DESIGN, SYNTHESIS, CHARACTERIZATION OF COUMARIN DERIVATIEVES AND ITS PHARMACOLOGICAL EVALUATION

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ABSTRACT

Novel Coumarin derivatives (CM-1 to CM-6) were synthesized, characterized by the spectral analysis and screened for Anti-inflammatory activity by Egg Albumin protein denaturation method and Anti-ulcer activity by acid neutralizing capacity method. The synthetic route involves the mixture of 3-Amino phenol(22.0g) and 3-Oxo butanoate (25.0ml) were dissolved in (18ml) of zinc chloride with conc sulphuric acid to give 7-amino-4-methyl coumarin (I).

Different substituted aromatic aldehydes were added to the 7-amino-4-methyl coumarin (I) and dissolved in ethanol. The reaction mixture was taken stirred for 30 min, kept for reflux for 2-3 hrs ,filter and recrystallized with ethanol to give 6 novel Coumarin derivatives (CM-1 to CM-6). The compounds show the mild to moderate anti- inflammatory activity when compared with standard diclofenac sodium and also show the mild moderate anti-ulcer activity when compared to aluminium hydroxide & magnesium hydroxide, and.all the derivatives (CM-1 to CM-6) were screened for docking targeted for anti – tuberculosis activity. Docking results of compounds of 3a shows more binding energy against 3f targeting phospholipaseA_2 (PDB ID- IQG6) for potential Anti-tuburculosis.

Key Words: Coumarin derivatives, aromatic aldehydes Anti-Ulcer, Anti-inflammatory, Docking

INTRODUCTION

Coumarin is a simple molecule and many of its derivatives have been known for more than a century. Coumarin and coumarin-related compounds have been proved for many years to have significant therapeutic potential[1-3]. Normally Coumarin is synthesised by many of the named reactions with perkins reaction between salicylaldehyde and acetic anhydride as popular example. But in the present work 3-amino phenol and 3-oxo butanoate is used in presence of conc.H_2SO_4 as solvent and Zinc chloride used as base to obtain coumarin. Their physiological, anti-microbial, anti-cancer and anti-inflammatory activities make these compounds attractive for further backbone derivatisation and screening as novel therapeutic agents[19-22].

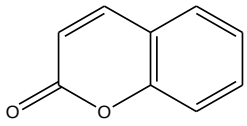


Fig :1 Coumarin

Materials and methods:

Compounds are weighed under weighing balance in precise amount. By using melting point apparatus were determining the melting point for newly synthesized compounds by using only one capillary tube. The reaction was monitored by using pre-coated with silica gel TLC plates. After completion of the reaction recrystallized with a suitable solvent and the purity of the new compound were checked by using TLC plates are run in a solvent system like Ethanol Water and Ethyl acetate (3:1:3) the completion of reaction and purity of the product were evaluated. The spots are visualised under UV chamber.

Finally newly synthesised compounds are undergo for spectral characterization by IR, MASS AND NMR.

STEP- 1 **Synthesis of 7-amino-4-methyl Coumarin :**

Weigh the (22.0g,202mmol) of 3- amino phenol, and (18.0ml,234mmol) of Zinc chloride, followed by the addition of ethyl 3-oxo butanoate (25.0ml) and slowly add concentrated sulfuric acid with continuous stirring . the product was collected by filtration, washed twice with ethanol and then dried invaccum for characterization with very carefull operation to avoid the destruction. The resulted will be 7- Amino-4-methyl coumarin.

STEP-2 Preparation of (Z)-7-(benzylidene amino)-4- methyl-2H-chrome-2-one:

The above synthesized product was weighed about 0.1 M of 7-Amino- 4-methyl coumarin was taken into a round bottom flask. Then add 0.1M of substituted different aromatic aldehydes was dissolved in 35-40 ml of ethanol. Then the mixture was transferred into a round bottom flask. The reaction mixture was stirred for 1-2 hours, and refluxed for 3 hours. Finally obtained product was collected and cooled at room temperature and poured into ice water. After cooling the product was filtered and dried at room temperature. Recrystallized with ethanol.

