IJBPAS, March, 2024, 13(3): 1221-1231

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

RESEARCH ARTICLE: EVALUATION OF ANTIDEPRESSANT ACTIVITY OF LEAF PARTS OF *NYCTANTHES ARBOR TRISTIS* LINN.

PRATAP S AND SHARMA N

Ph.D. Scholar, Department of Pharmacy, Bhagwant University, Rajasthan, Sikar Rode, Ajmer - 305004, Rajasthan, India

***Corresponding Author: Mr. Sushil Pratap: E Mail: piramalsushil@gmail.com**

Received 4th Feb. 2023; Revised 15th April 2023; Accepted 8th July 2023; Available online 1st March 2024

https://doi.org/10.31032/IJBPAS/2024/13.3.7854

ABSTRACT

Nyctanthes arbor-tristis Linn. commonly known as Harsingar (English:Night Jasmine), is a well documented plant. The decoction of the leaves of *Nyctanthes arbor-tristis* Linn. is widely used in Ayurvedic System of medicine for treatment of arthritis, fevers, various painful conditions and as a laxative. It is considered as an important plant that yields not only unique medicinal products but also has industrial importance. It has several medicinal properties such as anti-helminthic and antipyretic, anti-inflammatory and anti-oxidant activities, hepatoprotective, anti-leishmaniasis, anti-viral, antifungal, anti-pyretic, anti-histaminic, anti-malarial, anti-bacterial besides it is used as a laxative, in rheumatism, skin ailments and as a sedative. Moreover, none of the medications assessed in randomized controlled studies are effective in sciatica pain. NSAIDS are less than ideal as most of the NSAIDs are known to causes the gastric irritation, gastrointestinal ulceration reduces renal blood flow, platelet dysfunction, exacerbates asthma, allergic reactions and skin rashes. Sciatica pain requires chronic drug treatment and NSAIDs are not recommended for long-term administration. *Nyctanthes arbor tristis* is also called the "tree of sorrow", because the flowers lose their brightness during daytime; the scientific name arbor-tristis also means "sad tree". The flowers can be used as a source of yellow day for clothing. Similarly the animals treated with standard drug (Imipramine HCl, 15mg/kg) exhibited significant decrease in immobility time as

expected. The p values for ethanol at 100mg/kg and 150mg/kg are 0.0008 and 0.0009after 30 minutes, methanol extract at 150mg/kg and 200mg/kg are 0.0003 and 0.0004 after 60 minutes.

Keywords: *Nyctanthes arbor-tristis*, antidepressant activity, tail, suspension, glycoside, etc.

INTRODUCTION:

Medicinal plants represent a rich source of antimicrobial agents. Wide range of different parts of medicinal plants was used for extract as raw drugs and they posses varied medicinal properties. Some of these raw drugs are collected in larger quantities and traded in market as raw material for many herbal industries **[1]**. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity **[2]**. *Nyctanthes arbor-tristis*is commonly known as Harshinghar or Night Jasmine. It belongs to the family Oleaceae **[3]**. It has also been reported to possess hepatoprotective, anti-leishmanial, anti-viral and anti-fungal activities and analgesic, antipyretic and ulcerogenic activities. The plant also possess anti-allergic anti-malarial **[4]** anti-helminthic **[5]**, activities and recently reported heapatoprotective **[6]**, anti-spermatogenic and antioxidant activities **[7]**.

Vernacular Names:

Family: Oleaceae; Nyctanthaceae.

Unani: Harasingaar.

Sanskrit: Parijatha.

Siddha: Pavazhamattigai.

Hindi: Harsingar.

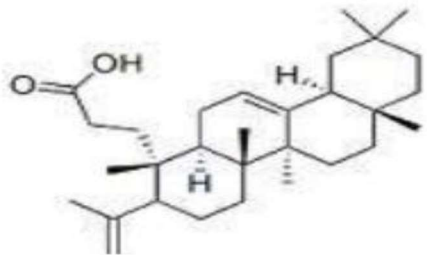
Ayurvedic: Paarijaata, Shephaali, Shephaalikaa, Mandaara.

English: Tree of Sorrow, Night Jasmine, Coral Jasmine.

