Research Article

Study of anti ulcer activity of *Ficus religiosa* leaf extract on experimentally induced gastric ulcers in rats

B. Divya, V. Palanivel and KL. Senthil Kumar

Padmavathi College of Pharmacy and Research Institute, Periyanahalli, Dharmapuri, Tamilnadu, India.

ABSTRACT

To investigate the gastroprotective activity of ethanolic leaf extract of *Ficus religiosa* (*F. religiosa*) in different experimental models of gastric ulcer in rats.The ethanolic leaf extract of *F. religiosa* were studied at two dose levels (250 and 500 mg/kg, oral) in rats against absolute cold stress induced ulcer and pyloric ligation induced gastric ulcer. Ranitidine (50 mg/kg, oral) was used as a standard drug. Mean ulcer indices and oxidative stress were measured. Phytochemical tests and acute toxicity tests were also carried out. Administration of *F. religiosa* to rats significantly decreased the ulcer index value when compared with the control treated group. Ranitidine (50 mg/kg, oral) also produced a significant decrease the ulcer index value when compared with the control treated group. Phytochemical analysis revealed the presence of tannins, sterols, saponins, flavonoids, carbohydrates and proteins.The results suggest that the leaves of the *F. religiosa* possess sinificant anti ulcer activity.

Keywords: Antiulcer, *Ficus religiosa*, stress induced, Pyloric ligation.

INTRODUCTION

Over the last decade there has been a growing interest in drugs of plant origin and such drugs formed an important class for disease control. The traditional heritage of India includes many true tested medicinal plants/drugs for various diseases and to which there is no answer in modern medicine till today. Indian traditional medicine is based on various system including Ayurveda, Siddha, Unani and Homeopathy. Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. Herbalism is also known as botanical medicine, medicinal botany. Peptic ulcer disease is ulceration of mucous membrane penetrating through the muscularis mucosa exposed to acid and pepsin in stomach and duodenum. If ulceration occurs in Stomach, it is known as Gastric ulcer and when it is in duodenum, it is known as duodenal Ulcer. Peptic ulcer disease is a common medical emergency with an annual incidence of approximately 100 per 100,000 adults and overall mortality of 10 to 15% in recent studies. *F. religiosa* is a large, deciduous tree. This big and old tree is of 30m long. The leaves are shiny, thin, and bear 5-7 veins. Fruits are small, about ½ inch in diameter, similar to that of eye pupil. It is circular in shape and compressed. When it is raw, it is of green color and turns black when it is ripe. The tree fruits in summer and the fruits get ripened by rainy season The leaves show more or less sigmoid growth pattern, each leaf increases in size in 9 days from about 425 to 4025mm^2 (as judged by the average mature leaf size) after its emergence from the spathe. The leaf is hypostomatic and has paracytic and anomocytic stomata between polygonal epidermal cells. The frequency of stomata per square millimeter increases from 33.3 to 400 per mm ^{2} with the growth of the leaves, while the number of upper epidermal cells decreases from 5600 to 1110. The vasculature comprises a single main vein (the midrib), secondaries, tertiaries, quaternaries, and intermediaries. The number of areoles per square millimeter decreases from 15.5 to 2.7, while the number of vein endings and vein tips per areole show no correlation either with one another or with leaf size.

It has a heart shaped leaves. It shed its leaves in the month of March and April. Leaves are bright green, the apex produced into a linear lanceolate tail about half as long as the main portion of the blade.

Phytochemistry

Leaves

campestrol, stigma sterol, isofucosterol, α-amyrin, lupeol, tannic acid, arginine, serine, aspartic acid, glycine, threonine, alanine, proline, tryptophan, tyrosine, methionine, valine, isoleucine, leucine, n-nonacosane, n-

hentricontanen, hexa-cosanol and n-octacosan.

MATERIALS AND METHODS

Collection of plant material

The leaf of *Ficus religiosa* (moraceae) was collected from Padmavathi college botanical garden. The plant was identified and authenticated by Prof.P.Jayaraman, Ph.D, Director, National Institute of Herbal Sciences, Sakthinagar, and Chennai. The voucher specimen number is PARC/2011/1025.

