ISSN 2581-6217

World Journal of Pharmaceutical Science & Technology

Journal homepage: www.wjpst.com

Original Research Article

EVALUATION OF BIOACTIVE CONSTITUENTS AND ANTIPYRETIC ACTIVITY OF LEAVES EXTRACT OF *CASSIA TORA*

Miss Priyanka Bharti*[1], Dr. C.K. Tyagi[2], Dr.Prabhakar Budholiya[3]

1. College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M. P.)
2. College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M. P.)
3. College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M. P.)

Address for correspondence:

Priyanka Bharti, College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M. P.)

Email Id- bhartipriyanka250@gmail.com

Received: 15-02-2021, Revised: 25-02-2021, Accepted: 28-02-2021

ABSTRACT

Cassia tora is well known drug for its kustaghna, dadrughna karma as per literature available in Ayurvedic classics. Phyto-chemical study offered to determine active ingredient which are accountable for bringing out drug action. It also provides introductory information on the quality of the drug. The alcoholic extract of Cassia tora leaves were collected from coarse powder of Cassia tora leaves by using of extraction. Alcoholic extract of Cassia tora was then subjected to phytochemical screening to test for presence of metabolites such as alkaloids, flavonoids, phenol, tannins, saponins, sugar, glycosides, steroids, Carbohydrates, glycosides which were qualitatively analyzed. This study would provide preliminary research-based evidence for Cassia tora as potent drug, because of Cassia tora leaves have more active concept like alkaloids, flavonoids, phenol, tannins, saponins, sugar, glycosides, steroids, Carbohydrates, glycosides. Hence phyto-chemical study of Cassia tora leaves is essential in order to evaluate active ingredient accountable for its medicinal actions.

KEYWORDS: Cassia tora Linn, Hydroalcoholic extract, antipyretic activity, Brewer's yeast-induced

1. INTRODUCTION

Pyrexia or Fever is defined as an elevation of body temperature. It is a response due to tissue damage, inflammation, malignancy or graft rejection. Cytokines, interleukin, interferon and Tumor Necrosis Factor α (TNF- α) are formed in large amount under this condition, which increase PGE2 which in turn triggers

hypothalamus to elevate body temperature. Fever is associated with symptoms of sickness behavior which consist of lethargy, depression, anorexia, sleepiness, & inability to concentrate. This increase in set point triggers increased muscle tone & shivering. However antipyretic medication can be effective at lowering the temperature which may include the affected person's comfort. Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive. Normally the infected or damaged tissue initiates the enhanced formation of proinflammatory mediator's (Cytokines like interleukin 1β, α, β and TNF- α), which increase the synthesis of prostaglandin E2 (PG E2) near peptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature [Spacer CB *et al*., 1994]. As the temperature regulatory system is governed by a nervous feedback mechanism, so when body temperature becomes very high, it dilate the blood vessels and increasing sweating to reduce the temperature; but when the body temperature become very low hypothalamus protect the internal temperature by vasoconstriction. High fever often increases faster disease progression by increasing tissue catabolism, dehydration and existing complaints, as found in HIV [Veugelers PJ *et al* 1997]. Drugs having anti-inflammatory activity generally possess antipyretic activity (e.g) non-steroidal anti-inflammatory drugs (NSAIDs). It has been suggested that prostaglandin (PGE) mediates pyrogen fever; the ability of NSAIDs, to inhibit prostaglandin synthesis could help to explain their antipyretic activity. Fever is one of the most common presenting signs of illness in office-based primary care pediatric practice, accounting for 19% to 30% of visits [Eskerud *et al*., 1992 and Baucher *et al*., 2001]. Infants and young children are particularly susceptible to fever because of their small body size, high ratio of body surface area to weight, and low amount of subcutaneous fat. Although most experts consider fever a beneficial physiologic response to the infectious process, it can lead to patient irritability and stress as well as high parental anxiety. Therefore, physicians usually prefer to prescribe antipyretic agents in addition to nonpharmacologic, physical fever-reducing modalities [Baraff *et al*., 1993]. Fever is a complex physiologic response triggered by infections or aseptic stimuli. Elevation in body temperature occurs when the concentration of prostaglandin E2 (PGE2) increases within parts of the brain. Such an elevation contributes to a considerable alteration in the firing rate of neurons that control the thermoregulation process in the hypothalamus. It is now evident that most of the antipyretic drugs exert their action by inhibiting the enzymatic activity of cyclooxygenase and consequently reducing the levels of PGE2 within the hypothalamic region. High fever often increases faster disease progression by increasing tissue catabolism, dehydration, and existing complaints, as found in HIV. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PgE2 biosynthesis. These synthetic agents irreversibly inhibit COX-2 with a high selectivity and are toxic to the hepatic cells, glomeruli, cortex of brain, and heart muscles. Natural COX-2 inhibitors have lower selectivity with fewer side effects [Cheng L *et al*., 2005].

