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In-Vitro and in-Vivo Evaluation of Anti Asthmatic Activity of Rhizomes Extract of *Acorus Calamus (Linn.)* in Guinea Pigs

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Abstract

The aim of the paper is to evaluate the anti-asthmatic possessions of ethanolic extract of Rhizomes of Acorus calmus (Linn) by retaining in-vivo and in-vitro screening models in Guinea pigs. In vivo experiment was done by inducing bronchus asthma in guinea pigs by exposing them to 0.2 % histamine aerosol produced by an ultra-sound nebulizer in an aerosol chamber. sacrifice of animals In vitro were done by cervical dislocation and carotid bleeding. The trachea was dissected out and transferred to a dish containing kerb's solution (composition (g/l): NaCl (6.5), KCl (0.33), $CaCl_2$ (0.26), $MgSO_47H_2O$ (0.28), $NaHCO_3$ (2.5), KH_2PO_4 (0.19) and glucose 5.0)and cut diagonally between the section of the cartilage and continuously aerated and maintained at 37 ± 1°C. The trachea was adjourned and allowed to stabilize for at least 40 minutes. During stabilization, the bath was full with fresh kerb's solution for every 15 minutes. As a results the ethanolic extract produced significant dose-dependent antiasthmatic activity. The extracts also inhibited the contraction induced by histamine, Primary phytochemical screening revealed the presence of flavonoids, steroids, saponins, and tannins. The present study confirmed that the Rhizomes extract of Acorus Calamus exhibited bronchodilator activity.

Keywords: Antiasthmatic activity, histamine induced bronchoconstriction, bronchodialaotr, preconvulsive dyspnea (PCD), histamine aerosol, preparation of tracheal chain.

Introduction

Acorus calamus Linn. Belongs to Family- Araceae, usually known as "sweet flag" or Waan-Nam, is a medicinal plant found application in ayurvedic medicine for several years. *Calamus* is a herb used for the appetite and as an aid to the digestion. It is used for fevers, stomach cramps and cholic. Their rhizomes were used for toothache and powdered rhizome for congestion. The rhizome part is also used to treat several diseases like asthma and bronchitis and as sedative. Native tribes treated cough by making a decoction of the plant as a carminative and also for cholic[1]. The rhizomes were employed widely by the Indians as well as by other cultures also. The Rhizomes and rhizomes of the plants are used in various diseases including hysteria, neurasthenia, recklessness, melancholia, epilepsy, insomnia, as well as in asthma. The various activities related to pharmacology of the plant such as analgesic, anticonvulsant, antispasmodic, anti-inflammatory, antibacterial, antiulcer and cytoprotective activity anti-sczhizophrenia, anti-anxiety), tranquilizer and CNS depressant activity, neuromodulatory effect in dopaminergic system have been reported. The Rhizomes and rhizomes of this plant found application in the structures of Indian and Chinese medicine for their valuable role in improving learning performance, and for their anti-aging effect[2]. The methanolic extracts of Rhizomes and rhizomes of a*corus calamus* reported to use in rat brain homogenate. The aqueous ethanol extracts of various traditional herbs like *Adhatoda vasica, Acorus calamus, Glyzyrrhiza glabra, Ocimum sanctum, Tylophora asthmatica, Piper longum* and *Solanum xanthocarpum* was assessed for its antihistaminic activity by the inhibition of histamine induced contractions on the guinea pig ileum.The cough syrup inhibited histamine induced contractions of guinea pig ileum at concentrations 2.8 to 28 µg/.[3] An adhuyashtyadi syrup used as ayurvedic medicine in which *acorus calamus used*as an ingredient, after administration showed a significant release in bronchospasm deprived of any side effect. Moreover, these drugs produce side effects. Therefore, there is a dire need to identify effective and safe remedies to combat bronchial asthma. The current accepted modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies. Herbal medicines are being used by nearly about 80% of the world population; primarily in developing countries for primary health care.

Material and Methods

Plant material: The Rhizomes of *Acorus calamus* were through the courtesy of Agricultural Department, Sagar and was authenticated by the Department of Botany, Dr. H.S. Gour University Sagar, MP. The herb specimen no. XXXIV has been deposited in the room no. 36 of the Chemistry Department, Dr. H.S. Gour University, Sagar MP.

Preparation of extract: Rhizomes of the *Acorus calamus* were air dehydrated and crinkled into powdered form. The crushed powder was extracted with 95% ethanol. The ethanolic extract

was concentrated under reduced pressure to get a brown viscous mass.

