

Anxiolytic and antidepressant activity of different methanolic extracts of *Melia azedarach* Linn.

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Abstract: Present study was planned to search for neuropharmacological properties of methanolic extracts of different parts of *Melia azedarach* Linn. because conventional medicine shows adverse effects. Recently natural products are studied worldwide, as safer alternatives, which are not duly supported with scientifically proved data. *Melia azedarach* (Bakayan, China berry tree) is medium sized tree belonging to family Meliaceae. Traditional use of plant is as anthelmintic, antilithic and diuretic. Topical application of oil for cramps and rheumatism is beneficial. Methanolic extract of flowers (BFM), twigs (BTM) and roots (BKB) were the test drugs to study different activities on NMRI mice. This is the first report regarding CNS activity of *Melia* flowers. Significant anxiolytic activity ($p=0.000$) of extract of flowers (BFM) and of roots (BKB) ($p=0.002$) was determined by “elevated plus maze” and “Light and dark activity box”. Antidepressant activity was seen by BFM ($p=0.000$) in “forced swim test”. Diazepam and Imipramine were used as positive controls respectively. In conclusion flower extract have shown very powerful anxiolytic and anti-depressant activity and cytotoxic potential of twigs cannot be ruled out.

Keywords: *Melia azedarach* Linn., Elevated plus maze, Light and dark box, Forced swim test, anxiety, depression.

INTRODUCTION

Stress disorders are common worldwide, estimating over 350 million people suffering from depression and quarter of them having anxiety as well (factsheet, 2013). A study conducted by Mirza & Jenkins (Mirza and Jenkins, 2004) showed 34% prevalence of these conditions in Pakistan. A more recent study on prevalence of depression (Gadit and Mugford, 2007) showed 53% in Lahore, 43.9% in Quetta and 35.7% in Karachi. Positive risk factors for these conditions include female sex, middle age, low income, minimal literacy and education, financial crisis and relationship problems. Conventional medicines are used to treat these conditions. Use of these agents is limited largely because of a number of side effects. Herbal medicines are therefore, given attention because of their low price and fewer side effects. Present work describes the neuro-pharmacological effectiveness of different extracts of parts of *Melia azedarach* Linn. that belongs to family Meliaceae. It is native of Himalayas and found naturally in China, Myanmar, Pakistan, Iran, Afghanistan and Turkey where it is known as China Berry or Margosa tree whereas locally it is known as Bakayn or Bakain. It is cultivated all over India. Whole of the plant is used medicinally but according to (Parajapati *et al.*, 2003), only bark is useful. Traditionally it is used as Antihelmintic, antilithic diuretic, astringent and wormicidal (Vishnukanta, 2008), whereas oil is used in cramps and rheumatism.

As a result of biological evaluation of this plant, isolation of a number of compounds summarized in table 1 was

seen. Careful sifting of the literature showed no reports of CNS activity therefore this study was planned to evaluate effectiveness of Methanolic extracts of different parts of *Melia azedarach* Linn in mental conditions like anxiety and depression. This is the first report of the evaluation of *Melia azedarach* Linn. for its neuro-pharmacological activity.

MATERIALS AND METHODS

Animals

Male female NMRI mice weighing 20-30grams were used in the experiments. Mice were bred in the animal house of “Dr. HMI Institute of Pharmacology and Herbal Sciences” with standard rodent diet and tap water when needed. Random allocation of 10 animals per appropriate cages of plexiglass and maintenance of temperature at $22\pm2^{\circ}\text{C}$ with 12hrs’ light and dark cycles was ensured. All the study procedures, animal handling and housing were approved by “Institutional animal ethics committee”, according to internationally accepted standard guidelines for laboratory animal use. All the experiments were done between 9.00 am - 13.00 pm.

Plant material

The plant was identified by Dr. Surriya of Dept of Botany, Karachi University with the voucher specimen number “63495-KUH”, deposited in the herbal museum of the same department. Different parts of plant were collected from the campus of Karachi University, in 1997.

