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Research Article

Evaluation of Anti-inflammatory and Analgesic Activity of Ethanolic extracts of *Inularacemosa* and *Albizia amara*

Khan A., Shah R. D.*, Pallewar S.

School of Pharmacy and Technology Management, SVKM's NMiMS, bank of River Tapi, Babulde, Shirpur, Dist. Dhule, Maharashtra- 425405

ABSTRACT
The present study was performed with the objective of dose determination and evaluation of Anti-inflammatory and Analgesic activity of the Ethanolic extracts of *Inularacemosa* root and *Albiziaamara*. The dose determination studies were carried out according to OECD guidelines and the safe dose determined was 2000 mg/kg. Hence, 200 mg/kg was given for the models for anti-inflammatory (carrageenan induced paw edema) and analgesic (hot plate) activity. The percentage reduction in paw volume observed against Carrageenan induced paw oedema for *Inula* was found to be 34 % whereas in *Albizia* it was15 %. In hot plate method, the percentage inhibition was 42% and 61.91% respectively.The data were found statistically significant by using one way ANOVA (P< 0.05).Thus, both the Extracts were able to show anti-inflammatory & analgesic activity as compared with standard drug Aspirin 100 mg/kg. But the effect of *Albiziaamara* extract was more statistically significant compared to extracts of *Inularacemosa*.
Keywords: anti-inflammatory, analgesic, *Inularacemosa* root ,*Albiziaamara*.

INTRODUCTION

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants,and the treatment that reduces this inflammation is called as anti inflammatory.Currently there are huge variety of anti inflammatory drugs are available in the market[1].These anti-inflammatory drugs are associated with someor the other side effects. Therefore, the development of potentanti-inflammatory drugs with fewer side effects isnecessary. Alternatively, we can use the herbal plant extracts for the treatment of inflammation, because these herbal plants shows the better activity and lesser side effect[2-6].

Inularacemosa(Pushkamoola) root is available as cylindrical, straight; surface rough due to longitudinal striations and cracks, scars of lateral rootlets and rhytidoma present, externally brownish-grey and internally yellowish-brown[7-10].The roots are used for the various treatments like angina pectoris[11], ischemic heart disease[12],anticancer[13], decreases cholesterol**,** anti bacterial, Asthma, bronchial disease, dyspnea[14-21].

*Albiziaamara*grows throughout southern India and in some parts of Madhya Pradesh[22-23]. This plantis a small to moderate-sized, much-branched deciduous tree with smooth, dark green, scaly bark. It resembles the acacias but lacks thorns. Leaf and flowers of this plant shows anti inflammatory activity usually used for boils and ulcers[24-26]. Leaves are used for erysipelas. Seeds showastringent, anti diarrhoeal and antibacterial effect whereas the extract showed DNA binding activity, antibacterial, and they inhibit platelet aggregation and human lymphocyte transformation. They also show anti-inflammatory and cytotoxic activity. The oil from the seeds is said to cure leprosy and leucoderma[27, 28].

No scientific report is available to date to validate these folkloric uses,so, we now report the anti-inflammatory and analgesic activities of Ethanolic extract of *Inularacemosa*and *Albiziaamara.*

MATERIALS AND METHODS

Plant material

Roots of *Inula* were purchased from local market in Aurangabad and Authenticated at the Agharkar Research centre, Pune. A voucher specimen of the root of *Inularacemosa*was deposited at the Agharkar Research centre, Pune.

Plant of *Albiziaamara*were collected from local market of Shirpur and Authenticated at Jai Hind Education Trust's, ZulalBhilajiraoPatil College Dhule,

Extraction of both the material was done at School of Pharmacy and Technology Management, NMiMs, Shirpur, Dhule.

Drugs and chemicals -

Aspirin was obtained as a gift sample from the Cadila pharmaceuticals Ltd., Gujrat. Carrageenan and Carboxy methyl cellulose was purchased from SD-Fine chem, Mumbai and CDH Lab, Mumbai respectively. Ethanol was used as solvent for extraction, and was obtained from Rankem, Mumbai.

