Asian Journal of Pharmaceutical Education and Research
Vol -8, Issue-4, October-December 2019
ISSN:2278 7496

RESEARCH ARTICLE Impact Factor: 5.019

STUDY OF PHYTOCONSTITUENTS AND EVALUATION OF ANTIPYRETIC ACTIVITY OF HYDROALCOHOLIC LEAVES EXTRACT OF *CARICA PAPAYA* LINN AND *TINOSPORA CORDIFOLIA* ON BREWER'S YEAST INDUCED PYREXIA IN WISTAR RATS

Ram Pyare Saket, Dr. R. B. Goswami, Dr. Surendra Jain, Priyanka Rathore

Sagar Institute of Research and Technology-Pharmacy, Bhopal (M.P.)

*Corresponding Author's Email: ph.ram2013@gmail.com

Received 18 Sept. 2019; Revised 19 Sept. 2019; Accepted 22 Sept. 2019, Available online 15 Oct. 2019

ABSTRACT

The Plant were one of the principal sources of drugs since beginning the starting of human civilization. The aim of the study was to evaluate the antipyretic activity of hydroalcoholic leaf extract of *Carica papaya* Linn and *Tinospora cordifolia* using Brewer's yeast-induced pyrexia model in Wister strain albino rats. The hydroalcoholic leaf extract at a dose of 100mg/kg & 200 mg/kg was evaluated for antipyretic activity. The extract of *Carica papaya* Linn and *Tinospora cordifolia* plant showed a significant ($P < 0.05$) dose-dependent of the antipyretic effect in yeast induced elevation of rectal temperature in experimental rats in comparison with the standard paracetamol. 36 no. of albino rats were weighing 150-200g rats were used. They were divided into six groups of six rats each groups. Group one serve as a control (n=6) and was given normal saline, group two serves as a standard group (n=6) was given 100mg/kg of paracetamol, while groups three-six serves as test groups were treated with 100mg/kg and 200mg/kg (n=6) of plant extract respectively. The body temperature of the rats was measured rectally over a period of 4hours. According to these data, therefore, suggest that extract of *Carica papaya* Linn and *Tinospora cordifolia* possesses the significant antipyretic activity and it mechanism would be by inhibition of release inflammatory mediators and prostaglandins.

Keywords: *Carica Papaya* Linn, *Tinospora Cordifolia*, Phytochemicals screened, Antipyretic activity, Yeast induced pyrexia

INTRODUCTION

India is an ironic source of therapeutic florae and a number of plant-derived oils and extracts are used against various ailments related to human health by traditional healers by different systems of medicine such as Ayurveda, Unani, and Siddha. Only a few of them have been scientifically explored. Secondary metabolites derived from plants as natural products such as flavonoids, terpenes, phenols, and alkaloids[1,2] have increased significant consideration by the researcher in recent years due to their diverse multi pharmacological properties these plants still represent an enormous cradle of natural antioxidants that might serve as leads for the development of novel drugs. Numerous anti-inflammatory, neuroprotective, antipyretic, analgesic activities digestive, hepatoprotective, anticancer, antidiabetic and antinecrotic medicines have lately been exposed to have an antioxidant and/or radical scavenging mechanism as part