Extraction

Flowers (40gm), Twigs (55gm) and Roots (149gm) of *Melia azedarach* were extracted with methanol at room

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temperature and the extract was evaporated under reduced pressure to provide 5.1gm crude extract of flowers, 3.9 gm from twigs and 9gm of extract from roots. HPLC data has been published for same extracts (Saleem *et al.*, 2008)

Drugs and chemicals

Diazepam (Rosch, Pakistan) and Imipramine HCl (Novartis, Pakistan) were bought from the local market. Normal saline was made by NaCl (Extra pure USP-BP from Scharlar S.A. LaJota 86-08016- Barcelona, Spain). All the solutions were freshly prepared immediately before experiment.

Behavioral procedures

Elevated plus maze

This apparatus has four identical arms (40x10cm) which radiate from the central platform to form a plus sign. Two opposite arms of the maze were open and the other two arms were closed with walls 17cm in height. Whole of the apparatus was 50cm elevated from the floor on a wooden stand. Animals were placed in the center of the apparatus facing the open arm (Peng *et al.*, 2000, Ishaq, 2014).

Light dark activity box

The apparatus used was a two compartment box with equal size (26x27x28cm) and a midway door of 10x10 cm. Both the compartments were different in their sensory properties that is one was dark and the other was transparent. Light box was brightly illuminated by a lamp placed 35cm above the floor of the box (Wei *et al.*, 2007). Drug administered animals were introduced to the light compartment with their back towards the door (Peng *et al.*, 2000, Ishaq, 2014).

Forced swim test

Most widely used test as pharmacological model for assessment of anti-depressant activity. It was first discovered by Porsolt and coworkers in 1977 (Porsolt *et al.*, 1977). Apparatus for forced swim was a plastic cylinder with 26cm height and 19cm diameter containing fresh water up to the height of 15cm at temperature $\pm 25^{\circ}\text{C}$. Behavior tested was Immobility time, when cessation of all the escape movements took place (Carlezon *et al.*, 2006, Ishaq *et al.*, 2013).

Experimental protocol

Anxiolytic activity of different parts of Melia

Animals were grouped randomly, with six animals per group. One saline group and one diazepam group served as controls for all the treatment groups. Three extracts of *Melia* flower (BFM), twigs (BTM) and Bark (BKB) were tested. Animals were treated with different doses of extracts one hour before the test session. After completion of one hour, individual animal was first placed in the light and dark box and then in the Elevated plus maze for five minutes each.

Effect of acute (Thrice a day) treatment of different parts of melia for their antidepressant activity in forced swim test

Animals were divided in control and treated groups. All the animals were subjected to prior pretest swim session for 10 minutes. After 10 minutes mice were removed from the water and dried with the towel and returned to their home cages. 24 hrs later mice were forced to swim again in the similar condition for 5 minutes (Norte *et al.*, 2005), which was the test session (Yu *et al.*, 2002). In all the treatment groups the treatment was given 23, 5 and 1 hour before the test session (Mendes *et al.*, 2002).

STATISTICAL ANALYSIS

All the results were analyzed by using one way ANOVA followed by LSD (Least significant difference) test by using "Statistical Product Selective solution" version 19 (SPSS-19). All the results were considered significant when $P < 0.05$

RESULTS

Anxiolytic activity

Anxiolytic activity of flower extract of plant (BFM)

Elevated plus maze

Three different parts of *Melia azedarach* viz. flowers (BFM), twigs (BTM) and roots (BKB) extracts were included in the study. Flower extracts (BFM) showed significant effects when animals were tested in EPM and LDB at doses of 75, 125 and 250mg/kg.

In elevated plus maze, one way ANOVA showed that there was significant difference between groups $F(7, 40) = 10.25$; ($p = 0.000$). Post hoc LSD analysis showed highly significant rise in % of time spent in the open arm by 75 mg/kg (35% rise, $p < 0.005$), 125mg/kg (48.25% rise, $p < 0.001$) and 250mg/kg (54.6% rise, $p < 0.001$) doses of BFM. The results were compared with 1mg/kg Diazepam which showed 47.43% rise in % of time spent in the open arm ($p < 0.001$) as shown in table 2. On comparison with diazepam there was non-significant difference between 75 mg/kg ($p = 0.313$), 125mg/kg ($p = 0.941$) and 250mg/kg ($p = 0.529$) doses of BFM which showed that the results from these three doses are comparable to diazepam in efficacy.

