihyperlipidemic effects of *Lagenariasiceraria* (Mol) fruit extracts. Ind J ExpBiol. P.; 44:905.
20. Gillian RB, Christopher N (2008). The exploratory behaviour of rats in the hole-board apparatus: is head-dipping a valid measure of neophilia? Behav Process 78(3):442–448.
21. Harvey AL (2008). Natural products in drug discovery. Drug Discov Today 13(19–20):894–901.
22. Hailu, E., Engidawork, E. and Asres, K., (2012). Essential oils of Myrtus communis L. produce a non-sedating anxiolytic effect in mice model of anxiety. *ZhongguoYaolixueyuDulixueZazhi - Chinese Journal of Pharmacology and Toxicology*, *26*(5)., p.729.
23. Helton, D.R., Berger, J.E., Czachura, J.F., Rasmussen, K. and Kallman, M.J., (1996). Central nervous system characterization of the new chloecystokinin-B antagonist LY288513. *Pharmacology Biochemistry and Behavior*, *53*(3), pp.493-502.
24. Herrera-Ruiz, M., García-Beltrán, Y., Mora, SN., Díaz-Véliz, G., Viana, G.S., Tortoriello, J. and J-Ramírez, G., 2006. Antidepressant and anxiolytic effects of hydroalcoholic extract from Salvia elegans. *Journal of Ethnopharmacology*, *107*(1), pp.53-58.
25. HossainM, UmaDevi P (2001). Effect of irradiation at the early fetal stage on adult brain function of mouse: learning and memory. Int J Radiat Bioliogy 77(5):581–585.
26. Ishola, I.O., Olayemi, S.O. and Idowu, A.R., (2013). Anticonvulsant, anxiolytic and hypnotic effects of aqueous bulb extract of Crinum glaucum A. chev (Amaryllidaceae): role of GABAergic and nitrergic systems. *Pak. J. Biol. Sci*, *16*(15), pp.701-710.
27. Jain NN, Ohal CC, Shroff RH, Somani RS, Kasture VS, Kasture SB (2003).Clitoriaternatea and the C-NS. PharmacolBiochemBehav 75:529–536.
28. K. Dhiman, A.Gupta, D.K.Sharma, N.S.Gill and A.Goyal. (2012). A Review on The Medicinally Important Plants of The Family Cucubitaceae. Asian Journal of Clinical Nutrition; Vol 4(1): 16-26.
29. Kahn, R.S., Van Praag, H.M., Wetzler, S., Asnis, G.M. and Barr, G., (1988). Serotonin and anxiety revisited. *Biological Psychiatry*, *23*(2), pp.189-208.
30. Kebebew, Z. and Shibeshi, W., (2013). Evaluation of anxiolytic and sedative effects of 80% ethanolic Carica papaya L.(Caricaceae) pulp extract in mice. *Journal of ethnopharmacology*, *150*(2), pp.665-671.
31. Kirtikar KR, Basu BD. The Indian medicinal plants. (2003), 2nd ed. Dehradun, Uttaranchal, India: Oriental Enterprises; v., p.1551-1554.
32. Kubde, M.S., Khadabadi, S.S., Farooqui, I.A. and Deore, S.L., (2010). Lagenaria siceraria: phytochemistry, pharmacognosy and pharmacological studie. *Rep Opin*, *2*(3), pp.91-98.
33. Kumar, A., Partap, S., Sharma, N.K. and Jha,KK., (2012). Phytochemical, ethnobotanical and pharmacological profile of Lagenaria siceraria:-A review. *Journal of Pharmacognosy and phytochemistry,1 1*(3), pp.24-31.
34. Lader M, Morton S. Benzodiazepine problems.Br J Addic, (1991), 86: 823-828.
35. Lister RG. The use of a plus-maze to measure an anxiety in the mouse. (1987). Psychopharmacology; 92: 180-85.
36. Lee, B., Sur, B., Yeom, M., Shim, I., Lee, H. and Hahm, D.H., (2012). Effect of berberine on depression-and anxiety-like behaviors and activation of the noradrenergic system induced by development of morphine dependence in rats. *The Korean Journal of Physiology &Pharmacology*, *16*(6),pp. 379-386.
37. M. Holm. (1988). Prescription of benzodiazepines in general practice in the county of Arhus, Denmark Dan Med Bull, (1988), 35: 495-9.
38. Mandrioli, R. and Mercolini, L., (2015). Discontinued anxiolytic drugs (2009–2014). *Expert opinion on investigational drugs*, *24*(4), pp.557-573.
39. Maqbool, S. and Younus, I., (2019). Anxiolytic and hypnotic effects of Cocculus laurifoliusleaf extract in mice. Bangladesh Journal of Pharmacology 14(1), pp.45-53.
40. MendonçaNetto, S., Warela, R.W., Fechine, M.F., Queiroga, M.N. and Quintans-Júnior, L.J., (2009). Anxiolytic-like effect of RauvolfialigustrinaWilld: ex Roem. &Schult., Apocynaceae, in the elevated plus-maze and hole-board tests. *RevistaBrasileira de Farmacognosia*, *19*(4), pp.888-892.
41. Michel B, Martine H (2003). The mouse light/ dark box test. Eur J Pharmacol.; 463:55-65.
42. Muhammad, A. and Rahila, N., (2013). Anxiolytic and memory enhancing activity of Lagenaria Siceraria in rodents. *Int J Biomed Adv Res*, *4*, pp.40-46.