Experimental animals: Approval for the use of animals in the experiments was obtained from the Institutional Animal Ethics Committee, Dr. H.S. Gour University Sagar, M.P. (Animal Eths. comm./DB-204) . Guinea pig of either sex, weighing (250-400 gm) and albino mice (25-30 gm) were used in this study. Animals were lodged under standard environmental conditions of temperature and humidity for 24 hrs and with water adlibitum.

Acute toxicity studies*:* Acute toxicity studies were implemented on Albino mice of either sex selected by sampling technique. The animals were fasted for 4hrs with free access to water only. The ethanolic extract of *Acorus Calamus* was administered orally with varying doses. The mortality was experimened for three days. If mortality was observed in 2/3 or 3/3 of animals, Then dose administered was considered as a toxic dose. However, if the mortality was observed in one mouse out of three animals then the same dose was repetitive to confirm the toxic effect. If mortality was not observed, then procedure repeated with higher dose such as 100, 200 and 400 mg.kg-1[4].

Evaluation of Antiasthmatic activity: Solated Guinea pig ileum preparation (in-vitro)[5-8]: Guinea pigs of either sex (250-400 gm) were grouped into four. Each group contains six animals and were allowed to starve overnight and free access to water. Animals were sacrificed by cervical dislocation and carotid bleeding. The trachea was dissected out and transferred to a dish containing kerb's solution (composition (g/l): NaCl (6.5), KCl (0.33), $CaCl_2$ (0.26), $MgSO_47H_2O$ (0.28), $NaHCO_3$ (2.5), KH_2PO_4 (0.19) and glucose 5.0) and cut crosswise between the sections of the cartilage of the trachea and continuously ventilated and maintained at 37 ± 1°C. The adjourned trachea was allowed to make steady for at least 40 minutes. On equilibrium, the bath was supplied with fresh kerb's solution for every 15 minutes. Then cumulative concentration response to histamine in the absence and presence of ethanolic extract of *Rhizomes of Acorus Calamus* were recorded with a (0.25 mm/sec) kymograph[9-11].

Histamine Aerosol induced bronchoconstriction in Guinea pigs (in-vivo)[12-14]: Histamine was dissolved in distilled water to prepare 0.2% w/v solution. Experimentally bronchial asthma was induced in *guinea pigs* by exposing histamine aerosol by an ultra-sound nebulizer in an aerosol chamber (30 x 15 x 15cm) made of Perspex glass. The required time for appearance of pre-convulsive dyspnoea produced by the histamine was noted for each animal. Each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The preconvulsion time (PCT), i.e. the time of aerosol exposure to the start of dyspnoea leading to the appearance of convulsion, was noted. As quickly as the preconvulsion dyspnoea (PCD) was recorded, the animals were removed from the chamber and positioned in fresh air for recover. This time for preconvulsive dyspnoea was recorded as basal value. *Guinea pigs* were then allowed to recover from dyspnoea for 2 days. After that, the animals were allotted to four different groups of 4-5 animals per group. Animals in group 1 served as control and received distilled water. The animals of group 2 and 3 were given, by oral intubation, 200 and 500mg/kg of the plant extract, respectively, while group 4 received the standard drug - Chlorpheniramine maleate, intraperitoneally. After receiving the drugs, all the animals were again exposed to histamine aerosol in the chamber, one hour, four hours and 24 hrs, to determine pre convulsive time (PCT).

The protection untaken by the treatment was calculated using the formula

Percentage protection

$$= \frac{\text{Eta} - \text{Etb}}{\text{Etb}} \times 100 \text{ (\% Protection offered by the extract)}$$

Where: Eta is the mean of PCT (preconvulsion time) before administration of test drugs. Etb is the mean of PCT (preconvulsion time) after administration of test drugs at 1 hr, 4 hr and 24 hrs.

Statistical analysis: All the values of *in vitro* and *in vivo* anti asthmatic activity were expressed as mean ± Standard error of mean (S.E.M) and was examined for significance by ANOVA (analysis of variance) and groups were compared by Dunnett's test for individual comparison of groups with control. P Value were measured moderate significant at P<0.01, <0.001 level.

Results and Discussion

Isolated Guinea pig ileum preparation: Ethanolic extract of Rhizomes of *Acorus Calamus (Linn)* displayed significant (***p*<0.001) percent decreased contraction at 100µg/ml in isolated Guinea pig ileum preparation (table 1).