Processing of the plant material and extraction -

The roots & the plant were separately dried in shade and were grounded to get a coarse powder and subjected to Soxhlet extraction using ethanol. Dried and powdered

**Author for Correspondence*
Email: parikhrima1@gmail.com

Table 1:Phytochemicalestimations on Ethanolic extract of *I.racemosa*&*A.amara*

Chemical constituent	*Inularacemosa*	*Albiziaamara*
Alkaloid	+ ve	+ ve
Glycoside	+ ve	+ ve
Essential Oil	+ve	+ ve
Saponins	+ ve	+ ve
Steroids	+ ve	- ve
Triterpenoids	+ ve	+ ve
Flavonoids	+ ve	+ ve
Carbohydrates	-ve	-ve
Amino Acids	+ve	+ve

+ve sign indicates Presence and –ve sign indicate Absence of compound

were placed in a Soxhlet apparatus, submerged with 95%

Table 2: Mortality Data of acute oral toxicity studies on Ethanolic extracts of *I.racemosa* and *A. amara*

Sr. No	Group	No. of animals	No. of animals dead	Mortality Ratio
1.	2000mg/kg*I. racemosa*	03	Nil	Nil
2.	2000mg/kg *A. amara*		Nil	Nil

ethanol and kept overnight at 50^0 C for 20 h. The percolate was collected and filtered. Ethanol was distilled off using Rota-evaporator under reduced pressure at 50° C. The final drying was done initially in vacuum desiccators. The yield of different extracts was calculated. The powder extracts obtained were then subjected to phytochemical analysis to detect the chemical constituents present in each extracts.

Animals

Studies were carried out using female *Wistar albino* rats (110–180 g).The animals were obtained from the Animal house of SPTM, grouped and housed in polyethylene cages with not more than six animals per cage and maintained under standard conditions with 12 hr natural light and dark cycle. They were fed with standard pellet diet and water *ad libitum*. All the experimental process and protocols used in this study were approved by the Institutional Animal Ethical Committee (IAEC). The experiments were performed as per the norms of CPCSEA.

Preliminary phytochemical investigation

The qualitative chemical tests of Ethanolic extracts of *Inularacemosa*&*Albiziaamara*was carried out using standard procedure.[29]

Acute Oral Toxicity

Acute oral toxicity studies were performed for ethanolic extracts of *Inularacemosa*&*Albiziaamara*according to the toxic class method 423 as per OECD guidelines. Firstly the dose of 2000mg/kg dose was given and studies were carried out. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. Additional observations will be necessary if the animals continue to display signs of toxicity[30].

Pharmacological Screening

After the successful determination of the dose, the animals were subjected for Anti-inflammatory and analgesic activity. For the same, animals were divided into 5 groups containing 6 animals each. The groups are as:

Group I: Control (1% CMC)
Group II: Standard (Aspirin –100mg/kg)
Group III: Test – I (Ethanolic extracts of *Inularacemosa* – 200mg/kg)
Group IV: Test – II (Ethanolic extracts of *Albiziaamara* – 200mg/kg)
Group V: Test – III (Ethanolic extracts of *Inularacemosa* & *Albiziaamara* (1:1))

Screening of Analgesic activity (Hot plate method)

Groups of 6 rats each with an initial weight of 110-170g were used for each dose. The hot plate, which is commercially available, consists of an electrically heated surface. The temperature is controlled for 55°C to 56 °C. The animals were placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch. The latency is recorded before and after - 30, 60 and 90 min following oral or i.p. administration of the the test or standard compound.The prolongation of the latency times comparing the values before and after administration of the test compounds or the values of the control with the experimental groups can be used for statistical comparison using the t-test[31, 32].