43. Nanumala, S.K., Shalini, S., Divya, N. and Singh, S.S., (2018). Anxiolytic Activity of Ethanolic Extracts of Tridax procumbens using Different Experimental Anxiety Models in Mice. The *Int J Pharma Res Health Sci*, *6*(2), pp.2359-63.

44. National Institutes of Health, (1985). Principles of laboratory animal care. *NIH publication*, pp.85-23.
45. O'Donovan A, Slavich GM, Epel ES, Neylan TC (2013). Exaggerated neurobiological sensitivity to threat as a mechanism linking anxiety with increased risk for diseases of aging. NeurosciBiobehav Rev 37:96–108.
46. Ojiako, O.A. and Igwe, C.U., (2007). Nutritional and anti-nutritional compositions of Cleome rutidosperma, Lagenaria siceraria, and Cucurbita maxima seeds from Nigeria. *Journal of medicinal food*, *10*(4), pp.735-738.
47. Organization, W. H. (2017). Depression and other common mental disorders: global health estimates.
48. Pertz, H.E.I.N.Z. and Eich, E.C.K.A.R.T., (1999). Ergot alkaloids and their derivatives as ligands for serotoninergic, dopaminergic, and adrenergic receptors. *Ergot: The Genus Claviceps.Amsterdam:Harwood Academic Publishers*, pp.411-40.
49. Pellow S, Chopin P, File SE, Briley M (1985). Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rats. *J Neurosci Meth 14:* 149-167.
50. Perveen, T., Haider, S., Kanwal, S. and Haleem, D. J.,(2009). Repeated administration of Nigella sativa decreases 5-HT turnover and produces anxiolytic effects in rats. *Pakistan Journal of Pharmaceutical Sciences*, *22*(2).
51. Prut L, Seo JJ, Lee SH, Lee YS, Kwon BM, Ma Y, Hwang BY et al (2007). Anxiolyticlike effects of obovatol isolated from Magnolia obovata. Involvement of GABA/benzodiazepine receptor complex.ProgNeuropsychopharmacol Biol Psychiatry, 31:1363–1369.
52. Rang HP, Dale MM, Ritter JN (2003) Pharmacology: Churchill Livingstone. 483–94 Rahman, A.H. Bottle gourd (Lagenaria siceraria) a vegetable for good health. *Natural Product Radiance*, *2*(5), pp. 249-250.
53. Rolland, A., Fleurentin, J., Lanhers, M.C., Misslin, R. and Mortier, F., (2001). Neurophysiological effects of an extract of Eschscholzia californica Cham. (Papaveraceae). *Phytotherapy Research*, *15*(5), pp. 377-381.
54. Shah BN, Seth AK, Desai RV. (2010). Phytochemical profile of *Lagenaria siceraria :* a review. Asia J Plant Sci. p. 9:152-157.
55. Shirwaikar A, Sreenivasan KK (1996). Chemical investigation and antihepatotoxic activity of the fruits of *Lagenaria siceraria* . Ind. J. Pharm. Sci. 1996; 58:197.
56. Shweta S. Saboo ,Priyanka K. Thorat , Ganesh G. Tapadiya ,S S Khadabadi (2003).Ancient and the Recent Medical Uses of Cucurbitaceae Family. The International Journal of Therapeutic Applications,Vol 9:11- 19.
57. Siddig Ibrahim Abdelwahab, Loiylsir Ahmed Hasan, HasnahMohdSirat, Sakina M. Ahmed Yagi, Waleed S. Koko,Syam Mohan, et al. (2011). Antiinflammatory activities of cucurbitacin E isolated from Citrulluslanatus var. citroides: Role of reactive nitrogen species and cyclooxygenase enzyme inhibition:p.1-8.
58. Siddiq, A. and Younus, I., (2018). The Radish, Raphanus sativus L. Var. caudatus reduces anxiety-like behavior in mice. *Metabolic brain disease,33*-(4), pp.1255-1260.
59. Sood, A., Kaur, P. and Gupta, R., (2012). Phytochemical screening &antimicrobial assay of various seeds extract of Cucurbitaceae family.
60. Soulimani, R., Fleurentin, J., Mortier, F., Misslin, R., Derrieu, G. and Pelt, J.M., (1991). Neurotropic action of the hydroalcoholic extract of Melissa officinalis in the mouse. *Planta Medica*, *57*(02), pp. 105-109.
61. Sonja S, Herman SS. (2000). Analysis of curcurbitacins in medicinal plants by HPLC-MS.Phytochem Anal; p. 11:121.
62. Takeda H, Tsuji M, Matsumiya T (1998). Changes in head-dipping behavior in the hole-board test ref lects the anxiogenic and/or anxiolytic state in mice. Eur J Pharmacol 350:21–29.
63. Wang HX, Ng TB. (2000).Lagenin, a novel ribosome inactivating protein with the ribonucleolytic activity activity from bottle-gourd (*Lagenaria siceraria*) seeds. Life Sci; p. 67:2631.
64. Wang HX, Ng. TB (2000). Lagenin: A novel ribosome inactivating protein with ribonucleolytic activity from bottle gourd (*Lagenaria siceraria)* seed. Life Sci; 67:2631-2638.