BIOLOGICAL ACTIVITIES:

***IN VITRO* ANTI-ULCER ACTIVITY:**

• ***The ability of the invitro approach to neutralized acids***:

The ability of the invitro approach to neutralise acids was determined for various concentrations (100 mg/ml, 200 mg/ml, 500 mg/ml, 1000 mg/ml) of coumarin derivatives .and it was compared to standard antacids, Aluminium hydroxide, and magnesium hydroxide - 500 mg/mL (AHMH). A precise amount (5 mL) of the drug solution was measured and Pour into a 250ml beaker, makeup to 70ml with non-carbonated distilled water, and stirred for 1 minute. A precise volume of 30 mL of 1.0 N HCl was pipetted into the above solution while stirring for 15 minutes. Excess HCl was titrated with 0.5N NaOH to reach a threshold pH of 3.5. Experiments were performed for gram of antacid was calculated. the ability of all concentrations of each batch at a temperature

of 37 °C ± 3 °C using a magnetic stirrer. The number of milliequivalents (mEq)of acid consumed per gram of antacid was calculated. the ability of the invitro approach to neutralize acids was calculated using[24-28]

Equation 1

Equation -1:THE MOLE OF ACID NEUTRALIZE IS**:-**

Moles of acid neutralized = (Vol. of HCl × Normality of HCl) - (Vol. of NaOH × Normality of NaOH)

Equation-2:- ACID NEUTRALIZING CAPACITY:-

Neutralise acids per gram of antacid = Moles of HCl Neutralized /Grams of antacid / extract …… …….2

IN-VITRO ANTI-INFLAMMATORY ACTIVITY :

Preparation of reagents :

Phosphate Buffer Saline P^H 6.3:

Dissolved 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 1.44 g of disodium hydrogen phosphate (Na_2HPO_4), 0.24 g of potassium dihydrogen phosphate (KH_2PO_4) in 800 ml distilled water. The P^H was adjusted to 6.3 by using 1N HCl and made up the volume to 100 ml with distilled water.[4-9]

Materials:

Egg Albumin, coumarin derivatives (Test), Diclofenac sodium (Standard drug), Phosphate buffer solution (P^H 6.3), Distilled water.

Procedure:

Protein Denaturation by egg albumin method

The reaction mixture (5mL) consisted of 0.2mL of egg albumin (from fresh hen's egg), 2.8mL of phosphate-buffered saline (PBS, pH 6.4), and

2mL of varying concentrations (10, 50, 100, 250, 500µg/mL) of test compounds. A similar volume of double-distilled water served as the control. The mixtures were incubated at 37°C in a BOD incubator for 15 minutes and then heated at 70°C for five minutes. After cooling, their absorbance was measured at 660 nm by using the vehicle as a blank. Diclofenac sodium in concentrations of 10, 50, 100, 250, and 500µg/mL was used as the reference drug and treated similarly for the determination of absorbance. The % percentage inhibition of protein denaturation by egg albumin is given in the table

Percentage inhibition of protein denaturation was calculated by using the following :

$$\% \text{ Inhibition} = 100 \times [V_t / V_C - 1]$$

Where,

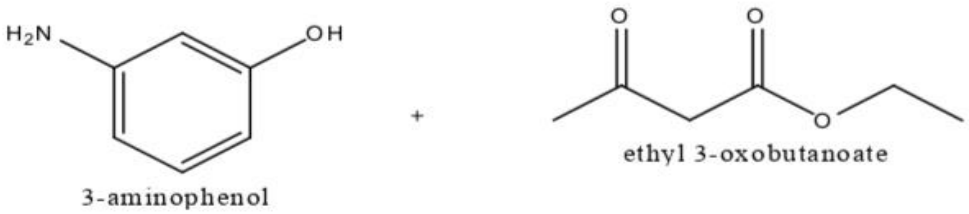
V_t = absorbance of the test sample,

V_c = absorbance of control

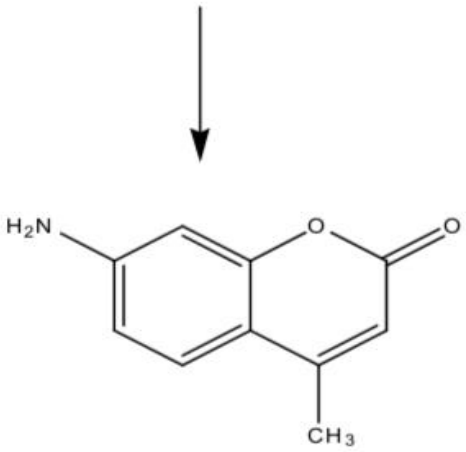
Synthetic scheme:

SCHEME

Step:1

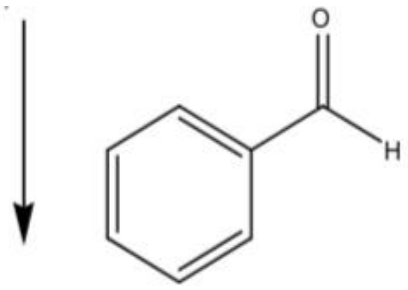


3-aminophenol + ethyl 3-oxobutanoate

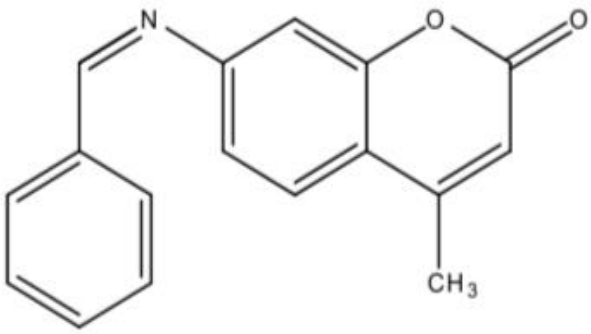


7-amino-4-methyl-2*H*-chromen-2-one

STEP-2



benzaldehyde



(*Z*)-4-methyl-7-(1-phenylethylideneamino)-2*H*-chromen-2-one

Results and Discussion:

Characterization:

Compound (CM-1):

IR (KBr in cm^{-1}): C- C stretch 1610**,** N-H stretch 3346**,** O-H bending 834, Aromatic C=C1511

NMR Chemical Shift: δ=7.961 (S, Secondary amide R2-NH),δ=7–7.6 (S, 10 Ar H),

δ=4.172 (S, 1C, Ar NH2)

Mass: Base peak 241, Molecular ion peak 367.05

Compound (CM-2)

IR (KBr in cm^{-1}): C-N Stretch 1209, C=O stretch 1629, O-H stretch 3371, C-H bending 831

NMR Chemical Shift: δ=7.961 (S, Secondary amide R2-NH), δ=7–7.6 (S, 10 Ar H), δ=4.172 (S, 1H, Ar NH2)

Mass: Base Peak 184.0500, Molecular ion peak 402.5500

Compound (CM-3)

IR (KBr in cm^{-1}): O-H stretch 3372, C=C stretch 1632, C-O stretch 1265, Aromatic C=C 1514

NMR Chemical Shift: δ=3.112 (S, 1H, NH2), δ=7.2 –7.9 (S, 10 Ar H)

Mass: Base peak 241, Molecular ion peak 383.05

Compound (CM-4)

IR (KBr in cm^{-1}): C-N Stretch 1231, O-H stretch 3352, C-C stretch 1601, N-O stretch 1454

NMR Chemical Shift: δ=3.167 (S, 1C, =CH),δ=7.213–9.437 (S, (S, 10 Ar H)

Mass: Base Peak 122, Molecular ion peak 410.05

Compound (CM-5)

IR (KBr in cm^{-1}): C-H stretch 2920, C-C stretch 1594,C-H rocking 1359,C-N stretch 1165

Compound (CM-6)

IR (KBr in cm^{-1}):C=C stretch 1517,N-H stretch 3367,C-C stretch 1623,N O stretch 1349

IN VITRO ANTI- INFLAMMATORY ACTIVITY

The anti-inflammatory activities of the synthesized derivatives were evaluated by egg albumin protein denaturation method against the standard, diclofenac. The results of this method were represented in the Table1& 2.

S.no	Concentration (µg/ml)	Absorbance (nm)	% Inhibition
1.	10	0.107±0.005	69.6±0.811
2.	50	0.115±0.0023	81.6±3.457
3.	100	0.119±0.0012	89.3±1.891
4.	250	0.122±0.0015	93.0±2.325
5.	500	0.123±0.0015	94.3±2.325

Table.no 1: Anti- inflammatory activity of diclofenac by Egg albumin denaturation method

Egg Albumin method:

Con (µg/ml)	CM-1		CM-2		CM-3		CM-4		CM-5		CM-6	
	Abs	%Inh	*Abs*	%Inh	*Abs*	%Inh	*Abs*	%Inh	*Abs*	%Inh	*Abs*	%Inh
10	*0.070 ±0.006*	*24.5 ±2.61*	*0.318 ±0.236*	22.5 ± *2.51*	*0.074 ±0.002*	*33.4 ±2.71*	*0.088 ±0.022*	*22.5 ±3.69*	*0.086 ±0.003*	*21.5 ±3.99`*	*0.075 ±0.003*	*36.43±3.99*
50	*0.072 ±0.0024*	*33.5 ±2.08*	*0.092 ±0.002*	*30.9 ±3.29*	*0.076 ±0.002*	*29.0 ±3.21*	*0.095 ±0.003*	*28.7 ±4.20*	*0.093 ±0.003*	*31.5 ±4.30*	*0.080 ±0.001*	*32.0 ±4.30*
100	*0.085 ±0.002*	*41.8 ±3.29*	*0.095 ±0.002*	*42.8 ±2.39*	*0.086 ±0.003*	*43.9 ±4.70*	*0.108 ±0.002*	*43.6 ±3.83*	*0.113 ±0.003*	*44.8 ±4.41*	*0.090 ±0.001*	*44.5 ±4.41*
250	*0.097 ±0.001*	*52.9 ±3.99*	*0.109 ±0.004*	*51.50±6.99*	*0.093 ±0.002*	*52.0 ±3.21*	*0.118 ±0.003*	*51.8 ±4.70*	*0.118 ±0.001*	*51.7 ±1.03*	*0.098 ±0.002*	*52.7 ±1.03*
500	*0.103 ±0.001*	*92.4 ±2.41*	*0.115 ±0.004*	*81.4 ±5.03*	*0.102 ±0.102*	*63.3 ±3.46*	*0.119 ±0.003*	*62.5 ±4.61*	*0.122 ±0.001*	*61.6 ±1.84*	*0.112 ±0.003*	*90.6 ±1.84*

Table no: 2 Anti-inflammatory activity of test compounds by Egg albumin denaturation method

INVITRO ANTI-ULCER ACTIVITY

The ability of the invitro approach to neutralise acids:

The neutralizing effect of Coumarin derivative was measured at four concentrations (100mg,200mg,250mg,500mg) and standard aluminium hydroxide + magnesium hydroxide [Al $(OH)_2$+Mg $(OH)_2$] (500 mg) was studied. The results obtained show that drug concentrations of 100mg,200mg, and 500 mg showed a significant decrease in acid capacity. Compared to standard Al (OH) 3+ Mg (OH) 2 (500 mg). The drug at a concentration of 500mg was found to neutralize acids significantly more than the standard were represented in table 3

CONCENTRATION Mg/ml	ANC per gram of drug					
	CM-1	CM-2	CM-3	CM-4	CM-5	CM-6
100	97.5	93	87.5	91	92	96.3
200	63.5	43.75	37.5	42.5	45	35.5
500	51	13.25	7.4	6	5.8	5.6
1000	13.4	14	14	12	12.8	12.6
$Al(OH)_2$ &$Mg(OH)_2$ (500mg/ml)	13.8					

Table 3 *In vitro* anti-ulcer activity by Acid Neutralizing Capacity (ANC) method.

In silico Docking Results:

Docking results of compounds of 3a shows more binding energy against 3f targeting phospholipaseA_2 (PDB ID- IQG6) for potential Anti-tuburculosis activity on table 4**.**

S.NO	Drug Target	Compound name	Binding Energy in Kcal/mol
1.	**PDB-ID(IQG6)**	isoniazid	-8.7
2.		3a	-8.8
3.		3b	-7.7
4.		3c	-7.8
5.		3d	-8.2
6.		3e	-8.7
7.		3f	-8.5

Table: 4 Docking compounds of Coumarin derivatives

Conclusion

Six novels substituted Coumarin derivatives were synthesized by two steps simple procedure, characterized and all derivatives screened for *in-vitro* anti-inflammatory and *in-vitro* anti-ulcer activities respectively. Structural Characyerization was performed by FT-IR,NMR,MASS Spectroscopy

The present research work, involves the synthesis of series of 6 novel substituted compounds of Coumarin. Here 3-Amino phenol taken as a starting material. Treatment of 3-oxo butanoate were dissolved in 18ml Zinc chloride, 2ml Sulphuric acid it forms 7-Amino-4-methyl Coumarin. To this compound various aromatic aldehydes were added along with 30-40ml of ethanol and kept for reflux for 3hrs and it forms a novel various colored coumarin derivative. Purification was done by recrystallization. Characterization of all derivatives were done by FT-IR, NMR, and Mass Spectroscopy.