Screening of anti-inflammatory activity (Carragenan induced paw oedema method)

Groups of 6 rats each with an initial weight of 110-170g were used for each dose. Subsequently 30 min after above treatment - 0.1ml of 1% Carragenan was injected s
ubcutaneously into the subplanter region of left hind paw to induce oedema. The paw volume was measured initially and at 15, 30, 1 h, 2 h, 3 h and 5 h after Carragenan injection using Plethysmometer.[33-35]
Results were determined as the percentage inhibition by comparing with the control. Percentage inhibition of

Table 3: Effect of Ethanolic extracts on latency time observed rats (time expressed in seconds and values are expressed in Mean ± SEM, n=6)

Groups	0 min	15 min	30 min	60 min	120 min
Control	9.94 ± 3.28	6.03 ± 1.89	6.14 ± 1.7	7.00 ± 3.42	6.0 ± 2.21
Standard	6.01 ± 1.74	34.16 ± 3.14	55.13 ± 1.95	62.12 ± 1.81	65.47 ± 1.92
Test-I	6.76 ± 2.32	17.03 ± 3.60	21.51 ± 3.66	33.08 ± 2.26	42.99 ± 1.95
Test-II	3.76 ± 1.34	28.83 ± 5.04	50.58 ± 2.68	56.7 ± 1.97	61.91 ± 1.9
Test-III	8.99 ± 2.35	20.07 ± 2.50	37.52 ± 3.13	38.77 ± 6.99	47.35 ± 9.42

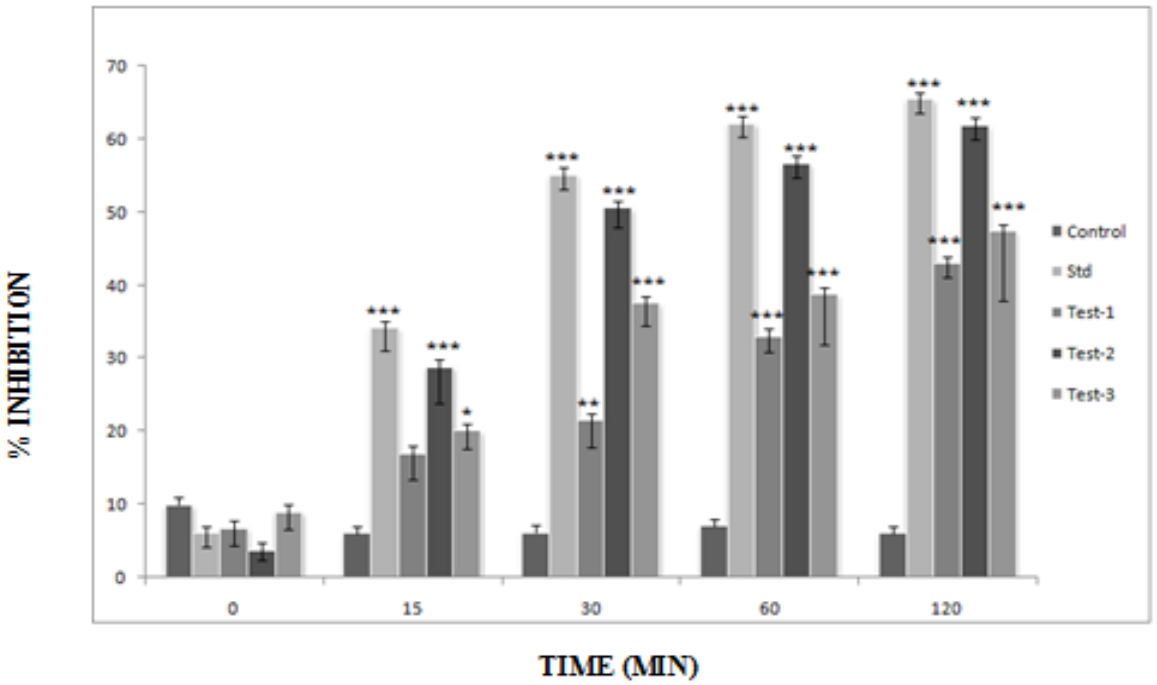

Figure 1: Effect of Ethanolic extracts on increase in latency time by using Hot plate method (time expressed in seconds and values are expressed as Mean ± SEM, n=6, where; *P<0.05, **P<0.003, ***P<0.0002 compared with control group)

latency time between treated and control group was calculated as follows:

Percentage inhibition = $\frac{Vc-Vt}{Vc} \times 100$

Where, $V_c$and V_t represent mean increase in latency time in control and treated groups respectively.