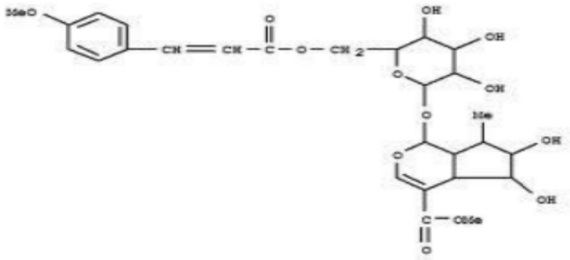
Figure 1: *Nyctanthes arbor-tristis* Linn

Chemical Constituents: [9-11]

Leaves contain D-mannitol, β-sitosterole, Flavanol glycosides, Astragaline, Nicotiflorin, Oleanolic acid, Nyctanthic acid, Tannic acid, Ascorbic acid, Methyl salicylate, Amorphous glycoside, Amorphous resin, Trace of volatile oil, Carotene, Friedeline, Lupeol, Mannitol, Glucose, Fructose, Iridoid glycosides, Benzoic acid.



Nycthintic acid



Arbortristoside Acid

MATERIALS AND METHODS:

Selection of Plants:

Selection of plants has been based on their ethnomedical & traditional uses. The plant *Nyctanthes arbor-tristis* Leaves were chosen for the present investigation. *Nyctanthes arbor-tristis* is reported to have been used for a number of diseases. Traditionally it was used in diarrhea, dysentery, leprosy, piles, cancer, inflammatory swellings and epilepsy but nothing is on record or the record is inadequate regarding its cytomorphology and pharmacological activity. Literature review revealed that a limited number of studies have been done on the leaf of this plant. As discussed with tribal people these plants were widely used by them for treatment of diarrhoea, dysentery, inflammation, tooth ache, and to cure other disorders, therefore the plants were selected.

Collection and Authentication of Plants:

Nyctanthes arbor-tristis leaves obtained locally from Gorakhpur and azamgarh region of Uttar Pradesh. Identification of plant samples were done by Professor Dr. N. K. Dubey Taxonomist, centre of advanced study in botany, institute of science, Banaras Hindu University, Varanasi (India).

Pharmacological Evaluation:

Detremination of Moisture Content:

The percentage of active constituents in crude drug is mentioned on air dried bases. Hence, the moisture content of the crude drugs should be determined and should also be controlled. The moisture content should be minimized in order to prevent decomposition of crude drugs either due to chemical changes or microbial contamination.

Procedure:

The powdered sample of leaves of *Nyctanthes arbor-tristis* weighed 5gm accurately and kept in IR moisture balance. The loss in weight was recorded as percentage (%) moisture with respect to air-dried sample of crude drug.

Determination of Ash Value:

The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts, naturally occurring

in drugs or adhering to it or deliberately added to it as a form of adulteration. Many a time the crude drugs are admixed with various mineral substances like sand, soil, calcium oxalate, chalk powder or other drugs

Pharmacological evaluation:

Macroscopic examination:

Colour:

Untreated samples were examined under diffuse day light. An artificial light source with wavelength similar to those of day light may also be used. The colour of sample was observed. Surface Characteristic, Texture and Fracture Characteristics Materials was touched to determine if it is soft or hard bend and ruptured it to obtain information on brittleness and the appearance of the fracture plane-whether it is fibrous, smooth, rough, granular etc.

Odour:

A small portion of the sample was placed in the palm of the hand and slowly and repeatedly, the air was inhaled over the material.

Taste:

A small amount of drug powder was kept over the tongue and the taste was observed.

Loss on Drying:

For estimation of loss on drying, it was dried at 105°C for 5 hours in a hot air oven, cooled in a desiccator for 30 minutes, and weighed without delay. The loss of weight was calculated as the content of in mg per g of air-dried material.

Extraction of Plant Material:

The dried powdered crude drugs 50 gm were kept for maceration in 200 ml ethanol for 7 days. These drugs were re-macerated and obtained extracts were further used for chemical evaluation. Same process has been repeated with water as a solvent.

Preliminary Phytochemical Evaluation of Extract

The extracts obtained were subjected to various qualitative tests to reveal the presence or absence of common phytopharmaceuticals. The extracts obtained were subjected to various qualitative tests to reveal the presence or absence of common phytopharmaceuticals.

a. Alkaloids

Small portion of alcoholic extract stirred separately with a few drops of dilute hydrochloric acid and then filtered. The filtrate is then tested carefully with various alkaloid reagents such as:

Mayer's Reagents

Alkaloids give precipitate with Mayer's reagents. One ml of Mayer's reagent (Potassium mercuric iodide solution) was added to 1 ml extract, whitish yellow or cream-colored precipitate indicated the presence of alkaloids.