1.2 The pathogenesis of fever:

Many of the mediators underlying pyrexia have been described in recent years. The critical "endogenous pyrogens" involved in producing a highly regulated inflammatory response to tissue injury and infections are polypeptide cytokines. Pyrogenic cytokines, such as interleukin-1b (IL-1b), tumor necrosis factor (TNF), and interleukin-6 (IL-6), are those that act directly on the hypothalamus to affect a fever response [Luheshi GN et al., 1998]. Exogenous pyrogens, such as microbial surface components, evoke pyrexia most commonly through the stimulation of pyrogenic cytokines. The gram-negative bacteria outer membrane lipopolysaccharide (endotoxin), however, is capable of functioning at the level of the hypothalamus, in much the same way as IL-1b [Dinarello CA *et al*., 1999]. Microbial tissue invasion sparks an inflammatory response and activates local vascular endothelial cells and leukocytes. The extravasation of white blood cells into inflamed areas depends on a multistep interaction with endothelial cells regulated by a variety of cytokines, chemokines, and adhesion molecules. Activated leukocytes release the pyrogenic cytokines interleukin-1b (IL-1b), tumor necrosis factor (TNF), and interleukin-6 (IL-6). Hematogenous dissemination (depicted here) allows these endogenous pyrogens to stimulate vascular endothelial cell production of prostaglandin E2 (PGE2) within the central nervous system. Peripheral inflammatory signals may also travel along neural

connections (such as the vagus nerve) to trigger central nervous system PGE2 production. Neurons within the preoptic area of the anterior hypothalamus (POAH) bearing specific E-prostanoid receptors orchestrate the febrile response after the PGE2 signal. PGE2 alters the firing rate of these neurons, resulting in an elevated thermoregulatory set point. The febrile set point body temperature is reached through the regulated evocation of behavioral and physiologic changes aimed at enhancing heat production and reducing heat dissipation. Fever is believed to augment the peripheral and systemic inflammatory response to infection in part by modulating the expression of inflammatory cytokines and enhancing leukocyte function

1.2 Antipyretics

Antipyretics are drugs which can reduce elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus which regulate the set point of body temperature. Drugs like paracetamol do not influence body temperature when elevated by factors such as exercise or increase in ambient temperature. Antipyretics have been shown to suppress fever by inhibiting prostaglandin synthetase, resulting in the blockade of the synthesis of prostaglandin in the brain or suppressing the rise of interleukin-1α production subsequent to interferon production Flavanoids like baicalin have been shown to exert antipyretic effect by suppressing TNF-α and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in reduction of prostaglandin levels thus reducing the fever and pain.