of their activity [3-5]. The hypothalamus regulates body temperature with a delicate balance between heat production and heat loss through the set-point control. Infection, tissue damage, inflammation, graft rejection, malignancy, and another disease may elevate the setpoint to induce fever [6]. Fever is a complex physiologic response triggered by abnormalities in the brain, toxic substances that affect temperature regulation, bacterial infections, brain tumors, and dehydration. Elevation of the body temperature occurs when the concentration of prostaglandin E_2 (PGE_2) increases within parts of the brain. The mechanism of antipyretic drugs is inhibiting the cycloxygenase (COX) activity and consequently reducing the levels of PGE_2. Synthetic antipyretic drugs have side effects [7]. Therefore, it is worthed to searching herbal medicines that are equally efficacious and comparatively side effects free, as substitutes for synthetic drugs. *Tinospora cordifolia* (*T. cordifolia*), a shady climbing shrub belongs to the family *Manispermaceae*, is found in the tropical areas of India, Pakistan, Sri Lanka, Burma, Africa, Australia and China [8]. In the Hindi language, the plant is called *giloya*, which means a divine thing that prevents aging. The phytochemical constituents of *T. cordifolia* include aliphatic compounds, alkaloids, steroids, glycosides, sesquiterpenoid, polysaccharides, different types of fatty acids and essential oils [8, 9]. Panchabhai et al. have provided a detailed summary of the pharmacological properties of *T. cordifolia* [8]. Experimental studies conducted on *T. cordifolia* have shown that *T. cordifolia* has significant therapeutic effects on diabetes and its associated complications, hepatotoxicity, different types of infections, gastrointestinal related complications and different types of cancers. Moreover**,** this plant extract has traditionally been used for the treatment of fever [8, 10]. *Carica papaya* Linn belonging to family Caricaceae is commonly known as papaya in English, Papita in Hindi and Erandakarkati in Sanskrit. Papaya is a powerhouse of nutrients and is available throughout the year. It is a rich source of threes powerful antioxidant vitamin (C A & E); the minerals(magnesium and potassium;) the B vitamin pantothenic acid and folate and fiber [11]. The plant is native to tropical America and was introduced to India in the 16th century. The papaya tree is basically a short-lived Indian tree. In historic times, it was considered as an exotic fruit because of its buttery taste and appearance [12]. The plant is recognized by its weak and usually unbranched soft stem and yielding copious white latex and crowded by a terminal cluster of large and long-stalked leaves, is rapidly growing and can grow up to 20m tall. Traditionally leaves have been used for the treatment of a wide range of ailments, like in the treatment of malaria, dengue, jaundice, immunomodulatory and antiviral activity [13]. Young leaves are rich in flavonoids (kaempferol and myricetin), alkaloids (carpaine,pseudocarpaine, dehydrocarpaine I an d II), phenolic compounds (ferulic acid, caffeic acid,chlorogenic acid), the cynogenetic compounds (benzylglucosinolate) found in leaves[14]. Since the antipyretic activity of *Carica papaya* Linn and *Tinospora cordifolia* leaves has been

experimentally not confirmed. So the present study has been carried out to evaluate and compare the in vivo antipyretic activity of the hydroalcoholic extract of *Carica papaya* Linn and *Tinospora cordifolia* leaves by yeast induced pyrexia method.

MATERIALS AND METHODS:

Plant material

Leaves of *Carica papaya* Linn and *Tinospora cordifolia* was collected from the local area of Bhopal in the month of April, 2019.

Chemical reagents

All the chemicals used in this study were obtained from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction of plant material

Dried powdered leaves of *Carica papaya* Linn and *Tinospora cordifolia* has been extracted with Hydroalcoholic solvent (Ethanol: Water: 80:20) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C [15]. % yield was calculated for each extract after drying under vacuum.

Preliminary screening for phytoconstituents

The freshly prepared hydroalcoholic extracts of leaves of *Carica papaya* Linn and *Tinospora cordifolia* were qualitatively tested for the presence of phytochemicals by using standard procedures [16, 17].

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined. Extracts obtained from leaves of *Carica papaya* Linn and *Tinospora cordifolia* plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso et al [18]. A volume of 2 ml of extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (75g/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. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/g).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso et al [18]. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/g).

Experimental 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 experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute toxicity studies

The acute toxicity was performed according to OECD guidelines no. 423 [19]. 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 *Carica papaya* Linn and *Tinospora cordifolia* leaves was given orally to rats at the graded doses like 100, 300, 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.

Yeast-induced hyperpyrexia in rats

Yeast induced pyrexia was used to evaluate the antipyretic activity of the extract. The rats were divided into six 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. 18 hour after yeast injection, the rats were again restrained in individual cages to record their rectal temperature. Immediately the hydroalcoholic extract of *Carica papaya* Linn and *Tinospora cordifolia* leaves were administered orally at doses of 100 and 200 mg/kg to the treatment control groups animals, the normal control group received distilled water and standard control groups animals received 100mg/kg of paracetamol. Pre-drug control temperatures of all the rats were recorded at 18h immediately before the extract or paracetamol administration and again at 1h interval up to 4h after yeast injection [20]. The followings are group distribution.

Group –1: Control (15% w/v suspension of Brewer's yeast)

Group –2: Paracetamol (100 mg/kg, bw, Standard)

Group –3: Hydroalcoholic extract of leaves of *Carica papaya* (100mg/kg, p.o.)