For the purpose of loco-motor activity, the total arm entry was recorded to see whether there is sedative potential of the drug or not. One way ANOVA shows that the difference between groups was highly significant, $F(7, 40) = 2.288$ ($p < 0.05$). Significant rise was observed by 125 mg/kg dose of BFM (26%) in total arm entry ($p < 0.05$). When post hoc analysis was done 75mg/kg (8.2%) and 250mg/kg (22.47%) however showed non-significant rise in loco-motor activity. This was compared with diazepam which showed no rise at all. Total arm entry by saline and diazepam were equal.

Table 1: Isolated active compounds from different parts of *Melia azedarach* Linn

Part of plant	S. No.	Compound	Activity	Reference
Seeds	1	Pinoresinol	Antifungal activity	(Zhao <i>et al.</i> , 2010)
	2	Vanillin	Antifungal activity	
	3	4-hydroxy-3-methoxy-cinnom-aldehyde	Antifungal activity	
	4	Fatty oil (Stearic acid, Palmitic acid etc)	Insecticide activity	
Root bark	5	Azaridine (Margosine)	-	(Parajapati <i>et al.</i> , 2003)
	6	Sterols	-	
Stem bark	7	Tannins	Astringent	(Khare, 2007)
	8	Gedunin	Anti-malarial	
Heart wood	9	Bakayanin	-	
	10	Bakalactone	-	
Leaves	11	Quercitrin	Anti-nociceptive and anti-oxidant	(Hasan <i>et al.</i> , 2006)
			Anti-inflammatory	(Camuesco <i>et al.</i> , 2006)
	12	Rutin (Flavonoid)	-	(Parajapati <i>et al.</i> , 2003)
	13	Paraisine(Alkaloid)	-	
	14	Vilasinin	-	
	15	Salanin	-	(29)
	16	Meliacine	Anti-viral activity against HSV-1	(Laura <i>et al.</i> , 2002)
Fruits	17	Azadrine	Fungicidal and anti-bacterial activity	(Khare, 2007)
	18	Meliatannic acid		
	19	Melianoninol	Strong anti-feedant activity	
	20	Melianol		
	21	Melianone		
	22	Meliandiol		
	23	Vanillin		
	24	Vanillic acid	Micro/macro filaricidal activity	
	25	Toosandanin	Anti-viral/anti-cancer activity	(28)
Insecticidal			(Hong <i>et al.</i> , 2007)	
Flowers	26	Stigmasterol	Anti-bacterial activity	(6)
	27	Kulactone		
	28	p-O-β-glucosyle benzoic acid		
	29	Benzoic acid		
	30	p-O-β-glucosyle gallic acid		
Roots	31	Kulactone	Cytotoxic activity in CB cell lines	(Faizi <i>et al.</i> , 2002)
	32	β-sitosterol		

In case of light and dark activity box most promising response was % of time spent in lighted compartment. One way ANOVA showed that there is significant difference between groups F (7, 40) =9.95 (p<0.001). LSD (post hoc) showed that 75 mg/kg dose showed 25% rise (p<0.001) in % of time spent in lighted compartment whereas diazepam showed 40.6% rise (p<0.001) rise in the same parameter (table 2). Other two doses did not showed significant difference.

Anxiolytic activity of twig extract of plant (BTM)

Significant difference between groups F (4, 25) =5.414 (p<0.005) was observed. Post Hoc analysis with LSD showed that 125 mg/kg dose had significant (25.73%, p<

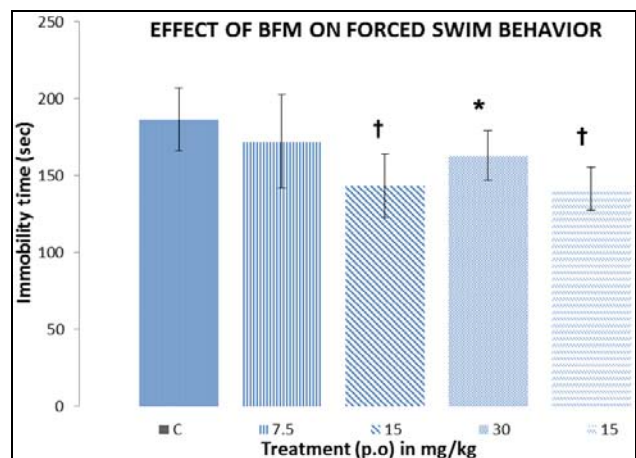
0.05) rise in open arm entry. Diazepam in contrast showed 47.49% rise (p<0.001) in open arm entry. The result however was not supported by concomitant rise in the % of time spent in the open arm.