Dragendroff's Reagents

Alkaloids give orange brown precipitate with Dragendorff's reagents. One ml of Dragendorff's reagent (Potassium bismuth iodide solution) was added to 1 ml extract, an orange-red precipitate indicated the presence of alkaloids.

Hager's Reagents

Alkaloids give yellow coloured precipitate with Hager's reagents. In to the 1 ml extract, 3 ml of Hager's reagent (saturated aqueous solution of picric acid) was added, a yellow-coloured precipitate indicated the presence of alkaloids.

Wagner's Reagents

Alkaloids give reddish brown precipitate with Wagner's reagents. In to 1 ml extract, 2 ml of Wagner's reagent (iodine in potassium iodide) was added and the formation of reddish-brown precipitate indicated the presence of alkaloids.

b. Carbohydrates and Glycosides

A small quantity of each extract dissolved separately in distilled water and was filtered. The filtrate is subjected to the following test for Carbohydrates.

Molisch's Test

One ml of α-naphthol solution and concentrated sulphuric acid was added in 2 ml of the extract, through the side of the test tube. The formation of purple or reddish violet color at the junction of the two liquids reveals the presence of carbohydrates.

Fehling's Solution

Equal volume of Fehling's A (copper sulphate in distilled water) and Fehling's B (potassium tartrate and sodium hydroxide in distilled water) reagent was mixed along with few drops of extract solution and boiled, a brick red precipitate of cuprous oxide forms.

Benedict's test

Extract solution was treated with few drops of Benedict reagent (alkaline Solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate forms, if reducing sugar is present.

Tollen's test

To 100 mg of compound add 2ml of Tollen's reagent and heat gently; a silver mirror is obtained inside the wall of the test tube. It indicates the presence of aldose sugar.

Keddes reagent test

Cardenolides give blue or violet color with this reagent which fads after 1-2 hour. This reagent is prepared by mixing equal volume of 0.2% solution of 3, 5 dinitro benzoic acid in 100ml of 0.5 N KOH solution in 50% methanol.

Legal's test

Treat the extract with pyridine and add alkaline sodium nitroprusside solution, blood red color appears.

Keller Killiani Test

1gm of powdered drug extracted with 10ml of 70% alcohol for few minutes and filtered. To 5 ml of this filtrate 10 ml of hydrogen peroxide solution and 0.5 ml of strong solution of lead acetate is added. Precipitate thus obtained is filtered. Filtrate is shaken with 5 ml of chloroform layer is separated and to this 1ml of mixture of 1 volume of 5% ferricsulphate and 99 volume of glacial acetic acid is added. To this mixture 1 or 2 drops of concentrated sulfuric acid is added. Appearance of blue color confirms the presence of deoxy sugars.

Test for flavonoids

Zinc hydrochloric reduction test:

Take the sample add a mixture of zinc dust and conc. hydrochloric acids. It gives red color after few minutes.

Alkaline test:

To the test solution add few drops of sodium hydroxide solution. Formation of an intense yellow color, which turns to colorless on addition of few drops of dilute acid indicate presence of flavonoids.

Acute Toxicity Study of Extract (LD50)

Acute oral toxicity studies have been conducted on an individual basis followed by using OECD guideline 423. The method used defined doses of 5, 50, 300, 2000 mg/kg p.o. body weight. Results were allowed substance rank and classify according to the Globally Harmonized System (GHS) for classification of chemicals which causes acute toxicity. From LD50 determination, 1/10th of the dose was focused as the medical for pharmacological screening. Since all the animals were alive; no mortality, no toxicity and no significant changes in the body weight between the control and treated group were observed at a dose of 2000 mg for duration of 72 hours. This finding probably suggests that the ethanol and aqueous extract are relatively safe or non-toxic in rats at the doses used for this study. The present study has been carried out to evaluate the LD50 and all Pharmacological activities of ethanolic extract & aqueous extract of *Nyctanthes arbor-tristis.* Stem & leaves. All drugs have been obtained from Pallav Chemicals Pvt. Ltd., Bombay. All extracts were suspended with the help of gum acacia in distilled water at the time of oral administration.