Preparation of the extract

A weighed quantity (250g) of the crushed leaves extracted with ethanol (90%) in a Soxhlet extractor. The Ethanolic extract was concentrated to dryness under reduced pressure and controlled temperature (48°C–50°C) with a rotavapour. The extract was dried in order to produce a dark green solid extract. The dark green extract was then subjected to various qualitative phytochemical studies.

Experimental animals

Adult male and female rats of wistar albino strain weighing between 180-200g were obtained from the Animal House, Padmavathi College of pharmacy. They were kept in polypropylene cages and allowed to get acclimatized to a standard laboratory diet. The animals were adapted to laboratory condition for prior to the experiments and constant room temperature at 22°C–24°C with 12 hour day and night cycle. Feed and drinking water were provided ad libitum. The studies were performed with the approval of Institutional Animal ethical committee (IAEC) following the guide lines of CPCSEA.

Acute oral toxicity study and selection of doses

A safe oral dose of ELFR was determined through the acute oral toxic test in rats as described by the Organization of Economic Co-Operation and Development (OECD) as per 423 guidelines (OECD Guidelines for the Testing of Chemicals). The ELFR, at different doses up to 2000 mg/kg, was prepared by dissolving the extract in distilled water and the concentration was adjusted in such a way that it did not exceed 1 ml/100 g of the rat. The extract was then administered (p.o.) and animals were observed for behavioural changes, any toxicity and mortality up to 48 h. Two different doses (250 and 500 mg/kg, p .o) of ELFR were later chosen for this study based on the acute toxicity testing.

Anti-ulcer assays

Pylorus ligation induced ulcer model:

Albino rats were fasted in individual cages with measures taken to avoid corpophagy for 24hrs prior to the experiment with free access to water. The animals were divided into 4 groups, each consisting of six rats. After 30 min of oral administration of the vehicle/standard/extracts rats are anaesthetized with anesthetic ether. Secure the rat on operating table. Give an incision of 1 cm. long in the abdomen just below the sternum. Expose the stomach pass a thread around the pyloric sphincter and apply a tight knot. Ligation was done without causing any damage to the blood supply of the stomach. Close the abdomen wall by putting the sutures. Clean the skin from any blood spot and bleeding. Apply collodion over the wound. Keep the rat in a separate cage and allow it to recover. Animals were allowed to recover and stabilize in individual cages and were deprived of water during post-operative period. After 4 hrs. Of surgery, rats were sacrificed, abdomen is opened and stomach was removed, and the stomach contents are drained in centrifuge tube for biochemical estimation. Along the greater curvature the stomach is opened for ulcer scoring.

Table 1: Animal protocols for Pylorus ligation

S. No.	Group	Dose	Animals
1.	Control (Vehicle)	1ml/100gm	6
2.	Standard (Ranitidine)	50mg/kg	6
3.	Ethanolic extract	250mg/kg	6
4.	Ethanolic extract	500mg/kg	6

Cold Stress induced ulcers (cold water immersion method).

Albino rats between (180-200 gm.) and each group containing 6 animals were divided into 4 groups. After 30 min of oral administration of the vehicle/standard/extracts rats are placed in cold water vertically for 4hrs in individual restraint cages maintained at 22^{o}c Then, they were taken out ,dried and injected with 30 mg/kg Evans blue i. v via the tail vein.10 min later , sacrificed with ether and stomachs are removed . Formal –saline (2%v/v) is then injected into the totally ligated stomachs for overnight storage. The next day, the stomachs opened along the greater curvature, were washed in warm water, and examined. Microscopically for ulcers with the help of hand lens (10x) Mean ulcer score for each animal is expressed as ulcer index. Gastric juice collected into centrifuge tubes and centrifuged at 1000 rpm for 10min and the volume was noted .the pH of the gastric juice was recorded

by pH meter and the gastric content is subjected for analysis of free and total acidity.

Table 2: Animal protocols for cold stress induce ulcer

S. No.	Group	Dose	Animals
1.	Control (Vehicle)	1ml/100gm	6
2.	Standard (Ranitidine)	50mg/kg	6
3.	Ethanolic extract	250mg/kg	6
4.	Ethanolic extract	500mg/kg	6

Ulcer index will be then calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach.