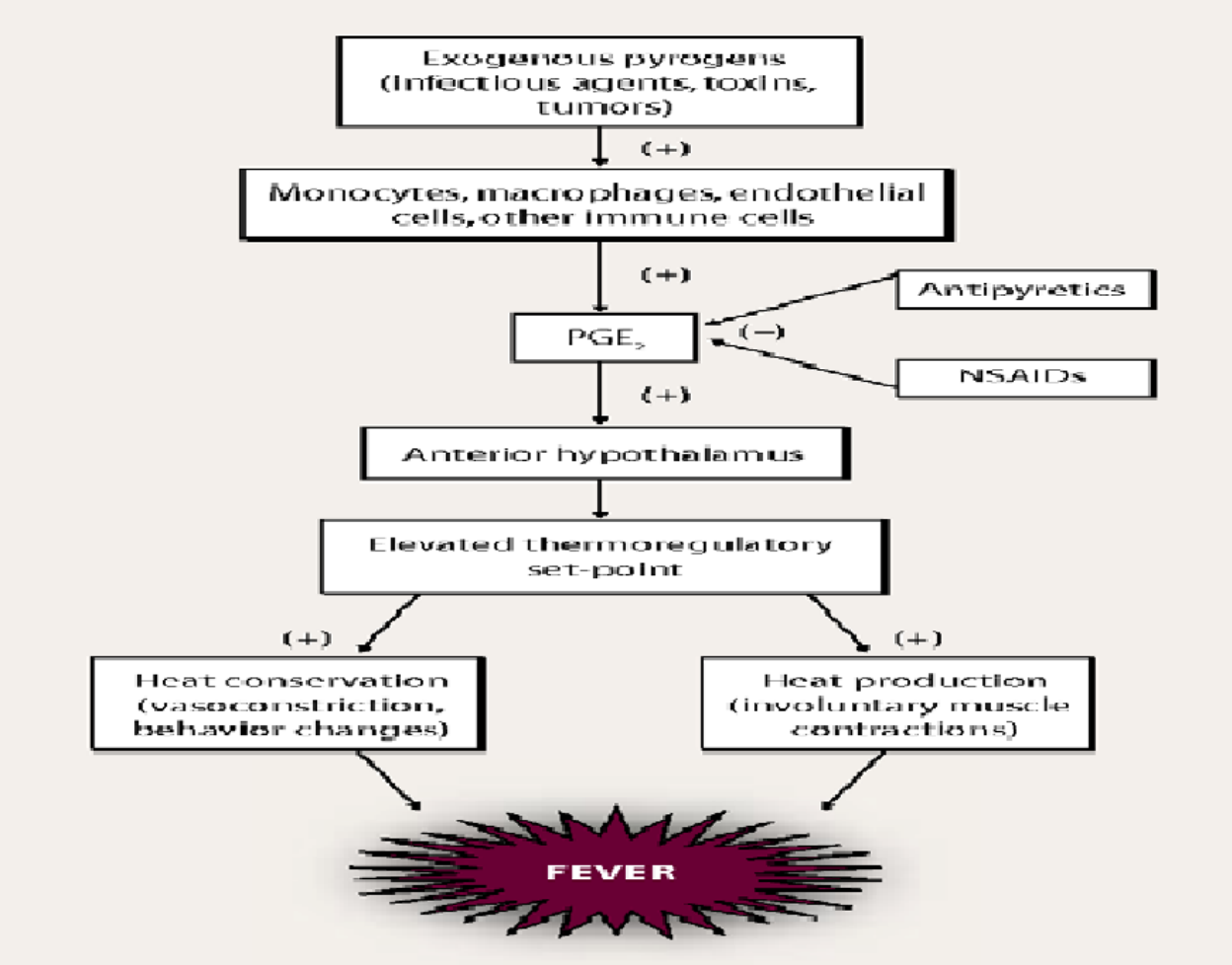

Fig.1 : Pathogenesis of fever

2. Material and Method

2.1 Plant material collection

Leaves of *Cassia Tora* Linn. was collected from Vindhya herbals Bhopal (M.P.) in the month of September 2020.

2.2 Extraction of plant material

Dried powdered leaves of *Cassia Tora* Linn. has been extracted with hydroalcoholic using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40^0C.

2.3 Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

2.4 Phytochemical Screening

The *Cassia Tora* extract acquire was subjected to the precursory phytochemical analysis following standard methods by Khandelwal and Kokate. The extract was screened to identify the presence of various active principles of alkaloids, glycosides, phenols, flavonoids, Amino acid, Cabohydrates, Terpenoids, Saponins, Steroids.

2.5 Estimation of total Phenolic, flavonoid and alkaloid Content

2.5.1 Total Phenolic content estimation

Procedure: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol.10 mg of dried extracted dissolve in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenols. 2 ml of extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

2.5.2 Total flavonoids content estimation

Procedure: Determination of total flavonoids content was based on aluminium chloride method.

10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of extract dissolved in 10 ml methanol and filter. Three (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

2.5.3 Total alkaloid content estimation

Procedure: Bromocresol green solution was prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2M sodium phosphate (71.6 gm Na_2HPO_4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 gm citric acid in 1 L distilled water). The plant extract (20mg) was dissolved in 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 μg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract [Shamsa *et al.,* 2008; Rao *et al.,* 2012].