Table–1
Effect of ethanol extract of Rhizomes of Acorus Calamus Linn (100µg/ml) on histamine induced contraction on isolated guinea pig ileum

Dose (ml)	Control (Distilled water) (10µg/ml)	Acorus Calamus Ethanolic extract (100µg/ml)	Chlorpheniramine maleate (CPM) (10µg/ml))
0.1	44.12±2.16	18.26±0.98**	19.26±1.36**
0.2	65.43±3.04	30.15±1.67**	32.67±1.76**
0.4	67.94±2.28	32.17±1.14**	41.22±2.72**
0.8	78.5±2.53	35.22±1.31**	41.22±2.33**
1.6	83.4±1.72	47.60±0.81**	56.20±1.99**
3.2	100±1.69	49.24±0.98**	65.00±2.26**

n=5, values are mean ± SEM. Control = DRC of histamine in absence of ACEE extract. ACEE (100µg/ml) = DRC of histamine in presence of ethanolic extract of *Acorus Calamus,* CPM (10µg/ml) = DRC of histamine in presence of chlorpheniramine maleate which is standard (10µg/ml). Statistical analysis done by using Student's t-test and ANOVA followed by Dunnet's test. $p<0.05$, ** $p< 0.01$, *** $p< 0.001$ significantly different from control.

Histamine induced bronchoconstriction in Guinea pigs: The ethanolic extract of Rhizomes of *Acorus Calamus (Linn)* expressively extended the latent period of spasms followed by exposing to histamine aerosol at the dose of 400mg/kg, p.o. which showed extreme protection of 48.9 % at time 4 hour as compared to chlorpheniramine maleate (standard) 1mg/kg, p.o. which untaken maximum protection of 63.2% at time 4 hour (tables 2 and 3).

Table – 2

Effect of ethanol extract of Rhizomes of *Acorus calmus (Linn)* against Histamine induced bronchoconstriction in guinea pigs

Groups	Latent period of convulsion (in sec.)(Mean ± SEM)			
	Before	**1 hr**	**4hr**	**24hr**
STD (Standard) chlorpheniramine maleate (1mg/kg,)	16.2±0.87	55.6±2.61**	63.2±2.01**	28.7 ±1.06**
Acorus Calamus Rhizomes ethanolic extract (100 mg/ Kg)	14.7±1.02	27.6±2.19**	37.3±2.09**	26.2±0.89**
Acorus Calamus Rhizomes ethanolic extract (200 mg/Kg)	15.2±0.83	42.3±1.67**	49.26 ±2.01**	27.8 ±1.40**
Acorus Calamus Rhizomes ethanolic extract (400 mg/Kg)	16.6±0.73	35.6±1.43**	48.9±1.55**	24.2±1.57**

Table – 3

% Protection of Rhizomes of *Acorus calmus (Linn)* against histamine induced Bronchoconstriction in guinea pigs Groups

Groups	% Protection		
	1 hr	**4hr**	**24hr**
STD (Chlorpheniramine maleate (2 mg/kg, i.p.)	73.5	77.40	47.91
Acorus Calamus Rhizomes ethanolic extract (100 mg/Kg)	55.24	67.82	38.12
Acorus Calamus Rhizomes ethanolic extract (200 mg/Kg)	67.07	64.90	40.70
Acorus Calamus Rhizomes ethanolic extract (400 mg/Kg)	58.51	60.90	39.40

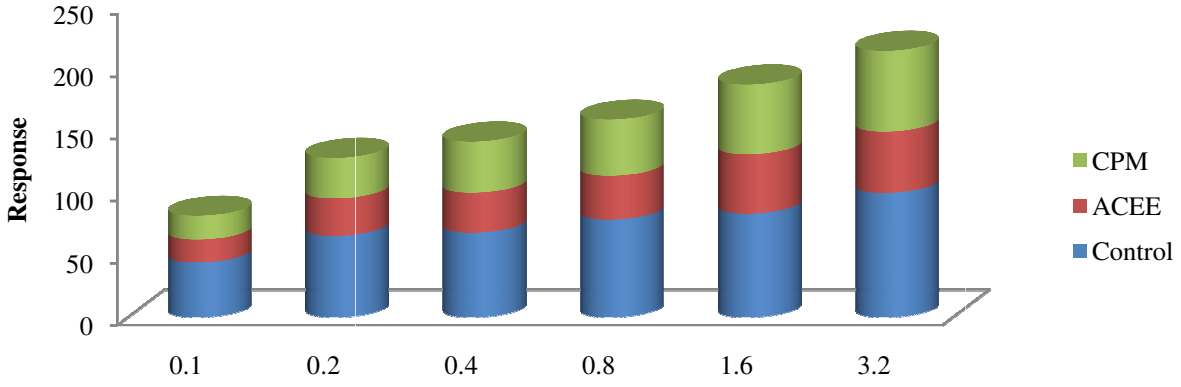

Figure-1

Effect of ethanolic extract of rhizomes of Acorus Calamus Linn. (100µg/ml) on histamine induced contraction on isolated guinea pig ileum