In the light and dark activity box, BTM at the doses of 75 showed 28.62% (p<0.05) rise in the % of time spent in the lighted compartment. Comparison with Diazepam however showed significant difference between two groups i.e. Diazepam and 75mg/kg dose of BTM (p<0.05).

Anxiolytic activity of Root extract of plant (BKB)

Three different doses of root extract showed significant difference, F (4, 25) =4.717 (p<0.025). 60mg/kg dose of

root extract was found effective on post hoc analysis i.e. significant rise (36%, $p < 0.01$) in % time spent in the open arm was observed where as that of Diazepam was 47.43% ($p < 0.001$). When compared with Diazepam, there was non-significant difference between Diazepam and 60 mg/kg BKB ($p = 0.394$) showing comparable activity of the two drugs. In case of light and dark box, significant difference between groups, $F(4, 25) = 7.34$, ($p < 0.001$) was observed however Post Hoc analysis showed 32.78% rise ($p < 0.001$) in the percent of time spent in the lighted compartment by 30mg/kg dose of whereas Diazepam showed 40.6% ($p < 0.001$) rise. Comparison with Diazepam showed no-significant difference between Diazepam and 30 mg/kg dose of BKB ($p = 0.371$) (table 2).



Results are shown as average $\pm$ S.D.* = $P < 0.05$ † = $P < 0.005$. (n=6)

Fig. 1: Effect of BFM on forced swim behavior

Anti-depressant activity

Immobility time was compared by one way ANOVA, which revealed that there was significant difference between groups, $F(4, 25) = 4.0803$ ($p < 0.005$). Post hoc analysis showed 15 mg/kg dose significantly (22.7%, $p < 0.005$) decreased immobility time which was compared with same dose of IMI i.e. 15mg/kg which showed 23.70% ($p < 0.01$) fall in immobility. When these two groups were compared, non-significant difference ($p = 0.871$) was seen in the immobility time produced by two drugs thus proving equally potent anti-depressant effect both in dose as well as efficacy.

When swimming behavior was compared by one way ANOVA, it revealed significant difference between groups, $F(4, 25) = 16.097$ ($p < 0.001$). Post Hoc analysis showed 50% rise in the swimming behavior ($p < 0.025$) by 15mg/kg dose of BFM, which was compared by Imipramine (IMI) which showed 99% rise in swimming ($p < 0.001$). There was however significant difference between ($p = 0.02$) IMI and BFM showing that the rise by 15mg/kg dose of BFM was not comparable with that of IMI.

DISCUSSION

Major neuropsychiatric disorders include anxiety, feeling of constant and inappropriate worry and restlessness (Graeff and Junior, 2010) and depression which is associated with low mood, feeling of helplessness and tendency of suicide (Mao *et al.*, 2009).

Despite of extensive research, etiology of neuropsychiatric disorders is still unclear. After a long time of study, involvement of free radicals in the pathophysiology of neuropsychiatric disorders has become evident (Sattayasai *et al.*, 2008). Brain tissue is vulnerable to lipid peroxidation therefore anti-oxidant supplementation is thought to be beneficial for the treatment of mental disorders (Sattayasai *et al.*, 2008).

Stressful situations also triggers Hypothalamo-hypophyseal axis (HPA axis) and continuous triggering of HPA axis is very important for pathogenesis of psychiatric disorders. Activation of HPA axis for longer and extended period of time is associated with chronically increased levels of cortisol in blood (Barden, 2004, Ishaq, 2014) which leads to anxiety (Graeff and Junior, 2010) and depression (Piato *et al.*, 2008).

Melia azedarach Linn. has many medicinal properties. Its flowers and leaves are diuretic whereas seeds are anti-rheumatic (Jain *et al.*, 2006) and leaves have anti-ulcer activity (Bahuguna *et al.*, 2008). Many chemical compounds have been isolated from this plant. Recently isolated compounds are mentioned in table 1.