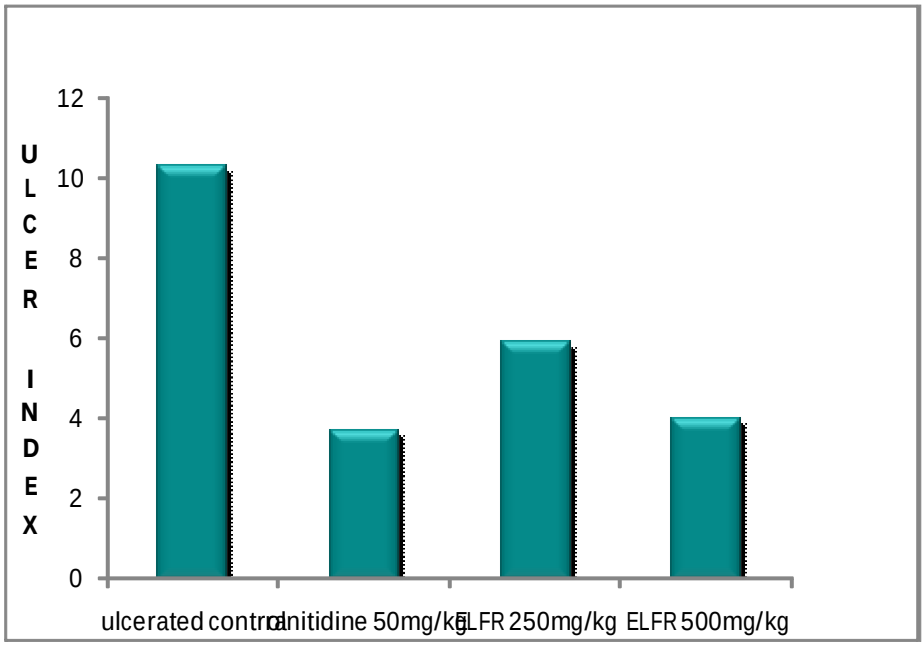
PHARMACOLOGICAL SCREENING
Pylorus ligation induced ulcer

Table 3: Effect of *F. religiosa* leaf extract on ulcer index in pylorus ligation induced ulcer

Group	Treatment	Dose (mg/kg)	Ulcer index (mm^2/rat)	% Protection
I.	Control	Pylorus ligation	10.29±0.02	-
II.	Ranitidine	50	3.7±0.05***	64.04
III.	Ethanolic extract	250	5.9±0.07*	42.66
IV.	Ethanolic extract	500	4.03±0.07**	60.08

Values are mean ± SEM (n=6) one way ANOVA followed by Student- Newman-keuls test.
*represents significant at p<0.05 , **represents more significant at p<0.01,
*** represents most significant at p<0.001.when compared to control group.

RESULTS



Graph 1: Effect of ELFR on ulcer index in pylorus ligation Induced ulcer model

Table 4: Effect of *F. religiosa leaf* extract on volume of gastric juice, Total acidity and Free acidity in pylorus ligation induced ulcer.

Group	Treatment	Dose (mg/kg)	Volume of Gastric Juice (ml)	Free acidity (m/eq/1)100g	Total acidity (m/eq/1)100g
I.	Control	Pylorus ligation	3.3±0.17	31.9±0.50	90.4±0.54
II.	Ranitidine	50	1.5±0.14**	6±0.177***	9.7±0.15***
III.	Ethanolic extract	250	1.9±0.17***	10.4±0.09***	21.7±0.24***
IV.	Ethanolic extract	500	1.5±0.28***	2.1±0.18***	15.1±0.27***

Values are mean ± SEM (n=6) one way ANOVA followed by Student- Newman-keuls test
** represents more significant at p<0.001. When compared to control group.
*** represents most significant at p<0.001. When compared to control group.

6.3.2 Cold Stress induced ulcer

Table 5: Effect of ELFR on volume and pH of gastric content in stress induced rats

S. No.	Treatment(mg/kg)	Volume of gastric juice (ml)	pH of gastric juice
1.	Ulcerated control	5.28±0.12	1.97±0.67
2.	Ranitidine(50mg/kg)	3.92±0.71	2.48±0.05
3.	Ethanolic Extract(250mg/kg)	4.04±0.07*	3.94±0.08*
4.	Ethanolic Extract(500mg/kg)	4.32±0.06**	3.24±0.10*

Values are expressed as Mean ± SEM. *p<0.05, **p<0.01,
as compared with ulcerated control using one way ANNOVA followed by Dunnet test.