Statistical analysis

The data are reported as mean ± standard error of the mean (SEM) and were compared using one way analysis of variance (ANOVA), followed by the Dunnett's multiple comparison test using Graph Pad PRISM software ,and *p*- values < 0.05 was considered significant.

Pharmacological Screening

Analgesic Activity (Hot Plate Method) - Analgesic studies were determined by using hot plate method. The analgesic studies of both the ethanolicextract and the combination of both the extract has been carried out at dose level of 200 mg/kg body wt. The dose of both extracts has shown significant analgesic activity (P<0.05) when compared with standarddrug aspirin. Among the 3 test groups, the Ethanolic extract of Albiziaamara hassignificantly higher activity compared to other groups.The percentage inhibition of I. racmosa and A.amara are 42.99 and 61.91 respectively. Table 3 shows the details of the analgesic activity as shown by the extracts. Anti-inflammatory Activity - Anti-inflammatory activity was carried out by Carrageenan induced rat paw oedema method. The anti-inflammatory studies of both the ethanolicextracts individually and the combination of both the extracts has been carried out by Carrageenan induced rat paw oedema method at dose level of 200 mg/kg body wt. The dose of both extracts has shown significant analgesic activity (P<0.05) when compared with standard drug Aspirin. Among the 3 test groups, the Ethanolic extract of Albiziaamara has significantly higher activity compared to other groups.The percentage reduction in

Table 4:Effect of Ethanolic extracts on Carrageenan Induce Paw Oedema(values are expressed in volume and they are shown in Mean ± SEM, n=6)

Groups	0 min	15 min	30 min	60 min	120 min	180 min	240 min	300 min
Control	2.5 ± 1.70	7.5 ± 1.7	14.17 ± 2.02	19.17 ± 2.0	40.83 ± 10.83	47.5 ± 7.04	45.83 ± 7.12	40.83 ± 3.27
Standard	24.14 ± 2.38	32.5 ± 1.11	25.83 ± 1.53	25.67 ± 0.95	17.5 ± 1.70	12.5 ± 1.70	8.33 ± 1.05	4.16 ± 0.83
Test-I	10 ± 1.82	19.17 ± 2.713	23.33 ± 3.33	26.67 ± 1.66	34.17 ± 6.76	28.38 ± 3.65	21.67 ± 3.5	17.5 ± 2.5
Test-II	18.33 ± 2.47	31.16 ± 3.08	37.5 ± 5.73	33.17 ± 1.04	15 ±4.47	12.5 ± 3.81	6.66 ± 3.03	5 ± 2.23
Test-III	8.33 ± 1.66	27.5 ± 2.14	33.33 ± 1.66	36.64 ± 2.108	21.67 ± 1.2	27.5 ± 4.03	19.17 ± 3.0	13.33 ± 4.04