All derivatives were screened for their *in-vitro* anti-inflammatory and *in-vitro* anti-ulcer activities.

Anti-inflammatory activity:

Anti-inflammatory activity was performed by Egg albumin protein denaturation method diclofenac as reference standard. All the titled compounds (CM-1 to CM-6) were evaluated for in-vitro anti - inflammatory activity. The effect of the synthesized titled compounds was tested with different concentrations (10, 50,100,250,500µg/ml). All derivatives were able to inhibit anti-inflammatory activity. The most effective was CM-6, CM-3 shows more potent activity. The results were tabulated in table no. 1, 2. The order of anti-inflammatory activity of synthesized compounds as follows.

CM-6>CM-3> CM-5> CM-4>CM-1 >CM-2

Anti-ulcer activity:

From all the derivatives, CM-1, CM-2, CM-3,CM-4,CM-5&CM-6 were performed by Acid Neutralizing Capacity ANC method. For theoretical prediction of anti-ulcer activity using "Synthesized derivatives were compared against the standard drug Aluminium hydroxide and Magnesium hydroxide. Results revealed that the synthesized derivatives has shown some moderate inhibition. The most effective was CM-4 shows more potent activity. The results were tabulated in table no. 3. The order of anti-ulcer activity of synthesized compounds as follows.

CM-4>CM-6> CM-5> CM-1>CM-3>CM-2

In-silico anti-tuberculosis screening:

In order to prove the alternative hypothesis, in silico estimation of activity for the synthesized derivatives had been performed using PASS online web resource. The results showed that the title compounds were having good anti-tuberculosis activity to that of current marketed drugs. This had given the confidence to take the research to further level in future for screening the anti-tuberculosis activity using other targets. Docking results of compounds of 3a shows more binding energy against 3f targeting phospholipaseA_2 (PDB ID- IQG6) for potential Anti-tuburculosis isoniazid by using PDB-ID IQG6.

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REFERENCES

1.Sarmah M, Chutia K, Dutta D, Gogoi P. Overview of coumarin-fused-coumarins: Synthesis, photophysical properties and their applications. Org Biomol Chem. 2021;20:55–72.10.1039/D1OB01876KSearch in Google Scholar

2.Review Peptic Ulcer Disease: A Brief Review of Conventional Therapy and Herbal Treatment Options Lucija Kuna 1 , Jelena Jakab 2,3, Robert Smolic 2,4,5, Nikola Raguz-Lucic 1 , Aleksandar Vcev 2,5 and Martina Smolic 1,4,*

3.Review Article A Review on Antiulcer Activity of Few Indian Medicinal Plants G. Vimala and F. Gricilda Shoba.

4.Grivennikov SI, Greten FR, Karin M. Immunity, inflammation and cancer. *Cell.* 2010;**140**:883–899. [PMC free article] [PubMed] [Google Scholar]

5.Kankala S, Kankala RK, Gundepaka P, Thota N, Nerella S, Gangula MR. Regioselective synthesis of isoxazole–mercaptobenzimidazole hybrids and there *in vivo* analgesic and anti-inflammatory activity studies. *Bioorg Med Chem Lett.* 2013;**23**:1306–1309. [PubMed] [Google Scholar]

6.Bansal Y, Sethi P, Bansal G. Coumarin: a potential nucleus for anti-inflammatory molecules. *Med Chem Res.* 2013;**22**:3049–3060. [Google Scholar]

7.Hwu JR, Singha R, Hong SC, Chang YH, Das AR, Vliegen I. Synthesis of new benzimidazole–coumarin conjugates as anti-hepatitis C virus agents. *Antiviral Res.* 2008;**77**:157–162. [PubMed] [Google Scholar]

8. Fylaktakidou KC, Hadjipavlou-Litina DJ, Litinas KE, Nicolaides DN. Natural and synthetic coumarin derivatives with anti-inflammatory/antioxidant activities. *Curr Pharm Des.* 2004;**10**:3813–3833. [PubMed] [Google Scholar]

9. Rosskopf F, Kraus J, Franz G. Imunological and antitumor effects of coumarin & some derivatives. *Pharmazie.* 1992; **47:139**. [PubMed] [Google Scholar]