Group –4: Hydroalcoholic extract of leaves of *Carica papaya* (200mg/kg, p.o.)

Group –5: Hydroalcoholic extract of leaves of *Tinospora cordifolia* (100mg/kg, p.o.)

Group –6: Hydroalcoholic extract of leaves of *Tinospora cordifolia* (200mg/kg, p.o.)

Statistical Analysis

All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

RESULTS AND DISCUSSION:

The crude extracts so obtained after the maceration extraction process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using hydro alcohol as solvents are depicted in the Table 1. Preliminary phytochemical screening of leaves of *Carica papaya* Linn and *Tinospora cordifolia* extracts revealed the presence of various components such as carbohydrates, diterpenes, flavonoids and saponins and the results are summarized in Table 2.

Table 1 % Yield of hydroalcoholic extract of *Carica papaya* Linn and *Tinospora cordifolia*

S. No.	Plant	% Yield (w/w)
1.	*Carica papaya* Linn	7.88
2.	*Tinospora cordifolia*	9.45

Table 2 Result of phytochemical screening of *Carica papaya* Linn and *Tinospora cordifolia*

S. No.	Constituents	Hydroalcoholic extract of Leaves	
		***Carica papaya* Linn**	***Tinospora cordifolia***
1.	Alkaloids		
	i. Mayer's test	-ve	-ve
	ii. Dragendorff's test	-ve	-ve
	iii. Hager's test	-ve	-ve
	iv. Wagner's test	-ve	-ve
2.	Carbohydrates		
	i. Fehling's test	+ve	+ve
3.	Flavonoids		
	ii. Alkaline reagent test:	+ve	+ve
	ii. Lead acetate Test:	+ve	+ve
4.	Proteins		
	i. Xanthoproteic Test:	-ve	+ve
5.	Saponins		
	i. Foam test	+ve	-ve
6.	Diterpenes		
	i. Copper acetate Test:	+ve	+ve
7.	Amino acid		
	i. Ninhydrin Test:	-ve	+ve
8.	Glycosides		
	i. Legals test:	+ve	-ve
9.	Tannins		
	i. Gelatin test	-ve	-ve
10	Phenol		
	i. Ferric-chloride test:	-ve	+ve

Quantitative phytochemical assay was performed by calculating total phenolic content (TPC), and total flavonoid content (TFC). The TPC was calculated with respect to gallic acid (standard) and the TPC in hydroalcoholic extract of *Tinospora cordifolia* was found to be 0.971mg/g equivalent to gallic acid. Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: $Y = 0.06X+0.019$, $R^2= 0.999$, where X is the absorbance and Y is the quercetin equivalent (QE). Results were shown in Table 3 and Fig. 1, 2.

Table 3 Total phenolic and total flavonoid content of *Carica papaya* Linn and *Tinospora cordifolia*

S. No.	Hydroalcoholic Extract	Total Phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	*Carica papaya* Linn	-	0.878
2.	*Tinospora cordifolia*	0.971	0.765