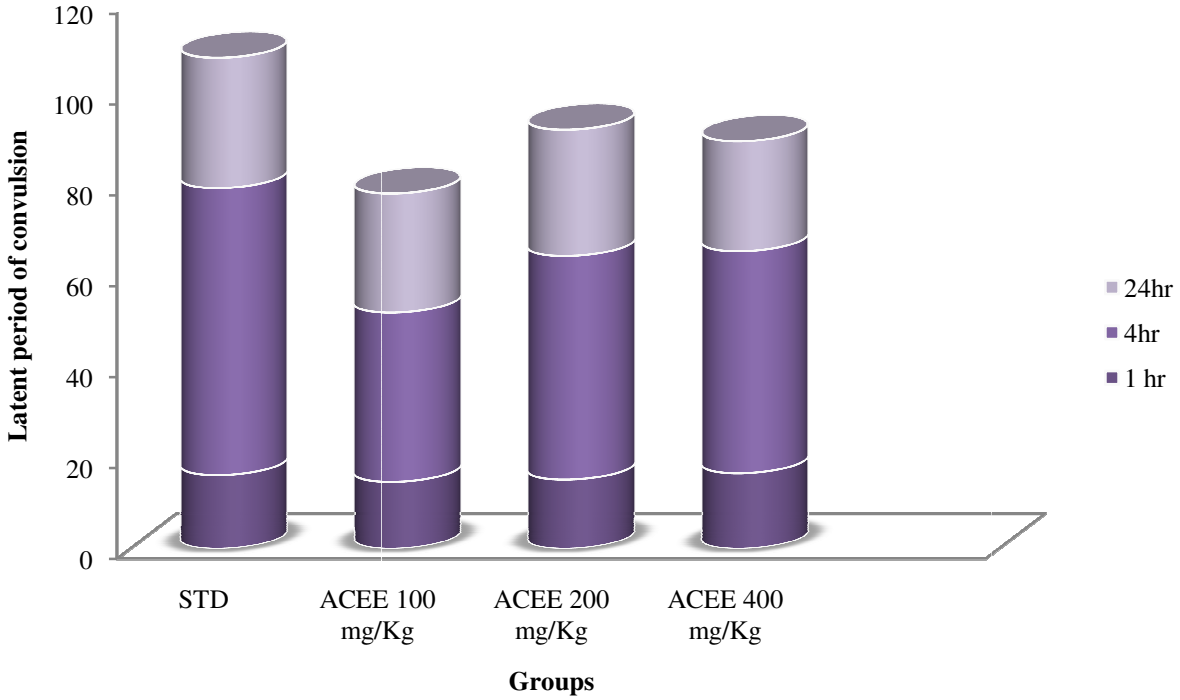

Figure-2
Effect of ethanolic extract of rhizomes of Acorus calamus (Linn) against Histamine induced bronchoconstriction in guinea pigs