10. Patel RV, Kumari P, Rajani DP, Chikhalia KH. Synthesis of coumarin-based 1, 3, 4-oxadiazol-2ylthio-*N*-phenyl/benzothiazolylacetamides as antimicrobial and antituberculosis agents. *Med Chem Res.* 2013;**22**:195–210. [Google Scholar]

11.Roussaki M, Kontogiorgis CA, Hadjipavlou-Litina D, Hamilakis S, Detsi A. A novel synthesis of 3-aryl coumarins and evaluation of their antioxidant and lipoxygenase inhibitory activity. *Bioorg Med Chem Lett.* 2010;**20**:3889–3892. [PubMed] [Google Scholar]

12.Sreeja S, Mathan S, Kumaran J. Design, synthesis and pharmacological evaluation of new coumarin derivatives. *Int J Adv Pharm Biol Sci.* 2012;**2**:80–91. [Google Scholar]

13.Rodríguez SA, Nazareno MA, Baumgartner MT. Effect of different C3-aryl substituents on the antioxidant activity of 4-hydroxycoumarin derivatives. *Bioorg Med Chem.* 2011;**19**:6233–6238. [PubMed] [Google Scholar]

14.Stefani HA, Ueogjan KG, Manarin F, Farsky SH, Zukerman-Schpector J, Caracelli I. Synthesis, biological evaluation and molecular docking studies of 3-(triazolyl)-coumarin derivatives: effect on inducible nitric oxide synthase. *Eur J Med Chem.* 2012;**58**:117. [PubMed] [Google Scholar]

15.Bansal Y, Silakari O. The therapeutic journey of benzimidazoles: a review. *Bioorg Med Chem.* 2012;**20**:6208. [PubMed] [Google Scholar]

16.Patil A, Ganguly S, Surana S. A systematic review of benzimidazole derivatives as an antiulcer agent. *Rasayan J Chem.* 2008;**3**:447–460. [Google Scholar]

17.R.O.Kennedy, and R.D. Thornes, Coumarins: Biology, Applications and Mode of Action, John Wiley and Sons, Chichester, 143(1997)

18. Pratibha, S. and P. Shreeya, Synthesis, Characterization and antimicrobial studies of some novel 3-arylazo-7-hydroxy-4-methyl-coumarin, Indian J. Chemistry, 1999, 38B, 1139-1142.

19.atonay, T., G. Y. Litkei, R. Bognar, J. Erdei and C. Misztic, Synthesis, antibacterial and antifungal activity of 4- hydroxy-coumarin derivatives, Analogues of Novobiocin, Pharmazie, 1984, 39 :2, 86-91

20. Nofal, Z.M.; M. El-Zahar, and S. Abd El-Karim, Novel Coumarin derivatives with expected biological activity, Molecules, 2000, 5: 99-113.

21. Vekariya R.H., Patel H.D. Recent advances in the synthesis of coumarin derivatives via *Knoevenagel condensation*: A review. *Synth. Commun.* 2014;44:2756–2788.

doi: 10.1080/00397911.2014.926374. [CrossRef] [Google Scholar]

22.Reppel L. About natural coumarins. Pharmazine. 1954;9:278–99.Search in Google Scholar

23.Dandriyal J, Singla R, Kumar M, Jaitak V. Recent developments of C-4 substituted coumarin derivatives as anticancer agents. Eur J Med Chem. 2016;119:141–68.10.1016/j.ejmech.2016.03.087Search in Google ScholarPubMed

24.Vyawahare N, Deshmukh V, Gadkari M, Kagathara V. Plants with antiulcer activity. Pharmacognosy Reviews. 2009;3(5):118.

25.Wormsley KG. Aetiology of ulcers. Bailliere's clinical gastroenterology. 1988 Jul 1;2(3):555-71.

26.G. Vinothapooshan And K.Sundar , Anti-Ulcer Activity Of Mimosa Pudica Leaves Against Gastric Ulcer In Rats, Research Journal Of Pharmaceutical, Biological And Chemical Sciences.

27.N. L. Dashputre And N. S. Naikwade , Evaluation Of Anti-Ulcer Activity Of Methanolic Extract Of Abutilon Indicum Linn Leaves In Experimental Rats, International Journal Of Pharmaceutical Sciences And Drug Research 2011; 3(2): 97-100.

28.P. Thirunavukkarasu, L. Ramkumar And T. Ramanathan, Anti-Ulcer Activity Of Excoecaria Agallocha Bark On Nsaid-Induced Gastric Ulcer In Albino Rats,