Experimental Protocols:

All experimental protocols were reviewed and accepted by the Institutional Animal Ethical Committee (IAEC) prior to the initiation of allied experiments. Protocol approval reference number (PBRI/IAEC/PN-17047a).

Pharmacognostical Evaluation

Macroscopic Examination

Size-10-12cmlong

Shape-Cylindrical, Irregular, elliptic, Oblong

Colour – Greenish Yellowishbrown

Odour - odourless

Taste -Acrid

The macroscopic examination of the plant *Nyctanthesarbor-tristis* were carried out. The results are reported as-

1. Loss on drying (%) -5.6
2. Total ash (%) - 3.5
3. Acid insoluble ash (%) -0.96
4. Foreign matter (%)- 0.8
5. Moisturecontent (%) - 7.2
6. Extraction of methanol (%)- 4.25, green color, semi solid and 7.1 pH value

Extraction of Plant Material

The shade dried coarsely powder of the plant viz., ***Nyctanthes arbor-tristis*** (Leaves) was extracted with ethanol and water in a Soxhlet apparatus. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator. The percentage yields of various extract along with their color, nature and pH were presented.

Preliminary Phytochemical Screening of Extract

The extract obtained after extraction of the plant material viz., ***Nyctanthes arbor-tristis*** (Stem & Leaves) was subject to phytochemical screening which revealed the present of various active phytoconstituents. The results were presented in **(Table 1)**.

Table 1: Preliminary Phytochemical Screening of Extract

S. No.	Chemical Constituents	Methanolic
1	Alkaloids	+
2	Carbohydrates	+
3	Glycosides	+
4	Steroids	+
5	Flavonoids	+
6	Saponins	+
7	Fixed oils and fats	-
8	Tannins	_
9	Proteins and amino acids	+
10	Terpenoids	-

Acute Toxicity Study of Extract (LD50)

The aqueous and ethanolic extracts the plant material viz., ***Nyctanthes arbor-tristis***. (Stem & Leaves) was screened for acute toxicity study by OECD guideline no. 423 for determination of LD50. The result indicates 200 mg/kg dose has been considered as effective dose (ED50), ***Nyctanthes arbor-tristis***. Results are presented.

LD 50- 2000mg/kg

ED 50 -200mg/kg

Pharmacological Screening of Extract

Anti-depressant activity

Tail Suspension

Malemice weighing 20–25garm used preferentially. They are housed in plastic cages for atleast 10days prior to testing in a 12 hlightcycle with food and water freely available. Animals are transported from the housing roomto the testing are in their own cages and allowed to adapt to the new environment for 1hr before testing. Groups of 10 animals are treated with the test compounds or the vehicle by intraperitoneal injection 30 min prior to testing. For the test the mice are suspended on the edge of a shelf 58cm above table to by adhesive tape placed approximately 1cm from the tip of the tail. The duration of immobility is recorded for periods of 5min. Mice are considered immobile when the hang passively and completely motionless for atleast 1min.

Experimental Animals and treatment regimens

Before one day of the experiment, the animals were divided and only into control, standard and

Experimental Groups (n=6). The first group (Group I) served as control group and receive vehicle, distilled water. The second group (Group II) has served as reference standard. The third group (Group III) was used to served standard drug mix with methanol for better analysis. Five groups (Group III, IV, V, VI and VII) and other five groups (Group IV, V, VI, VII and VIII) served test groups and received chloroform and methanolic extract of *Nyctanthes arbor-tristis* leaves respectively at five different doses such as 50,100,150,200 and 250mg/kg per orally. On the basis of our preliminary screening these five doses were selected.

Experimental protocol

Tail suspension test was first given by Steru.et.al. is commonly used animal behavioural model for screening of antidepressant-like activity in the rat or mice. So the present study was based on this model with some modification. For adaptation of laboratory condition, animals were transported from their housing colony to laboratory in their own cages before 1-2hr. Animals were individually hung on the edge of the shelf 50cm by above the floor by the using of adhesive tape placed approximately 1cm from the tip of the tail. The duration of immobility was recorded for 5min by using stop-watch. Animal was considered to be immobile when the hung passively and completely motionless. The changes in immobility were studies after 30min of administration of extracts, standard imipramine and vehicle. The test was conducted dim light and noise free room.