Quercitrin, a very useful component, has been isolated from the leaves of *Melia azedarach* and is found to be potent anti-nociceptive and anti-oxidant agent (Hasan *et al.*, 2006). anti-inflammatory (Camuesco *et al.*, 2006). Gedunin, another very important compound has been isolated from roots and stem barks of the plant. It has been found to be anti-malarial (Mackinnon *et al.*, 1997) also it decreases allergic infections (Ferraris *et al.*, 2012). Vanillic acid isolated from fruits have radical scavenging activity (Kang *et al.*, 2006).

Anxiolytic behavioral paradigms as mentioned above are EPM and LDB to investigate newer anti-anxiety agents (Raginatto *et al.*, 2006). EPM reduces natural fear of rodents to open arm and enhance exploration (Raginatto *et al.*, 2006) whereas LDB demonstrate the natural decreased activity in bright light (Wei *et al.*, 2007). Our study showed very promising results of all the extracts of the plant *Melia azedarach*, especially 75, 125 and 250 mg/kg doses of flower extract i.e. BFM and 30- 60mg/kg dose of BKB in both the paradigms. Additionally non-sedative potential was also found in BFM as locomotor activity was also enhanced significantly thereby making BFM a good candidate for further study as anxiolytic

Table 2: Different anxiety parameters studied by three extracts of *Melia azedarach*

Extract	Doses	% time spent in open arm (Elevated plus maze)	% open arm entry (Elevated plus maze)	% time spent in lighted compartment (Light and dark box)
Saline	-	29.16±2.37	42.62±3.14	46.06±1.43
Diazepam	1 mg/kg	42.98±2.12***	62.82±1.86***	64.83±2.06***
BFM	30 mg/kg	25.33±2.96	41.87±1.87	51.95±2.59*
	75 mg/kg	39.6±1.67** ‡	52.96±2.65**	58±2.03***
	125 mg/kg	43.23±1.88*** ‡	52.1±1.2**	50.87±1.7
	250 mg/kg	45.09±2.28*** ‡	54.49±1.83***	54.06±1.65*
	500 mg/kg	30.74±2.39	52.2±1.41**	46.39±2.3
	1000 mg/kg	32.98±2.79	48.5±1.04*	48.71±2.04
	F value	10.25 (P = 0.000)	11.47 (p = 0.000)	9.954 (p = 0.000)
BTM	75 mg/kg	34.56±3.09	48.55±1.95	59.23±2.64**
	125 mg/kg	34.11±2.39	53.57±2.21*	53.94±1.43*
	250 mg/kg	17.77±2.76**	43.48±6.46	42.11±3.85
	F value	12.95 (P = 0.000)	5.414 (P = 0.003)	14.34 (p = 0.000)
BKB	30 mg/kg	34.17±3.31	46.58±1.72	61.16±3.14**
	60 mg/kg	39.67±3.45*	46.55±1.47	51.67±3.77
	125 mg/kg	30.66±1.91	41.89±1.15	51.49±3.16
	F value	4.71 (P = 0.006)	18.35 (P= 0.000)	7.338 (P = 0.000)

Results given as Average ± S.E.M. (N=6 in every group) * = p<0.05; **=p<0.005; ***=p=0.000. ‡ =Non-significant difference when compared with diazepam

agent without sedative adverse activity as compared to adverse effects of diazepam namely loss of coordination and fine motor activity.

According to Porsolt (Porsolt *et al.*, 1977) forced swim paradigm is the most reliable test for newer anti-depressant agents. Anti-depressant activity was only seen by 15mg/kg dose of BFM other extracts like BKB and BTM did not show the response at all in lowering the immobility time. This makes BFM a very useful drug candidate as anti-depressant agent.

From above discussion it is evident that Quercitrin, Gedunin and vanillic acid shows radical scavenging activity along with most probably other compounds with similar activity. It is clear from the literature that radical injury is one of the major causes of depression as it prevents BDNF formation resulting in depression and decrease in the brain structure. Presence of Quercitrin, Gedunin and vanillic acid in the plant may be the cause of anti-depressant activity via free radical scavenging property of chemicals present. Flavonoids enriched plants have tranquilizer property (Grundmann *et al.*, 2009) without sedative and amnesic property (Guaraldo *et al.*, 2000). *Melia azedarach* is rich in flavonoids and from the result it is evident that non-sedative property of the extract it, may be due to these flavonoids present in the extract. Keeping this in mind it can be postulated that mode of action of extracts from different parts of *Melia azedarach* may be through its radical scavenging property. Analytical work is in progress for further evaluation.