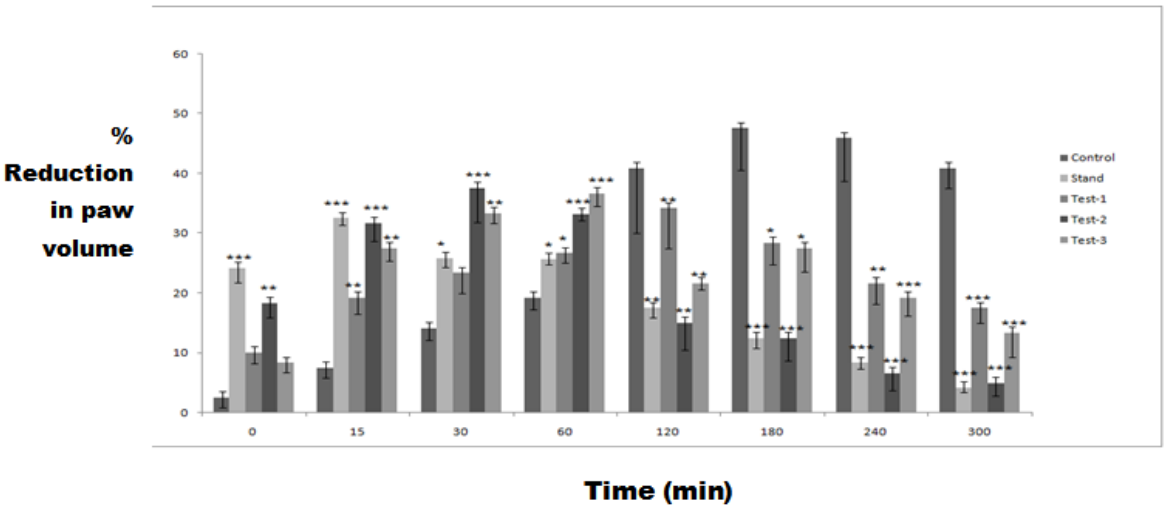

Figure 2: Effect of Ethanolic extracts on % Reductionin volume in Carrageenan induced paw edema method (values are expressed in volume and they are shown in Mean ± SEM, n=6, where; *P<0.05, **P<0.003, ***P<0.0002 compared with control)

paw volume observed against Carrageenan induced paw oedema for Inula was found to be 34 % whereas in Albizia it was15 %. Table 4 depicts the results of Anti-inflammatory studies as studied by Carragenan induced paw oedema in rats.

RESULTS AND DISCUSSION

Preliminary phytochemical investigation - The preliminary phytochemical investigation of Ethanolic extracts of *Inularacemosa* and *Albiziaamara* are as shown in Table 1.

Acute Oral toxicity

Both the Ethanolic extracts were found to be safe at MTD >2000mg/kg as observed according to the OECD 423 guidelines. The data of the test is as shown in Table 2.

MTD_{50}> 2000mg/kg, drug was found to be safe and nontoxic, as no mortality occurs.The extract was found to be safe and nontoxic, no behavioral changes were observed. Hence the drug can be considered as safe at 2000mg/kg.

Thus, the final dose selected was 200mg/kg. (ED_{50})

DISCUSSIONS

After successful Ethanolic extraction, the Percentage yield found for *Inularacemosa&Albiziaamara*are 11.5% w/w and 6.72% respectively.The phytochemical studies performed in the present study confirmed that the extract of *Inularacemosa*and *Albiziaamara*possess Saponins, Steroids, flavonoids, alkaloids and glycosides.

After completion of Oral acute toxicity as per OECD guideline 423, it was found that MTD found is more than 2000mg/kg and drug was found to be safe. Thus, final dose selected was 200mg/kg.

For the pharmacological screening, Anti-inflammatory and Analgesic models were studied. From Table 3, it was seen that pretreatment by *Albiziaamara*plant increased the response latency in the hot plate test, which was significant. On the other hand pretreatment by *Inularacemosa*root extract slightly increase the response latency in the hot plate but less in comparison to the response that was produced by the *Albiziaamara*. And the combination of both the extract produces the intermediate response. From,Table 4,it was seen that pretreatment by *Albiziaamara*plant produce more reduction in paw volume, in Carrageenan induce model right from 120 min which was significant. On the other hand pretreatment by *Inularacemosa*root extract produce slight reduction in paw volume but less in comparison to the response that was produced by the *Albiziaamara*. And the combination of both the extract produces the intermediate response.

Statistically significant anti-inflammatory & analgesic activity was shown by the Ethanolic extract of *Albiziaamara* in comparison to the activity shown by *Inularacemosa*and combination.

Thus, further work needs to be carried for the exact mechanism responsible in Ethanolic extracts of *A. amara* for the anti-inflammatory & analgesic activity.