RESULTS AND DISCUSSION

The overall objective of the study was to compare the CNS activity of MENT explore on the rat. We have our attention focussed on CNS since the plant was used for the treatment of some psychic disorders. It is also widely used for the treatment of other disease, the neuro behavioural parameters were observed to see whether the plant is having any inherent toxicity which if present would make it unsuitable for any therapeutic promotion.

The present study was set about to evaluation of different pharmacological activities of ethanol, methanol, chloroform, n-hexane and petroleum ether extracts of the *Nyctanthes arbor-tristis* leaves.

The suggested worker presented compared antidepressant activity of *Nyctanthes arbor-tristis*. The selected medicinal plant was selected, authenticated and powdered. The powdered obtained was subjected for standardization with different parameters.

Evaluation of Antidepressant activity

EVALUATION OF ANTIDEPRESSANT ACTIVITY

Tail Suspension Test

The responses of control and all extract of different dose were compared. The result were found to be significant at 5% level of significance where P value<0.05. The effect of ethanol, chloroform, methanol, n-hexane and petroleum ether extracts were more propounced after 30, 60, 60, 60 and 60minutes respectively at all test doses which are showing the table I, II, III, IV and V respectively. It was observed that ethanol extract at 100mg/kg and 150mg/kg and chloroform extract at 150mg/kg and at 200mg/kg possess highly significant reduction in immobility time when compared to control in dose dependent manner. Similarly the animal is treated with standard drug (Imipramine HCl, 15mg/kg) exhibited significant decrease in immobility time as expected. The Pvalues for ethanol at 100mg/kg and 150mg/kg are 0.0008 and 0.0009 after 30minutes, methanol extract at 150mg/kg and 200mg/kg are 0.0003 and 0.0004 after 60 minutes.

Table 2: Anti-depressant effect of MENT by tail suspension method (mean±SEM)

Group	Dose (mg/kg)	Immobility Period (in second)								
		Pre Treatment	I (.30hr)	II (1.40hr)	III (2.50hr)	IV (4.0hr)	V (5.10hr)	VI (6.20hr)	VII (7.30hr)	VIII (24.0hr)
I	50	39.00±2.24**	40.00±0.00**	41.40±1.60**	40.42±1.58**	34.00±0.00**	40.45±0.45**	50.00±0.00**	54.59±2.41**	59.51±2.49**
II	100	70.97±3.03**	65.89±3.11**	71.91±3.09**	57.43±1.57**	52.45±1.55**	64.86±3.14**	52.48±1.52**	44.40±1.60**	58.52±2.48**
III	150	63.00±0.00**	55.00±1.00**	42.42±1.58**	52.54±2.46**	50.56±2.44**	49.77±0.23**	53.59±2.11**	42.41±1.59**	52.00±2.00**
IV	200	68.89±3.11**	49.00±1.00**	46.43±1.57**	61.00±1.00**	46.47±1.50**	50.00±1.00**	57.00±1.00**	46.42±1.58**	60.64±0.36**
V	250	64.6±0.40**	55.00±2.00**	57.54±2.46**	52.56±2.44**	52.58±2.40**	53.59±2.41**	61.00±1.00**	50.53±2.47**	55.55±2.45**
Std.	15	70.51±2.49	50.43±1.58	55.45±1.55	49.47±1.53	51.59±2.40	53.40±1.60	47.42±1.58	54.54±2.46	50.56±2.44
Cont.	15	86.85±3.15	84.47±1.53	88.39±0.61	79.41±1.59	82.53±2.47	76.44±1.56	78.46±1.54	81.58±1.42	82.50±1.50

All values are expressed in mean±standard error mean(n=6).