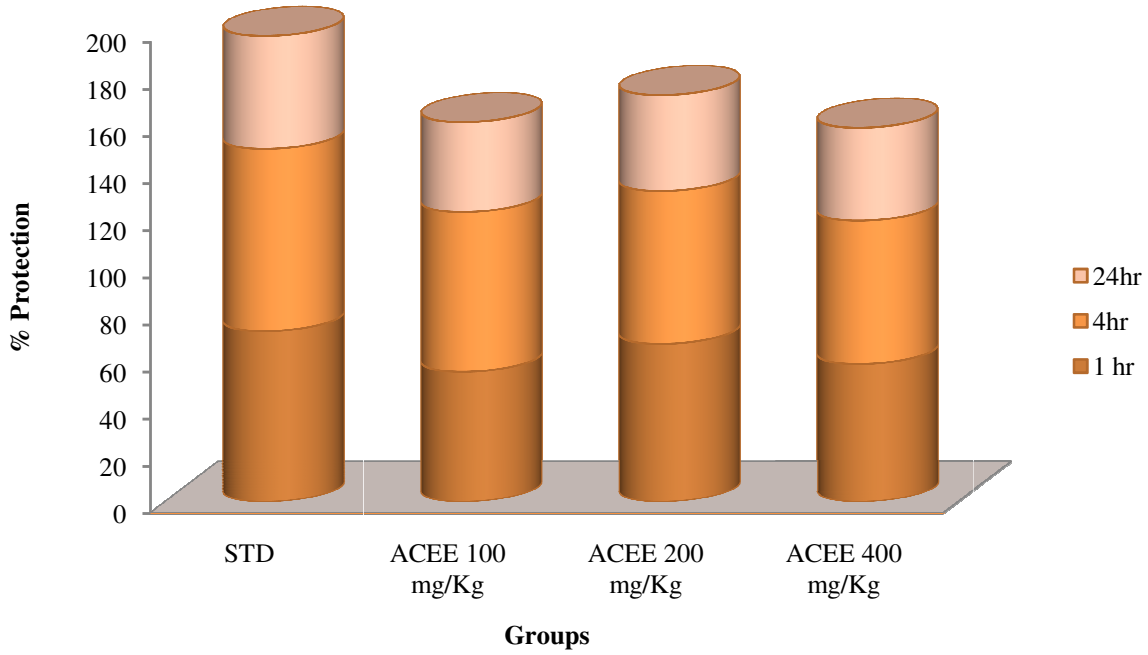

Figure-3
% Protection of rhizomes of Acorus calamus (Linn) against histamine induced Bronchoconstriction in guinea pigs Groups

Asthma is an allergic disease with the utmost clinical and economic effect is an allergic and inflammatory outward sign of respiratory disorders. Asthma is a respiratory disease. The illness and mortality of the disease it a worldwide concern. The symptoms of bronchial asthma is characterized by wide blowout narrowing of the bronchial tube due to contraction of the smooth muscle in reply to stimuli subsequently in the release of histamine. Ileum of Guinea pig is used for screening antihistaminic activity. The H^1 receptors after stimulation produces well-ordered dose related contraction of isolated Guinea pig ileum . In the current study, *Acorus Calamus (Linn)* (100 µg/ml) significantly inhibited the histamine induced contraction of isolated guinea-pig ileum preparation indicating its H^1 receptor antagonistic activity and supports the anti-asthmatic properties of the plant.

Bronchoconstriction induced by Histamine is the an immunological model of antigen induced airway obstruction. Histamine when inhaled causes hypoxia and leads to spasm in Guinea pigs and causes very strong smooth muscle contraction and capillary dilation in cardiovascular system. Bronchodilators can delay the occurrence of these symptoms. The study resulted in deep-rooted the bronchodilator properties of the plant, justifying its claiming in the treatment of asthma[15-18].

In asthma mostly drugs effective are steroid. Phytochemically the plant discloses the presence of steroidal, flavonoids, nucleus in form of triterpenoids as well various saponin glycosides. The antiasthmatic activity showed by the plant may be because of these chemical moieties. However this claim demands for further research and the studies are infact underway to isolate and characterize the active principles responsible for the anti-asthamatic activity.

Asthma is essentially characterized by the restriction of obstruction tracheal muscle. The disorder of bronchial asthma is characterized by wide spread narrowing of the bronchial tube due to contraction of the smooth muscle in response to stimuli causing in the release of chemical mediators such as histamine[19,20].

Conclusion

Current study resolved that *Acorus Calamus (Linn)* ethanolic extract possess highly substantial anti-asthmatic activity by significantly inhibited the histamine induced broncho constriction of guinea pig representing its H1 receptor antagonistic activity and support the plants by its anti-asthmatic properties. The results obtained in the study will provide basic data for further progress and application of these plants.

References

1. Achliya G.S., Wadodkar S.G., and Dorle A. K. Evaluation of CNS activity of Bramhi Ghrita, *Indian J. Pharmacol*., **37,** 33-36 (**2005**)

2. Aqil F., and Ahmad I., Antibacterial properties of traditionally used Indian medicinal plants, Methods Find, *Exp. Clin. Pharmacol.*, **29,** 79-92 (**2007**)

3. Elaya R. A., Vijayalakshmi M., and Devalara G., *Acorus calamus* linn.: Chemistry and Biology, *Res. J. Pharm. and Tech.,* **2 (2),** 256-261 (**2009)**

4. Harish M.S., Nagur M., and Badami S., Antihistaminic and mast cell stabilizing activity of Striga orobanchioide, *J Ethnopharmacol,* **76**, 197-200 (**2001)**