CONCLUSION

In conclusion, it can be said from the above research work that flowers of *Melia azedarach* have very strong neuro-pharmacological activity as they responded with fairly low doses of the drug and it had very strong anti-depressant activity which was shown by very remarkable comparison with the conventional medicine at same dose and same regimen. Roots extract also showed anxiolytic activity but anti-depressant activity was not seen.

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REFERENCES

- Bahuguna YM, Patil KS and Jalalpure SS (2008). Phytochemical and Pharmacological investigation of *Melia azedarach* leaves for Antirolithiatic activity. *J. Tropical Med. Plants*, 9(2): 344-352.
- Barden, N (2004). Implication of hypothalamic –pituitary-adrenal axis in physiopathology of depression. *J. Psychiatry Neurosci.*, 29: 185-193.
- Camuesco D, Comalada M, Concha A, Nieto A, Sierra S, Xaus J, Earzuelo A and Galvez J (2006). Intestinal anti-inflammatory activity of combined quercitrin and dietary olive oil supplemented with fish oil, rich in EPA and DHA (n-3) PUFA, in rats with DSS induced colitis. *Chem. Nutr.*, 25: 466-476.

- Carlezon WA, Beguin C, DiNieri JA, Baumann MS, Richards MR, Todtenkopf MS, Rothman RB, Ma Z, Lee DYW and Cohen BM (2006). Depressive-like effects of the κ - Opioid receptor agonist Salvinorin A on behavior and neurochemistry in rats. *J. Pharmacol. Exp. Ther.*, **316**: 440-447.
- factsheet W (2013). *Depression* (Online). WHO. Available: <http://www.who.int/mediacentre/factsheets/fs369/en/index.html>. (Accessed 11/7/2013 2013).
- Faizi S, Wasi A, Siddiqui BS and Naz A (2002). New terpinoids from roots of *Melia azedarach*. *Aust. J. Chem.*, **55**(4): 291-298.
- Ferraris FK, Moret KH, Figueirdo AB, Penido C and Henriques MD (2012). Gedinin, a natural tetranorterpinoïd, modulates T lymphocyte responses and ameliorates allergic inflammation. *Int. Immunopharmacol.*, **14**(1): 82-93.
- Gadit AAM and Mugford G (2007). Prevalence of depression among households in three capital cities of Pakistan: Need to revise the mental health policy. *PLoS ONE*, **2**(2): e209.
- Graeff FG and Junior HZ (2010). The hypothalamic-pituitary-adrenal axis in anxiety and panic. *Psychol. Neurosci.*, **3**(1): 3-8.
- Grundmann O, Nakajima J, Kamata K, Seo S and Butterweck V (2009). Kaempferol from the leaves of *Apocynum venetum* possesses anxiolytic activities in the elevated plus maze test in mice. *Phytomedicine*, **16**: 295-302.
- Guaraldo I, Chagas DA, Konno AC, Korn GP, Pffifer T and Nasello AO (2000). Hydroalcoholic extract and fractions of *Davilla rugosa* poiret: Effects on spontaneous motor activity and EPM behavior. *J. Ethnopharmacol.*, **72**: 61-67.
- Hasan MS, Ahmad MI, Mondal S, Jamaluddin S, Masud MM, Sadhu SK and Ishibasha M (2006). Anti-oxidant, anti-nociceptive activity and general toxicity study of *Dandrophthoe faleata* and isolation of Quercitrin as major component. *Pharm. Exp. Med.*, **6**(4): 355-360.
- Hong YZ, WANG HT, LI J, SU YZ and LIU YR (2007). The Extraction and Identification of Toosendanin from the Fruit of *Melia azedarach* Linn. *Journal-Xiamen University Natural Science*, **46**(3): 365.
- Ishaq H (2014). Anxiolytic effect of herbal medicine, *Khamira gaozaban ambri jadwar ood salib wala* (KGJ) in experimental rat models. *Pakistan Journal of Pharmaceutical Sciences*, **27**(2): 289-294.
- Ishaq H, Mahmood R, Javed I, Tariq T and Mahmood I (2013). Antidepressant activity on the herbal extract, *Khamira Gaozaban ambri Jadwar ood Salibwala*. *International Journal of Pharmacy*, **3**(3): 450-456.