2.6 *In vivo* antipyretic activity of *Cassia Tora* L

Animals

Swiss albino rats of either sex (150-200 g) were used for the experimental study. The animals were maintained under standard husbandry conditions in polypropylene cages and provided with food and water *ad libitum.* The animals were kept on fasting overnight prior to the experimentation. They are maintained at room temperature under suitable nutritional and environmental conditions throughout the experiment and all the procedures used in these studies were approved by the Institutional Animal Ethics Committee.

Acute toxicity studies:

The acute toxicity was performed according to OECD guidelines no. 423. The selected female albino rats were used for toxicity studies. The animals were divided into four groups of three in each. The animals were fasted overnight prior to the acute experimental procedure. Hydroalcoholic extract of *Cassia Tora* L. leaves was given orally to rats at the graded doses like 250, 500, 1000 and 2000 mg/kg body weight. Immediately, after dosing, The behavioral changes were closely observed for hyperactivity, ataxia, convulsion, salivation, tremors, diarrhoea, lethargy, sleep and coma. They were then kept under observation up to 14 days after drug administration to determine the mortality, if any.

Antipyretic activity of plant extract

A. Yeast-induced hyperpyrexia in rats (Mondal *et al*., 2016)

Yeast induced pyrexia was used to evaluate the antipyretic activity of the extract. The rats were divided into four groups of six animals and the body temperature of each rat was recorded by measuring rectal temperature at predetermined time intervals. Fever was induced by injecting 15% suspension of Brewer's yeast (*Saccharomyces cerevisiae*) in the back below the nape of the rat. In brief, the rats were allowed to remain quiet in the cage for sometimes. A thermistor probe was inserted 3-4 cm deep into the rectum, after fastened the tail, to record the basal rectal temperature. The animals were then given a subcutaneous (s.c.) injection of

10 ml/kg of 15% *w/v* Brewer's yeast suspended in 0.5% w/v methyl cellulose solution and the animals were returned to their housing cages. Twenty four hour after yeast injection, the rats were again restrained in individual cages to record their rectal temperature. Immediately the hydroalcoholic extract of *Cassia Tora* L. leaves were administered orally at doses of 250 and 500 mg/kg to the treatment control groups animals, the normal control group received distilled water and standard control groups animals received 45 mg/kg of paracetamol. Pre-drug control temperatures of all the rats was recorded at 24h immediately before the extract or paracetamol administration and again at 1h interval up to 4h after yeast injection.

Table 1: Treatment protocol:

Group	No. of animals in each group	Treatment/Dose
Group I Normal control	6	Brewer's yeast suspension (10 mL/kg b.w., s.c.)
Group II Standard Control	6	Brewer's yeast suspension (10 mL/kg b.w., s.c.) + Paracetamol (150 mg/kg *p.o.)*
Group III Treatment Group	6	Brewer's yeast suspension (10 mL/kg b.w., s.c.) + *Cassia Tora* hydroalcoholic extract at a dose of 250 mg/kg *p.o.*
Group IV Treatment Group	6	Brewer's yeast suspension (10 mL/kg b.w., s.c.) + *Cassia Tora* hydroalcoholic extract at a dose of 500 mg/kg *p.o.*

Statistical analysis

The data is expressed as mean ± Standard Deviation (SD). Results were analyzed using one-way ANOVA followed by Dunnet's test. Differences were considered as statistically significant at $P < 0.05$, when compared with control.

3. RESULTS AND DISCUSION

3.1 Result of Percentage Yield

Table No. 2: % Yield of hydroalcoholic extract

S. No.	Solvent	% Yield (w/w)
1.	Hydroalcoholic (30:70)	3.9 %

3.2 Phytochemical screening of extract

Table No. 3: Result of Phytochemical screening of hydroalcoholic extract

S. No.	Constituents	*Cassia Tora* L. (Leaves)
1.	**Alkaloids**	
	i. Mayer's test	+ve
	ii. Dragendorff's test	+ve
	iii. Wagner's test	+ve
2.	**Carbohydrates**	

	i Fehling's test *ii. Benedict's test*	-ve +ve
3.	**Flavonoids** *i. Ferric-chloride test:* *ii. Alkaline reagent test:* *iii. Shinoda's test*	 +ve +ve -ve
4.	**Proteins** *i. Biuret's test*	 +ve
5.	**Saponins** *i. Foam test*	 +ve
6.	**Steroids** *i. Salkowski test* *ii.Liebermann-burchard reaction*	 +ve +ve
7.	**Glycosides** *i. Legals test*: *ii. Borntrager's test*	 -ve -ve
8.	**Tannins** *i. Gelatin test*	 +ve
9.	**Phenol** *i. Ferric-chloride test:*	 +ve

