History of Medicine Studies
ISSN:1300-669
Volume 18 Issue 2 July 2022

In Vivo and In Vitro evaluation of Immunomodulatory Potential Of Cassia auriculata Linn's

G.BuelaPriyanka, M.Gobinath, Sk.Salma& V.Haribaskar

Abstract

The current research set out to examine Cassia auriculata Linn's immunomodulatory potential both in vitro and in vivo. The immunomodulatory ability of plant methanol extracts was examined in a number of in-vitro models, as well as in an in-vivo model of oxazolone-induced cell-mediated inflammation in rats. Scientifically, several activity models were used to screen methanolic extracts of roots and flowers. Methanolic floral extract has immunomodulatory activity, as shown by oxazolone-induced cell-mediated inflammation. On the other hand, methanolic floral extract exhibited dose-dependent stimulation in in-vitro immunomodulatory models such as the NBT reduction test using human PMN cells. The phagocytosis of Candida albicans by human PMN was also seen using the same extract. A dose-dependent rise in candidacidal activity was also seen in the methanolic floral extract. The methanolic floral extract showed stronger effects than the positive control in inducing chemotaxis of human PMN cells. This means that methanolic floral extract has an immunostimulant effect in vitro, and it dramatically decreased rat ear edema in a dose-dependent way in an in-vivo investigation of oxazolone-induced delayed type hypersensitivity. Because of its antioxidant capability, the in-vitro tests showed that methanolic floral extract has strong immunomodulatory activity. All things considered, the data shown here suggest that Cassia auriculata has strong immunomodulatory action with its purported cytoprotective benefits. In chronic inflammatory conditions like rheumatoid arthritis, for example, a methanolic floral extract high in flavonoids may be used to lessen the dosage and dose-related toxicities of conventional medications.

Keywords: Cassia auriculata, oxazolone, immunomodulatory, NBT test

Introduction

Wild in central and western India, the evergreen shrub Cassia auriculata Linn.has enormous, brilliant yellow blooms and is a member of the Leguminoseae family. Extreme drought and lengthy water stays were both documented as conditions in which plants not only survived, but also proceeded normally with ontogenetic processes including blooming and fruiting. Accordingly, our findings provided conclusive evidence that plants produce specific secondary metabolites that aid in survival under these stressful conditions. There are a number of things that the Cassia genus is famous for.(1) In traditional medicine, Cassia auriculata has many uses, including alleviating symptoms of chronic purulent ophthalmia, conjunctivitis, diarrhea, cough, asthma, and other conditions.(2) It's suggested for nocturnal emission, sore throat gargling, enemas, rheumatism, eye problems, and urinary disorders and skin ailments.(3) Diarrhea is treated with its decoction, and dysentery is treated with its fresh bark juice. Several species' aqueous extracts showed hypoglycemic effects.(4,5) Cassia auriculata herbal tea, made from dried flowers, is popular in Sri Lanka because to its favorable benefits on diabetes mellitus, constipation, and urinary tract problems. Furthermore, the plant's antibacterial, antiviral, antispasmodic, antioxidant, hypolipidemic, and oral hypoglycemic effects have been studied from a contemporary pharmacological perspective.(10) When exposed to cisplastin and gentamicin, the rat's kidneys were protected by the root's ethanolic extract.(11) Animal studies showed that the water-based seed extract had a hypoglycemic effect.(12) Flavonoids, triterpenoids, kaempferol, β-sitosterol, auricassidin, and anthracene derivatives were among the phytochemicals found in the plant.13) Polysaccharides, tannins, saponins, and flavonoids such as rutin and quercetin.(14) The purpose of this work was to examine the immunomodulatory potential of methanolic extracts of Cassia auriculata flowers and roots, since the literature review indicated that the plant is promising and has shown a broad range of pharmacological activity.

Department of Pharmacy
Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore Dt.524001 A.P. India

MaterialsandMethod

Collection and authentication of the plant material: The plant material including flowers, roots and othermorphologicalpartsof*Cassiaauriculata*werecollectedfromthedrystonyhillyareaofvillageHolnanthe, Dhule district of Maharashtra, with the helpoflocaltribes.Thespecimenwaspreparedandauthenticatedby Departmentof Botany,S.S.V.P.S'sL. K.Dr.GhogreyScienceCollegeofDhule,Maharashtra, India.Avoucherspecimen(MMR-03)has been preservedfor future reference.The flowersand roots were washed, cleaned; shade dried and thenpowderwasmadeandpassedthrougha40-meshsieve,

andkeptinawell-closedcontainerforextractionpurpose.

Preparationofstandardizedextracts: Thecoarsepowderofflowersandroots,3000gmeachwasextractedoutby coldmacerationmethodbyusingmethanol(80%)asasolvent.Theseextractswereconcentratedinrotaryvacuumevaporator(Roteva-Equitron,Mumbai)underreducedpressureandthendried by vacuum dryer. Then extracts were screened fortheir prime phytochemicalcontent.

Phytochemical analysis of the methanolic extracts: The conventional phytochemical tests were carried outon methanolic extracts of flowers and roots of *Cassiaauriculata*andconfirmedthepresenceofdifferent classesofsecondarymetaboliteslikeflavonoids,phenolics, andtriterpenoids.[15]

Animals: HealthyWistaralbinoratsofeithersexweighingabout180-200gmwereusedforimmunomodulatorypotential.Rats werefedwithstandarddiet,wateradlibitumandwerehousedinpolypropylenecagesmaintainedunderstandardcondition of 12/12 hrs. of light and dark cycles. Theethicalclearancewasobtainedbythe InstitutionalAnimalEthicsCommitteeR.C.PatelcollegeofPharmacy,Shirpur,Dist-Dhule(Maharashtra)(Registrationno.651/02/c/CPCSEA)beforetheexperiment.