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REFERENCES

1. Alam, K.; Pathak, D.; Ansari, S. H. Evaluation of Anti-inflammatory Activity of *AmmomumsubulatumFruit*, *Int J PharmaSciDr Res*. 2011, 3,1, 35-37
2. Vogel, H.; Vogel, W. H.; Bernward, A.; Sandow, J.; Müller, G. Drug Discovery and Evaluation, Pharmacological Assays, 2nd ed., Springer – Verlag: Berlin, 2002, pp 669-680
3. Saraswathi, R.; Upadhyay, B.; Venkatakrishnan, R.; Meera, R.; Devi, P. Phyto chemical investigation, analgesic and anti inflammatory activity of *Abutilon indicum*linn. *Int J Pharm Pharm Sci*. 2011,3, 2

4. Panda, B.B., Kalpesh, G., Kori, M.L.Anti-Inflammatory and Analgesic Activity of *Jatrophagossypifolia*in Experimental Animal Models. *Glo J Pharmacol*. 2009, 3,1,05
5. Amberkar, M. V.; Tara S.; Kumari, M. K. Evaluation of antiinflammatory and analgesic activities of alcoholic extract of *kaempferiagalanga*in rats. *Ind J Physiolpharmacol.* 2011, 55, 1, 13–24
6. Chauhan, N. S. Medicinaland Aromatic Plants of Himachal Pradesh. Indus Publishing Company:New Delhi, 1999, pp. 632
7. Enderberg, A. Taxonomy and Phylogeny of the tribe *Inuleae*(Asteraceae). *Pl. Syst. Evol*., 1991, 176, pp 75-123.
8. Patel, V.; Banu, B.; Ojha, J.K.; Malhotra, O. P.; Udupa,K. N. Effect of indigenous drug (*Pushkaramoola*) on experimentally induced myocardial infarction in rats. *Act Nerv Super (Praha)*, 1982, 3, 2, 387-94
9. Tripathi, S. N.; Upadhyaya, B. N.; Gupta, V.K. Beneficial effect of *Inularacemosa(Pushkaramoola)* in angina pectoris: a preliminary report. *Ind J physiol Paharmacol* , 1984, 28, 1, 73-5
10. Mehara, M.M.; Deshpande, K.G.; Ghatag, B.B.; Bhattacharya,S.K. Terpenoids CVTransformati on product of an Alantolactone, Tetrahendron 1967,23, 2469-80 .
11. Purushothanman, K.K.; Sarada, A. Chemical examination of substitutes for *Pushkaramoola, J Res Indian Med*, 1974, 9, 3, 30-32
12. Tripathi, S.N.; Upadhyaya, B.N.; Gupta, V. K. Beneficial effect of *Inularacemosa*(*Pushkaramoola*) in angina pectoris: a preliminary report. *Ind J physiol Paharmacol*, 1984, 28, 1, 73-5
13. Pal, H.C.; Sehar, I.; Bhushan, S.; Gupta, B.Activation of caspases and poly (ADP-ribose)polymerase cleavage to induce apoptosis in leukemia HL-60 cells by Inularacemosa, *Toxicol in Vitro*, 2010,24,1599–1609
14. Zafar, A. M.; Sualeh, S. B. Z. M. Herbal treatment for cardiovascular disease The evidence based therapy, *Pak J Pharm Sci*2010, 23, 1,119-124
15. Lignans and sesquiterpene lactones from Artemisia Sieversiana and inularacemosa, to check the anti fungal activity, TANG, SHUAI, Elsevier science Ltd, Phytochemistry\ Vol[38\ No[0\ pp[046050\ 088 .
16. Mahmood, M.,;Syyed, N. O.; Farzaneh, N. Cytotoxic Effects of Five Species of *Inula*Against Some Tumor, Cell Lines,*Iranian J Pharm Sci*, 2006, 2,4, 203-208.
17. Lokhande, P.D.;Gawai, K.R.;Kodam, K.M. Antibacterial Activity of Isolated Constituents and Extract of Roots of *Inularacemosa*,*Current science*, 2009, 93, 11, 1301-1305.
18. Sgreesh, O.; Nandave, M. Cardiop,rotection by *Inularacemosa*Hook in experimental model of myocardial ischemic reperfusion injury, *Ind J expt biology*, 2010, 48, , 918-924
19. Bhathal, S. S.; singh, D. Effect of crude root oils of inula and saussurealappa on feeding survival and development of spodopteralitura(Lepidoptera: Noctuidae)Larvae., .J Eur 1990,239-240.
20. R. Kaur, AditiKashyap, SadiqMajeed, *In vitro* propagation and conservation of *Inularacemosa*hook. F. AnEndangered medicinal plant of temperate origin, J. 88 Adv. Lab. Res. Biol. Vol.-I, Issue I | July-2010.
21. Sharma, S.; Sharma, R. K. Seed physiological aspects of pushkarmool(*Inularacemosa*), a threatened medicinal herb: response to storage, cold stratification, light and gibberellic acid,*Current science*, 2010,99, 12, 25,1801-1805.
22. Akilandeswari, S.; Senthamarai, R.; Valarmathi, R.; Savarinsha, J. A.; Selvan, A. Evaluation of anti-inflammatory and anti-arthritic activity of Albizialebbeck and *Albiziaamara* extracts, *Biomed* 2009 , 4, 3 295-302.
23. Mar, W., Tan, G.T., Cordell, G.A., Pezzuto, J.M., Jurcic, K., Offermann, F., Redl, K., Steinke, B., Wagner, H., 1991. Biological activity of novel macrocyclic alkaloids (budmunchiamines) from *Albiziaamara* detected on the basis of interaction with DNA. *J. Nat. Prod.* 54, 1531–1542.
24. Prasad, P.; Thippeswamy, S. D. Antimicrobial efficacy and phytochemical analysis of *Albiziaamara* (Roxb.) Boiv. anindigenous medicinal plant against some human and plant pathogenic bacteria and fungi, *J Pharm Res* 2011 .
25. Aliyu, A.B.; Musa, A.M.; Ibrahim, M.A.; Ibrahim, H.;Oyewale, A.O.Preliminary phytochemical screening and antioxidant activity of leave extract of albiziachevalieri harms, Bayero*J Pure and App Sci*, 2,1,149 – 153.
26. Kumar, S. P.; Sucheta, S.; Sudarshana, V.; Selvamani, P. Antioxidant activity in some selected Indian medicinal Plants, Af J Biotech, 7,12, pp. 1826-1828,
27. Gasper, B.; Nshimo, C. M. In vitro antimicrobial activity of albiziaamara leaves,*Eur.J*. 1988,89:240-243,
28. Harish Chandra Pal a, IrumSehar a, ShashiBhushan a, Bishan Gupta b.,Activation