Table 6: Effect of ELFR on free acidity and total acidity in Stress induced rats

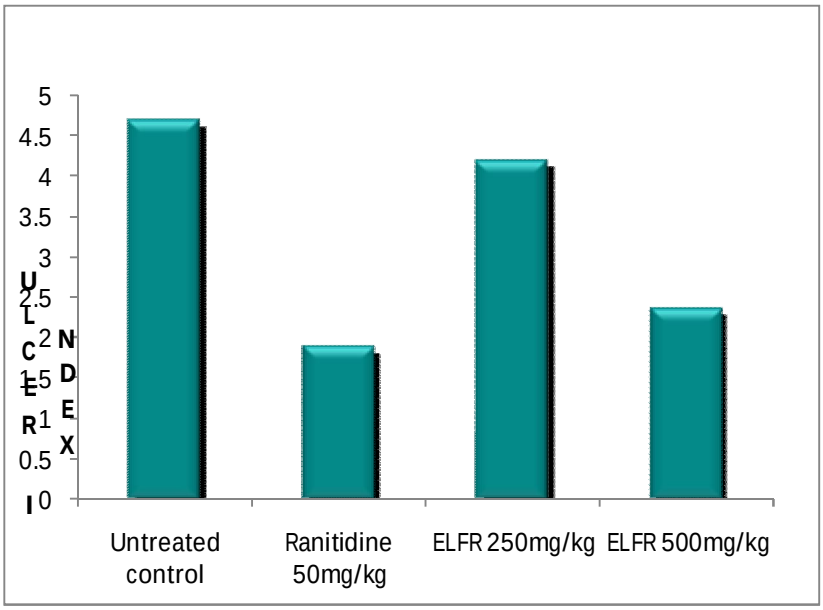
S. No.	Treatment (mg/kg)	Free acidity (meq/L/100gm)	Total acidity (meq/L/100gm)
1	Ulcerated control	37.33±1.80	74.13±1.70
2	Ranitidine(50mg/kg)	14.40±0.36	27.46±0.76
3	Ethanolic Extract(250mg/kg)	24.66±0.66	68.06±0.72
4	Ethanolic Extract(500mg/kg)	22.60±0.93**	39.83±1.31**

Values are expressed as Mean ± SEM. *p<0.05, **p<0.01, as
compared with ulcerated control using one way ANNOVA followed by Dunnet test.

Table 10: Effect of ELFR on ulcer index and % gastro protection in stress induced rats

S. No	Treatment (mg/kg)	Ulcer index (Mean± SEM)	% Gastro protection
1	Ulcerated control	4.7±0.29	-
2	Ranitidine(50mg/kg)	1.88±0.11	60
3	Ethanolic Extract(250mg/kg)	4.21±0.11	29.70
4	Ethanolic Extract(500mg/kg)	2.35±0.15**	62**

Values are expressed as Mean ± SEM. *p<0.05, **p<0.01, as compared
with ulcerated control using one way ANNOVA followed by Dunnet test.



Graph 2: Effect of Ethanolic leaf extract of *F. religiosa* on ulcer index in Stress induced ulcer

DISCUSSION

Peptic ulcer and gastritis have been associated with multipathogenic factors and could be due to disturbances in natural balances between the aggressive factors (e.g. of acid, bicarbonate, pepsin) and maintenance of the mucosal integrity through the endogenous defence mechanism. *F. religiosa* has been reported to exert several pharmacological properties such as anthelmintic, antibacterial, anti-diabetic and antioxidant, wound healing, anti-inflammatory, analgesic and antilipid peroxidation, anticonvulsant, and anti-amnesic activities. Despite claim of its potential in the treatment of gastric ulcer, this plant has so far not been screened for anti-ulcer activity. Thus, we take this opportunity to report the preliminary findings on anti-ulcer potential of *F. religiosa* leaf ethanolic extract for the first time here. The present study demonstrated the potential of ELFR to significantly reduced gastric ulceration as indicated by the reduction in ulcer index in the Pylorus induced and Cold stress induced assays. Based on further findings using the PL assay, the extract was suggested to act by reducing the volume of gastric juice secreted, gastric free and total acidities. These results suggested that ELFR possesses anti-secretory potency as well as acid neutralizing effect. It is also possible to suggest that the observed antiulcer activity associated with *F. religiosa* is the ability to exhibit antioxidant activity as cited above. Oxidative stress, resulting from the increase production of oxygen derived free radicals (e.g. superoxide anion, hydrogen peroxide and hydroxyl radicals), has been known to take part in the pathogenesis of various diseases including gastric ulcer and antioxidants help to protect cells from damage elicited by oxidative stress while enhancing the body's defense systems against degenerative diseases.