5. Bigoniya1 P., Shukla A., Agrawal G.P. and Rana A.C., Pharmacological Screening of *Wrightia tinctoria* Bark Hydro-Alcoholic Extract, *Asian J. Exp. Sci.,* **22(3),** 235-244 (**2008)**

6. Afreen A., Kashyap P., Sawarkar H., Deshmukh V., Upadhyay A. and Pal S., In- vitro and In- vivo Models for Evaluation of Anti-Asthmatic Activity: A Review, *International Journal of Herbal Drug Research***, I(1),** 19-27 **(2011**)

7. Surendra A., Pusapati M.R. and Harish M.S., Antiasthmatic Activity Of Aqueous Extract Of Pistacia Integerrima Galls, *International Journal of Pharmacy and Pharmaceutical Sciences*, **5(2),** 116-121(**2013**)

8. Kumar D., Bhujba S. S., Deoda R. S., and Mudgade S. C., *In-vitro* and *In-vivo* Antiasthmatic Studies *Ailanthus excelsa* Roxb. on of Guinea Pigs*, Journal of scientific research*, **2(1),** 196-202 (**2010)**

9. Kulkarni S.K. Hand book of experimental pharmacology, Vallabh Prakashan, New Delhi, II Edn, 22. **(1979)**

10. Tejas P. and Samir S., Anti asthmatic activity of aqueous extract of Myrica nagi bark, *Journal of Current Pharmaceutical Research,* **10 (1),** 34-39 **(2012)**

11. Kajaria D., Kajaria A., Tripathi J. S., and Tiwari S.K, In-Vitro and In-Vivo Assessment of Anti-Asthmatic Activity of Polyherbal Ayurvedic Drug, *Journal of Pharmacy and Biological Sciences,* **6(3)**, 60-70 (**2013)**

12. Stephen O Okpo, Gerald I Eze, Ifeanyi H Ajaanonwu1, Ogochukwu L Ijei,Dickson O Uwaya, Viona Ologe, Evaluation of the Anti-asthma Activity of Aqueous Root,Bark Extract of *Ficus exasperata* Vahl (Moraceae), *International Journal of Health Research*, **5(1)**, 5-12 (**2012)**

13. Sharma P., Hullatti K.K., Kuppasth I.J. and Sharma S., Studies on Anti-Asthmatic Property Of *Cyamopsis Tetragonoloba* (L.) Taub. Leaf Extracts, *Journal of Natural Remedies*, **10(1),** 81–86 **(2010)**

14. Garg M and Garg C, Effect of Phyllanthus Urinaria in Biochemical Profile of Experimental Hyperglycemic Albino Rats, *es. J. Pharmaceutical Sci,* **1(1),** 2-5**(2012)**

15. Sharma A., Bhatia S., Kharya M.D., Gajbhiye V., Ganesh N.A.G., Namdeo K.R. and Mahadik, Anti-inflammatory and analgesic activity of different fractions of *Boswellia*

serrate, International Journal of Phytomedicine, **2,** 94-99 (**2010)**

16. Nalamwar V.P., Khadabadi S.S., Aswar P.B., Kosalge S.B. and Rajurkar R.M. In vitro Licicidal Activity of Different Extracts Of Acorus calamus Linn. (Araceae) Rhizome. *International Journal of PharmTech Research* **1(1)**, 96-100 **(2009)**

17. Shelke M.E., S-Triazines: As Alternative Drugs for the Treatments of Typhoid, *Res. J. of Pharmaceutical Sci.,***2(1)**, 18-19**(2013)**

18. Parganiha R., Verma S, Chandrakar S., Pal S., Sawarkar H.A. and Kashyap P., In vitro anti- asthmatic activity of fruit extract of *Piper nigrum (Piperaceae), International Journal of Herbal Drug Research,* **1(I)**, 15-18 **(2011)**

19. Anbu Jeba Sunilson J.,Anandarajagopa , K., Abdullah khan, Khaja Pasha, Qusro Bin Hassan and Puspa V. Kuna Raja. Antihistaminic evaluation of formulated polyherbal cough syrup, *Journal of Medicinal Plants Research,* **4(14)**, 1482-1485, **(2010)**

20. Neelmani C., Saurabh R. and Dubey B.K., Antiasthmatic Effect of Rhizomes of *Clitorea Ternatea* Linn**,** *International Journal of Pharmaceutical and research,* **3(4),** 1076-1079, **(2012)**