of caspases and poly (ADP-ribose)polymerase cleavage to induce apoptosis in leukemia HL-60 cells by Inularacemosa, Toxicology in Vitro 24 (2010) 1600–1601.

29. Kokate, K.C.; Purohit, A.P.; Gokhale, S.B. Pharmacognosy.39th ed., Niraliprakashan: Pune, 2007, pp 607-611
30. OECD: Guidelines for testing of chemicals: Acute oral toxicity, Environmental Health and Safety Monograph Series on Testing and Adjustment No. 425, 2001.
31. Ghosh MN, Fundamentals of Experimental Pharmacology, 2nded,(Scientific book agency, Kolkatta, p.155,1984 .
32. Eddy NB, Studies of morphine and codine and their derivaties. J. Pharmacol. 1932; 45:339, 1932.
33. Pal SC and Nandy A, Anti‐inflammatory, analgesic and antipyretic activity of AchrasSapota Linn. leaf extracts and its isolated compounds, Indian Drugs, 1999;36:106.
34. Winter CA, Risley GA and NussGW.Carrageenan induced edema in hind paw of the rat as an assay for inflammatory drugs Proc.soc. Exp. BioMed. 1962; 3; 544‐547,
35. Kulkarni SK, Handbook of Experimental Pharmacology, VallabhPrakashan, Delhi, edn 3, ,1993, p.43.