F. religiosa leaf extract had been reported to possess antioxidant activity and to contain various types of compounds such as flavonoids, saponins, carbohydrates, proteins, glycosides and tannins. The anti-ulcer activity is probably due to the presence of bioactive compounds like flavonoids, and tannins. Tannins have astringent action, precipitating proteins of mucosal membranes and skin. Some tannins suppress the gastric secretion and having a local action of protecting the gastric mucosa. Statistical analysis revealed that Ethanolic extract of leaves of *F.religiosa* contains antiulcer activity due to the presence of flavonoids and sterol viz. Stigmasterol.

Effect of ELFR in pylorus ligated rats

Pylorus ligation in ulcerated control group had produced ulcer in all animals and the mean ulcer index was 10.29±0.02 indicating the ulcerogenic effect. Mean gastric content volume as 3.3±0.17, free acidity as 31.9±0.50, total acidity as 90.4 ± 0.54, indicating the ulcer production in animals. However, the ulcer index showed significant dose dependent reduction in the animal pre-treated with ELFR 250 mg/kg (UI;5.9 ± 0.07) and 500 mg/kg (UI; 4.03 ± 0.07).It indicated 42.66% gastro protection at 250 mg/kg and 60% gastro protection at 500 mg/kg as compared with ulcerated control. The results indicate that the higher dose of ELFR i.e. 500 mg/kg was effective in protecting ulcers in pylorus ligated rats. Pylorus ligation had produce ulcers in all animals pre-treated with Ranitidine 50 mg/kg. However, ulcer index (3.7 ± 0.05) showed significant reduction as compared with ulcerated control and showed 64 % gastro protection.

Effect of ELFR in Cold stress induced ulcer

Water Immersion stress is the one of the best model of stress in rats to induce ulcer. The model provides both emotional stress as well as physiological stress to the animal. In case of water immersion induced stress in rats, the ethanolic extract showed significant ulcer inhibition.

Stress induced ulcerated control group had produced ulcer in all animals and the mean ulcer index was 4.73 ± 0.29 indicating the ulcerogenic effect. However, the ulcer index showed significant dose dependent reduction in the animal pre-treated with ethanolic extract 250 mg/kg (UI; 4.21 ± 0.11) and 500 mg/kg (UI; 2.35 ± 0.15). It indicated 29.70% gastro protection at 250 mg/kg and 62% gastro protection at 500 mg/kg as compared with ulcerated control. The results indicate that the higher dose of ELFR i.e. 500 mg/kg was effective in protecting ulcers in cold stress induced model. Cold stress had produced ulcers in all animals pre-treated with Ranitidine 50 mg/kg. However; ulcer index (1.88 ± 0.11) showed significant reduction as compared with ulcerated control and showed 60 % gastro protection.

In conclusion, the present study provided preliminary data for the first time that the leafof *F. religiosa* possesses significant anti-ulcer activity in animal models.It has a gastric antisecretory and acid neutralizing effect that are comparable to reference drug ranitidine. The anti-ulcer activity is probably due to the presence of bioactive compounds like flavanoids, saponin and tannins. Further

studies are required to confirm the exact mechanism underlining the ulcer healing and protecting property of the extract and to identify the chemical constituents responsible for it.