3.3 Results of Estimation of Total Phenolic and flavonoid, alkaloid content estimation

Table No. 4 : Total Phenolic, Total flavanoid and Total alkaloid content of *Cassia Tora* Linn

S. No.	Extract	Total Phenol (GAE) (mg/100mg)	Total flavanoid (QE) (mg/100mg)	Total alkaloid (AE) (mg/100mg)
1.	Leaves (Hydroalcoholic)	2.87	1.88	0.72

3.4 Results of Acute toxicity studies

No mortality or morbidity was observed in animals through the 14 day period following single oral administration. Morphological characteristics (fur, skin, eyes and nose) appeared normal. No tremors, convulsion, salivation, diarrhea, lethargy or unusual behaviors such as self mutilation,walking backward etc. were observed. Gait and posture, reactivity to handling or sensory stimuli, grip strength was all normal. There was no significant difference in body weights between control and treatment groups. Food and water intake showed daily fluctuations within the range of control animals. This indicates that the hydroalcoholic extract of *Cassia Tora* leaves was safe to a single dose of 2000 mg/kg body weight. Hence, 250 and 500 mg/kg of body weight, of the maximum safe dose were selected for studying *in vivo* antipyretic activity.

3.4 .1 Antipyretic activity of plant extract

A. Yeast-induced hyperpyrexia in rats

It is well known that pharmaceutical companies around the world are interested in developing safer and more effective drugs to treat pain, inflammation and fever. Subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 24 h of administration. Treatment with the hydroalcoholic extract of *Cassia Tora* leaves at the doses of 250 and 500 mg/kg significantly decreased the rectal temperature of the rats. The antipyretic effect started as from the first hour and the effect was maintained for 4 h, after administration of the extract. The result obtained from both the standard paracetamol (150 mg/kg, p.o.) and hydroalcoholic extract of *Cassia Tora* leaves (250 and 500 mg/kg) treated rats were compared with that of control and a significant reduction (*P<0.05; **P<0.01; ***P<0.001) against yeast induced pyrexia was observed. Hydroalcoholic extract at a dose of 500mg/kg, after 4 h showed more effect as compared to standard drug.

Table No. 5: Effect of hydroalcoholic extract of *Cassia Tora* L. leaves on yeast induced pyrexia in rats

Group	Normal temperature before yeast administration	Pre-drug control, 1 h before drug admin.	Rectal temperature after drug administration (% decrease)			
			1h	2h	3h	4h
Group I Normal control	96.55±0.68	101.74±0.78	101.43±0.42 (0.31%)	101.34±0.38 (0.40%)	101.24±0.48 (0.52%)	101.16±0.56 (0.59%)
Group II Standard Control	96.65±0.84	100.39±0.69	98.64±0.59* (1.69%)	97.91±0.56** (2.42%)	96.12±0.40** (4.21%)	95.81±0.42** (4.98%)
Group III Treatment Group	96.85±0.69	100.98±0.49	99.41±0.29* (0.99%)	98.80±0.29** (2.11%)	97.84±0.27** (3.06%)	96.98±0.24** (3.89%)
Group IV Treatment Group	96.95±0.42	100.75±0.51	99.51±0.31** (1.18%)	98.21±0.67** (2.48%)	96.38±0.46** (4.29%)	94.91±0.67** (5.76%)

Each values represents the mean ± SEM; (n=6), *p<0.05, **p<0.01, ***p< 0.001 respectively when compared with toxicant control group (one-way ANOVA followed by Dunnett's test).Values in parentheses indicate percent decrease,