All data were found to be significantat 5% level of significance where$p$<0.05.**

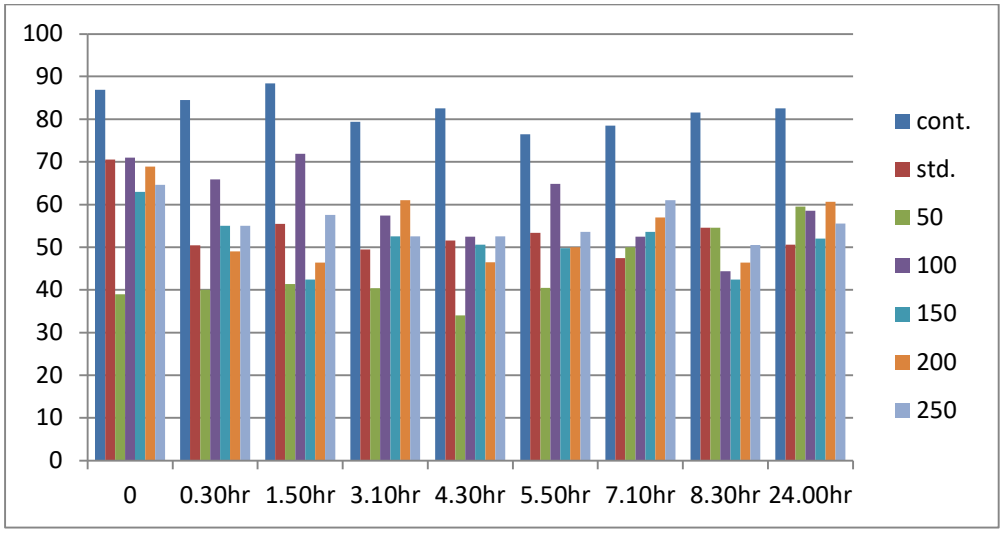


Figure 2: Effect of MENT in Immobility time

CONCLUSION:

These findings establish the potential of the selected plant as CNS activity and scientifically proved its traditional claim. Hence the present study concludes that the selected plant directs the importance of future development of some potential antidepressant drugs as well as their mechanism of action.

ACKNOWLEDGE:

The authors are thankful to Dr.Neeraj Sharma Prof. and Principal Bhagwant University Ajmer, Rajasthan for providing facilities, technical assistance, funding continuous encouragement and valuable guidance and facilities at all stages of this work support.

REFERENCES

[1] Uniyal SK, Singh KN, Jomwal P, Lal B. Traditional use of medicinal plants among the tribal communities of Chota Bengal, Western Himalaya. *Ethnobiol Ethnomed.* 2006; 2:1- 14.

[2] Elizabeth KM. Antimicrobial activity of *Terminalia bellerica. Indian ClinBiochem*2005; 20: 150-153.

[3] Shukla AK, Patra S, Dubey VK. Deciphering molecular mechanism underlying antileishmanial activity of *Nyctanthes arbor tristis*, an Indian medicinal plant. *J. Ethanopharmacology.* 2011; 134 (3):996-8.

[4] Badam L, Deolankar RP, Rojatkar SR, Nagsampgi, BA, Wagh UV. *Invitro* antimalarial activity of medicinal plants of India *Med. Res.* 1988; 87: 379-383.

[5] Lal J, Chandra SV, Prakash R, Sabir M. *In vitro* anthelminthic action of some indigenous medicinal plants on *Ascarisdiagall I* worms. *Ind. PhysiolPharmacol.* 1976; 20: 64-68.

[6] Kusum H, Sureban RR, Gopalakrishna B, Byahatti VV, Rajendra SV. Hepatoprotective activity of the leaves of *Nyctanthes arbortristis* Linn. *Ind. Pharmaceut*. Sci. 2006; 68: 542-543.

[7] Rathee JS, Hassarajani SA, Chattopadhyay S. Antioxidant activity of *Nyctanthes arbor-trisr-tis* leaf extract *Food Chem.*2007; 103: 1350-1357.

[8] Yang RY, lin S, kuo G. Content and distribution of flavonoids among 91 edible plant species. Asia Pac J ClinNutr 2008;17(S1):275-279.

[9] Grundmann O, Nakajima J, Kamata K, Seo S, ButterweckV.Kampferol from the leaves of *apocynum venetum* possesses anxiolytic activities in the elevated plus maze test in mice. Phytomedicine 2009 Apr;16(4):295-302.

[10] Mohapatra SP, Sahoo HP. An ethno-medico-botanical study of bolangir, Orissa, India: Native plant remedies against gynaecological diseases. Ethnobotanical leaflets 2008; 12: 846-850.

[11] Olowokudejo JD, Kadiri AB, Travih VA. An ethnobotanical survey of herbal markets and medicinal plants in lagos state of Nigeria. Ethnobotanical leaflets 2008; 12: 851-865.