- Jain JB, Kumane SC and Bhattacharya S (2006). Medicinal flora of madhya pradesh and chattisgarh-A review. *Indian Journal of Traditional Knowledge*, **5**(2): 237-242.
- Kang KS, Kim HY, Pyo JS and Yokozawa T (2006). Increase in the free radical scavenging activity of ginseng by heat-processing. *Biological and Pharmaceutical Bulletin*, **29**(4): 750-754.
- Khare CP (2007). *Indian Medicinal plants: An Illustrated dictionary*. Springer-Verlag, Berlin/Heidelberg, Germany.
- Laura EA, Andrea AB, Norberto AS and Celia EC (2002). An antiviral principle present in a purified fraction from *Melia azedarach* L. leaf aqueous extract restrains herpes simplex virus type 1 propagation. *Phytother Res.*, **16**(4): 348-352.
- Mackinnon S, Durst T, Arnason JT, Anqeraofer C, Pezzulo J, Sanchez Vindas PE, Poveda LJ and Gbeassor M (1997). Anti-malarial activity of tropical Meliaceae extracts and gedunin derivatives. *J. Nat. Prod.*, **60**(4): 336-341.
- Mao QQ, Ip SP, Ko KM, Tsai SH, Xian YF and Chi CT (2009). Effects of peony glycosides on mice exposed to chronic unpredictable stress: Further evidence for antidepressant like activity. *J. Ethnopharmacol.*, **124**: 316-320.
- Mendes FR, Mattei R and de Araujo Carlini EL (2002). Activity of hypericum brasiliense and hypericum cordatum on the central nervous system in rodents. *Fitoterapia*, **73**: 462-471.
- Mirza I and Jenkins R (2004). Risk factors, prevalence and treatment of anxiety and depressive disorders in Pakistan: Systematic review. *Brit. Med. J.*, **328**(7443): 794.
- Norte MCB, Cosentino RM and Lazarini CA (2005). Effect of methyl-eugenol administration on behavioral models related to depression and anxiety in rats. *Phytomedicine*, **12**: 294-298.
- Parajapati ND, Purohit SS, Sharma AK and Kumar T (2003). *A Handbook of Medicinal Plants: A complete source book*, Agrobios, India.
- Peng WH, Hsieh MT, Lei YS, Liu YC and Liao J (2000). Anxiolytic effect of seed of *Zizyphus jujuba* in mouse model of anxiety. *J. Ethnopharmacol.*, **72**: 435-441.
- Piato AL, Detanico BC, Jesus JF, Lhullier FLR, Nunes DS and Elisabetsky E (2008). Effects of marapuama in the chronic mild stress model: Further indication of antidepressant properties. *J. Ethnopharmacol.*, **118**: 300-304.
- Porsolt RD, Le Pichon M and Jalfre M (1977). Depression: A new animal model sensitive to antidepressant treatment. *Nature*, **266**: 730-732.
- Raginatto FH, De-Paris F, Petry RD, Quevedo J, Ortega GG, Gosmann G and Schenkel EP (2006). Evaluation of anxiolytic activity of spray dried powder of two South Brazilian passiflora species. *Phytother Res.*, **20**: 348-351.
- Saleem R, Rani R, Ahmed M, Sadaf F, Ahmad SI ul, Zafar N, Khan SS, Siddiqui BS, Lubna Ansari F, Khan SA and Faizi S (2008). Effect of cream containing *Melia azedarach* flowers on skin diseases in children. *Phytomedicine*, **15**(4): 231-236.

- Sattayasai J, Tiamkao S and Puapairoj P (2008). Biphasic effects of morus alba leaves green tea extract on mice in chronic forced swimming model. *Phytother Res.*, **22**: 487-492.
- Vishnukanta RAC (2008). *Melia azedarach*: A phytopharmacological review. *Pharmacogn Rev.*, **2**(3): 173 - 184.
- Wei XY, Yang JY, Wang JH and Wu CI (2007). Anxiolytic effect of saponins from *Panax quinquefolium* in mice. *J. Ethnopharmacol.*, **111**(3): 613-618.
- Zhao L, Huo CH, Shen LR, Yang Y, Zhang Q and Shi QW (2010). Chemical constituents of plants from the genus *Melia*. *Chem. Biodiversity*, **7**: 839-859.