REFERENCES

1. Elisabetsky E, Costa-Campos L. Medicinal plant genetic resources and international co-operation: the Brazilian perspective. J Ethanopharmacol.1996;51:111-19.
2. Joy PP, Thomas J, Mathew S and Skaria BP. MedicinalPlants. Tropical Horticulture Vol. 2. (eds. Bose, T.K., Kabir,J., Das, P. and Joy, P.P.). NayaProkash, Calcutta, 2001;449-632.
3. Arulmozhi S and Sathiya NL. Phcog Rev. 2007;1:163-170
4. Naira N, Rohini RM, Syed MB and Amit KD. Wound healingactivity of the hydro alcoholic extract of Ficusreligiosa leaves in rats. Internet J Altern Med. 2009;6:2-7.
5. Damanpreet S and Rajesh KG. Anticonvulsant effect of Ficusreligiosa: Role of serotonergic pathways. J Ethnopharmacol. 2009;123:330-4.
6. Panit R, Phadke A and Jagtap A. Antidiabetic effect of Ficusreligiosa extract in streptozotocin-induced diabetic rats. J Ethnopharmacol. 2010;128:462-6.
7. Agarwal V and Chauhan BM. A study on composition andhypolipidemic effect of dietary fibre from some plant foods. Plant Foods Hum Nutr. 1988;38:189-97.
8. Mallurvar VR and Pathak AK. Studies on immunomodulatoryactivity of Ficusreligiosa. Indian J Pharm Educ Res. 2008;42(4):343-347.
9. Kalpana G and Rishi RB. Ethnomedicinal Knowledge andhealthcare practices among the Tharus of Nwalparasidistrict in central Nepal. For Ecol Manage. 2009;257:2066-72.
10. Chopra RN and Chopra S. Indigenous Drugs of India.2 nd ed.Calcutta: Dhur and Sons; 1958;606.
11. Deepika Paliwal1, Krishna Murti, Yashpal Sangwan, Manish Kaushik and Divya Kiran. Preliminary and Pharmacological profile of ficusreligios a overview Pharmacologyonline. 2011;3:387-395.
12. Warrier PK, Indian medicinal plants-A compendium of 500 species, Orient Longman Ltd.Chennai, 1996;3:38-39.
13. Kapoor LD. Handbook of Ayurvedic Medicinal Plants, CRC Press, Boca Raton, 1990;149-150.
14. Khanom F, Kayahara H and Tadasa K. Superoxide-scavenging and prolylendopeptidase inhibitory activities of Bangladeshi indigenous medicinal plants. Biosci.Biotechnol. Biochem. 2000;64:837-840.
15. Babu K, Shankar SG and Rai S. Comparative pharmacognostic studies on the barks of four Ficusspecies. Turk J Bot. 2010;34:215-224.
16. . Swami KD and Bisht NPS. Constituents of Ficusreligiosa and Ficusinfectoria and their biological activity. J Indian Chem Soc. 1996;73:631.
17. Swami KD, Malik GS and Bisht NPS. Chemical investigation of stem bark of Ficusreligiosa andProsopisspicigera. J Indian Chem Soc. 1989;66:288–289
18. Joseph B and Justin SR. Phytopharmacological and Phytochemical Properties of Three Ficus Species - An Overview, International Journal of Pharma and Bio Sciences, 2010.
19. Margareth BCG and Miranda JS. Biological Activity of Lupeol. International journal of biomedical and pharmaceuticalsciences.2009:46-66
20. Husain A, Virmani OP, Popli SP, Misra LN, Gupta, MM, Srivastava, GN, Abraham Z and Singh AK.Dictionary of Indian Medicinal Plants, CIMAP, Lucknow, India, 1992:546.
21. Panda SK, Panda NC and Sahue BK. Effect of tree leaf tannin on dry matter intake by goats. Indian Vet J. 1976;60:660-664.Pharmacologyonline 2011;3:387-395 newsletter Paliwalet al.394.
22. Prasad S, Kalra N and Shukla Y. Hepatoprotective Effects of Lupeol and Mango Pulp Extract of Carcinogen Induced Alteration In Swiss Albino Mice, Molecular Nutrition & Food Research. 2007;51(3):352-9.
23. Suryawanshi K, Khakre S, Chourasia A, Chaurasiya PK, Pawar RS and Jhade D. Hepato-protectiveactivity of stem bark extract of FicusReligiosa Linn in Rat. International Journal of Biomedical Research. 2011;8:466-475

24. Grison L, Hossaert M, Greeff JM and Bessiere JM. Fig volatile compounds a first comparative study. Phytochemistry. 2002;61:61-71.
25. The Origin of the soxhletextractor,Willium B. Jenson , Journal of Chemical Education 1913, 2007;84(12).
26. Indian Pharmacopoeia 1996,Government of India. 947-949,A53-54,A70-71,A73,A76,A89,A105
27. Furis BS. Hannaford AJ Vogel's Text book of Practical organic chemistry 1978,Longman group publication p.400-402.