CONCLUSION

Phyto-chemical study helps to identify active constituents which are responsible for bringing out drug action. It also provides preliminary information on the quality of the drug. This study would provide preliminary scientific evidence for *Cassia tora* as potent drug, because of *Cassia tora* leaves have more active principles like alkaloids, flavonoids, phenol, tannins, saponins, glycosides, steroids, Carbohydrates, glycosides. Hence phyto-chemical study of *Cassia tora* leaves is essential in order to evaluate active constituents responsible for its medicinal actions. The plant based bio-active compounds have the effective dosage response with minimal side effects, when compared to the synthetic compounds. The presence of phytochemicals (secondary metabolites) is responsible for their therapeutic effects. It further reflects a hope for the development of many more novel therapeutic agents or templates from such plants which in future may serve for the production of synthetically improved therapeutic agents. Plant contains various phytoconstituents including phenols and flavonoid which can be responsible for antipyretic activity.

BIBLIOGRAPHY

1.World Health Organization: Quality control methods for medicinal plant materials. Published by WHO, Geneva, 1998.

2. Samy PR, Iushparaj PN, Gopalakrishnakone PA. Compilation of bioactive compounds from Ayurveda Bioinformation, 2008.

3. Subhose V, Narian A. Basic principles of pharmaceutical science in Ayurvĕda. Bull Indian Inst Hist Med Hyderbad, 2005, 35: 83.

4. Ballabh B and Chaurasia OP. Traditional medicinal plants of cold desert Ladakh--used in treatment of cold, cough and fever. J Ethnopharmacol, 2007, 112: 341.

5. Dev S Ethnotherapeutic and modern drug development: The potential of Ayurveda. Current Sci, 1997, 73: 909.

6. Perumal Samy R and Ignacimuthu S. Screening of 34 Indian medicinal plants for antibacterial properties. J Ethnopharmacol, 1998, 62: 173.

7. Perumal Samy R and Gnacimuthu SI. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. J Ethnopharmacol, 2000, 69: 63.

8. Kamboj V P. Herbal medicine – Some comments. Current Sci, 2000, 78: 35.

9. Rabe and Staden J V. Antibacterial activity of South African plants used for medicinal purposes. J Ethnopharmacol, 1997, 56: 81.

10. Nayar M P. The *ecological biogeography* of the lowland endemic tree flora. Bull Bot Surv Ind, 1987, 29: 319.

11. Cox PA, Ethnopharmacology and the search for new drugs Bioactive Compounds from PlantsCiba Foundation Symposium 154, Chichester, John Wiley & Sons, 1990, 40.

12. Cox P, Balick M. The ethnobotanical approach to drug discovery. Sci American, 1994, 82.

13. Tiwari S, Singh A. Toxic and sub-lethal effects of oleadrin on biochemical parameters of freshwater air breathing murrel, Chant punctatus (Bloch). Indian J Exp Biolo, 2004, 42: 413-18.

14. Tiwari S. Plants: A Rich Source of Herbal Medicine. Journal of Natural Products, Vol 1, 2008, 27-35.

15. Ved DK, Mudappa A, Shankar D. Regulating export of endangered medicinal plant species-need for scientific vigour. Curr Sci, 1998, 75: 341-4.

16. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. NISCIR, CSIR, Delhi 2002.

17. Singh J, Singh AK, Pravesh R. Product ion and trade potential of some important medicinal plants: an overview. In: Proceeding of first national interactive meet on medicinal and aromatic plants, CIMAP, Lucknow, India, 2003, 50-8.

18.Nadkarni AK. Indian Materia Medica.Popular Press Bldg. 2000.

19.Cardinali PD and Esquifino IA. Circardian disorganization in experimental arthritis.Neuro Signals. 2003;12:267-282.

20.Pervical M. Understanding the natural management of pain and inflammation, Clinical Nutrition insights. 1999:4:1-5

21. S. Kumar, BS. Bajwa, Singh Kuldeep and AN. Kalia 'Anti-Inflammatory Activity of Herbal Plants: A Review' IJAPBC – Vol. 2(2), Apr-Jun, 2013 ISSN: 2277 – 4688

22. Serhan CN, Yacoubian S, Yang R. Anti-inflammatory and proresolving lipid mediators. Annu Rev Pathol. 2008;3:279-312.

23. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. Nat Immunol. 2001 Jul;2(7):612-9.