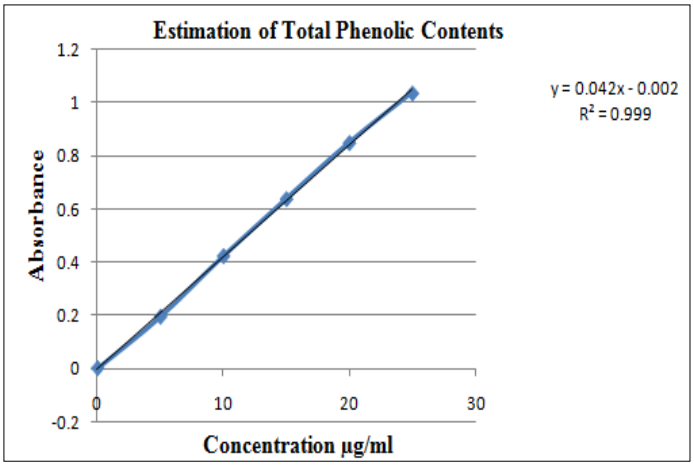

Fig.1 Graph of estimation of total phenolic content

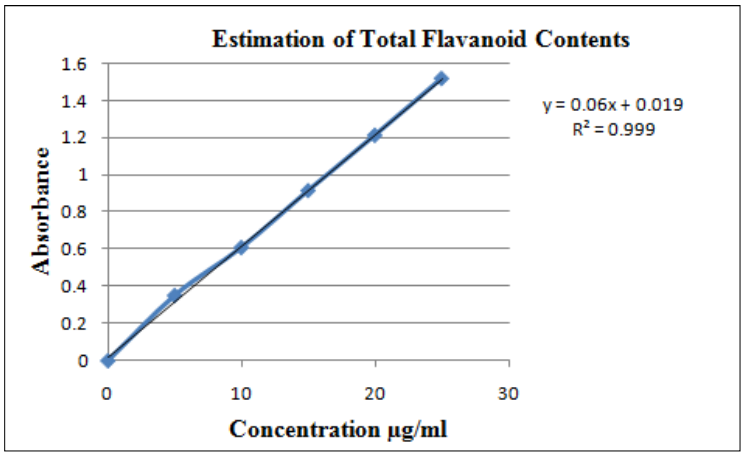

Fig. 2 Graph of estimation of total flavonoid content

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 *Carica papaya* Linn and *Tinospora cordifolia* leaves was safe to a single dose of 2000 mg/kg body weight. Hence, 100and 200 mg/kg of body weight, of the maximum safe dose were selected for studying *in vivo* antipyretic activity. 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 18h of administration. Treatment with the hydroalcoholic extract of *Carica papaya* Linn and *Tinospora cordifolia* leaves at the doses of 100 and 200 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 (100mg/kg, p o) and hydroalcoholic extract of *Carica papaya* Linn and *Tinospora cordifolia* leaves (100 and 200 mg/kg) treated rats were compared with that of control and a significant reduction (*P<0.05) against yeast induced pyrexia was observed Table 4 & Fig.3.

Table 4 Antipyretic effect of hydroalcoholic extract of leaves of *Carica papaya* and *Tinospora cordifolia* in yeast induced pyrexia in rats

Treatment	Dose (mg/kg)	Basal temp. °F	Rectal temperature (°F)				
			0 hour (after 18 hr)	1 hr	2 hr	3 hr	4 hr
Control	15% w/v suspension of Brewer's yeast (10 mL/kg)	99.0	100.5 ± 0.3	100.7 ± 0.1	100.7 ± 0.1	100.6± 0.1	100.6 ± 0.1
Paracetamol	100 mg/kg	98.50	100.5 ± 0.3	99.8 ± 0.1	100.5 ± 0.3	99.8 ± 0.1	100.5± 0.3
Hydroalcoholic extract of leaves of *Carica papaya*	100mg/kg	98.50	99.7 ± 0.2	99.5 ± 0.2	99.7 ± 0.2	99.5 ± 0.2	99.7 ± 0.2
Hydroalcoholic extract of leaves of *Carica papaya*	200mg/kg	98.40	99.6 ± 0.1	99.5± 0.2	99.0 ± 0.2	98.7 ± 0.2	98.3 ± 0.2
Hydroalcoholic extract of leaves of *Tinospora cordifolia*	100mg/kg	98.40	99.8 ± 0.1	99.6 ± 0.1	99.82± 0.1	99.6 ± 0.1	99.8 ± 0.1
Hydroalcoholic extract of leaves of *Tinospora cordifolia*	200mg/kg	98.50	99.8 ± 0.09	99.5 ± 0.11	99.8± 0.09	99.5 ± 0.11	99.8 ± 0.09

Values are expressed as mean ± SD. *P < 0.05-significant compared to Paracetamol treated group.

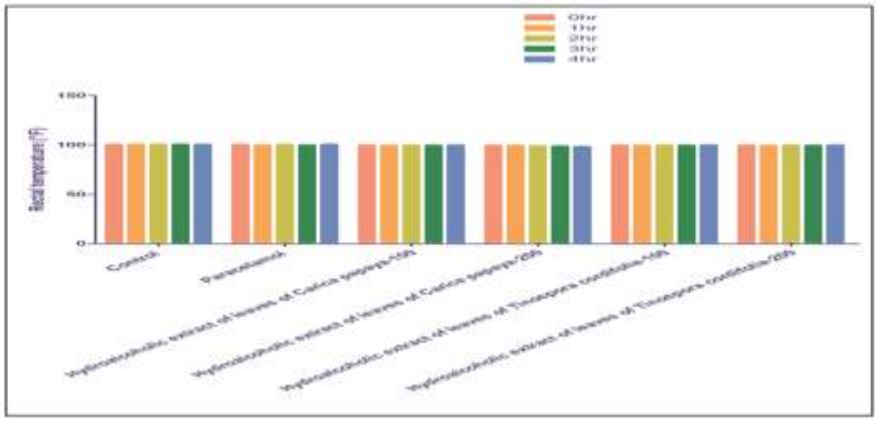

Fig.3 Antipyretic effect of hydroalcoholic extract of leaves of *Carica papaya* and *Tinospora cordifolia* in yeast induced pyrexia in rats

Conclusion

The present investigation it may be concluded that the hydroalcoholic extract of *Carica papaya* Linn and *Tinospora cordifolia* leaves have antipyretic activity. In this study no attempt was made to ascertain the mechanism of the observed antipyretic activity. However, it can be suggested that it may be acting through either the peripheral or central mechanism enumerated above. It is also possible that both the mechanisms may be involved. Further, study regarding isolation and characterization of active principle responsible for antipyretic activity are under planning in our laboratory.

References

1. Osawa T, Kawakishi S and Namiki, M., Antimutagenesis and Anticarcinogenesis Mechanism II. Plenum Press, New York. 1990; 139-153.
2. Keith MW, Sally AL, Michael WS, Thomas JG and Garry MM. Needles contain amounts of taxol comparable to the stem bark of Taxus brevifolia: analysis and isolation. J. Nat. Prod. 1990; 53: 1249-1255.
3. Perry EK, Pickering AT, Wang WW, Houghton PJ and Perru NS. Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy. Journal of Pharmacy and Pharmacology. 1999; 51: 527–534.
4. Lin C and Huang P. Antioxidant and hepatoprotective effects of Acathopanax senticosus. Phytotherapy Research. 2000; 14: 489- 494.
5. Repetto MG and Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Brazilian Journal of Medicine and Biological Research. 2002; 35: 523-534.
6. Goodman LS and Gilman AG. The Pharmacological Basis of Therapeutics, New York, The McGraw Hill Companies Inc.
7. Dipiro J, Talbert R, Yee G, Matzke G, Wells B and Posey L. Pharmacotherapy: a pathophysiologic approach, New York, The McGraw-Hill Companies Inc, 2008.
8. Panchabhai TS, Kulkarni UP and Rege NN. Validation of therapeutic claims of *Tinospora cordifolia*, a review.Phytother Res. 2008; 22: 425–441.
9. Patel MB and Mishra S. Hypoglycemic activity of alkaloidal fraction of *Tinospora cordifolia*. Phytomedicine. 2011; 18: 1045–1052.
10. Hussain L, Muhammad SH, Ain N and Rehman K, Muhammad I. The Analgesic, Anti-Inflammatory and Anti-Pyretic Activities of *Tinospora cordifolia*. Adv Clin Exp Med. 2015; 24 (6): 957–964
11. Milind and Gurditta. Basketful benefits of Papaya. International. Research. Journal of Pharmacy. 2011; 2(7): 6-12.

12. Bhowmik D, Aravind G, Duraivel S and Harish G. Traditional and Medicinal uses of *Carica papaya*. Journal Medical. PlantStudies. 2013; 1(1):7- 15.
13. Ayurvedic pharmacopeia of India, Govt of India.
14. . Arvind G, Debjit B, Duraivel S and Harish G. Traditional and Medicinal uses of *Carica papaya*, J Med Car Pap. 2013; 1(1) :2320-3862.
15. Mukherjee PK. Quality control of herbal drugs. 2nd Ed. Business Horizons; 2007.
16. Khandelwal KR. Ed. Practical Pharmacognosy Technique and Experiments, 23rd Edn: 2005; 15,29, 149,56.
17. Kokate CK. Ed. Practical Pharmacognosy, 4th Edn., Vallabh Prakashan. 1994; 112,120.
18. Olufunmis O, Olajuyigbe O and Anthony AJ. Phenolic Content and antioxidant property of the bark extract of *Ziziphus mucronata* wild. Subsp. Mucronata wild, BMC, Complementary and alternative medicine. 2011; 11: 130.
19. Guideline Document on Acute oral Toxicity Testing, Series on Testing and Assessment No. 423. Paris: Organization for Economic Co-Operation and Development, OECD Environment, Health and Safety Publications; 1996. Available from: http//www.oecd.org/ehs.