Immunomodulatoryactivity

***In-vivo* model: Oxazolone-induced immune type ofinflammation:**Following the protocol given, researchers examined the impact of 80% methanolic flower and root extracts on immunological type inflammation produced by 4-ethoxymethylene-2-phenyl-2-oxazolone (oxazolone).(16) A total of six groups were formed from Wistar albino rats weighing 180–200 gm. following the rats were sensitized with 300 µl of 2% oxazolone on their shaved abdomen, they were given 60 µl of 1.6% oxazolone to rub on both sides of their ears every three days beginning seven days following sensitization. The ear thickness was assessed 72 hours after each oxazolone administration using a micrometer (0.01-0.0 mm, Mitutoyo Corporation, Japan). A total of 60µl of betamethasone valerate was administered to each ear 30 minutes before and 3 hours after each oxazolone treatment. Thirty minutes before to and three hours after each administration of oxazolone, the groups that were treated with extracts received doses of 200 mg/kg of flower extract and 400 mg/kg of root extract, respectively. The experiment lasted for twenty-two days. The correct concentrations of oxazolone and betamethasone valerate (both from Sigma) were dissolved in ethanol. For the first sensitization, 2% oxazolone was dissolved in ethanol. For subsequent ear applications, 1.6% oxazolone was dissolved in a vehicle (acetone: olive oil, 4:1). Table 1 displays the results.

Table1:In-vivooxazoloneinducedinflammation(Delayedtypehypersensitivity)

Sr. No.	Group	Dose mg/kg p.o.	7th day	10th day	13th day	16th day	19th day	22nd day
1	Negative control	---	0.46±0.0076	0.59±0.012	0.65±0.010	0.69±0.0079	0.73±0.014	0.78±0.011
2	Positive Control[a]	60µl	0.46±0.011	0.52±0.0095**	0.50±0.012**	0.51±0.014**	0.49±0.010**	0.48±0.012**
3	CAMF	200	0.45±0.011	0.51±0.010**	0.55±0.0060**	0.60±0.0060**	0.64±0.010**	0.68±0.011**
4	CAMF	400	0.44±0.012	0.53±0.012**	0.55±0.014**	0.58±0.014**	0.62±0.019**	0.65±0.019**
5	CAMR	200	0.45±0.0095	0.55±0.017*	0.58±0.015**	0.62±0.011**	0.66±0.013**	0.71±0.0080**
6	CAMR	400	0.45±0.0067	0.56±0.010*	0.59±0.015**	0.62±0.013**	0.66±0.019**	0.69±0.019**

Valuesareexpressedasmean± SEM,n=6

Dataanalysed byOnewayANNOVAfollowed byDunnette'stest**$P<0.01$,*$P<0.05$CAMF-*Cassia auriculata*methanolicflowersextract.

CAMR-*Cassiaauriculata*methanolicrootsextract.a-Betamethasone valerate.

*In-vitro*immunomodulatory assays

NitroblueTetrazolium (NBT)test:A human volunteer in good health had her blood collected into tubes that contained the anticoagulant heparin. Using the dextran sedimentation technique, PMN cells were isolated from the blood sample. The

sterile centrifuge tubes were filled to capacity with a 6% w/v dextran solution in saline, and then the blood was added. For 15 minutes, the tube was left at room temperature. After collecting the liquid portion and the colored layer on top of the red blood cell layer using an automated pipette, the pellet was spun in a centrifuge, rinsed three times with phosphate-buffered saline, and then re-dissolved in methyl ethylcellulose (MEM) at a concentration of 5 X 10^6 cells/ml.(17)

A leucocyte suspension (5 X 10^6 cells/ml) and a 0.3% NBT in sucrose solution were made in MEM. From a stock solution of 10 mg/ml, it was modified to extract concentrations ranging from 2500εg/ml, 1250µg/ml, 625µg/ml, 250εg/ml, and 125µg/ml. A combination of MEM, PMN cells, NBT compound, and extract made up the final cocktail. In the positive control, endotoxin was substituted for the extract.(number one) Twenty minutes were spent incubating the tubes at 370C, followed by another twenty minutes at room temperature. Once the incubation period was over, the assay tubes were gently shaken, and 20 µl of the assay mixture was transferred to microscope slides in order to make smears. I used an oil immersion microscope (Labomed CXL Plus Sl. Microscope, Model No.-038086) to look for NBT positive cells that had phagocytosed NBT dye. After the slides were air dried, they were stained with Giemsa's stain.(19) The percentage of cells that tested positive for NBT is shown in Table 2.

Table 2: Effect of *Cassia auriculata*extracts on *invitro* stimulation of Human PMN cells for NBTreduction

Sr. No.	Concentration (µg)	CAMF	CAMR
1	2500	22±0.70	47.25±0.47*
2	1250	41±0.40*	43.75±0.75*
3	625	41.25±0.47*	40.75±0.47*
4	250	31.50±0.64*	40.50±0.64*
5	125	28.50±0.64*	30.00±0.40*

Valuesareexpressedas Mean±SEM,n=4
*p<0.01vsnegativecontrol
Negativecontrol21.25±0.47,positivecontrol48.00± 0.40
(CAMF*)Cassiaauriculata*methanolicflowersextract
(CAMR)*Cassiaauriculata*methanolicrootsextract

Phagocytosis: The PMN cell suspension was preparedas stated above. The count of PMN cells was adjustedapproximately to 5 X 106 cells/ ml. For preparation of*Candidaalbicans(C.albicans)*suspension,12-hourold, unicellular culture of *C. albicans*was used. ThecandidacellssuspensionwaspreparedinPBSataconc entrationofabout5X106cells/ml.Itwasadjusted to concentrations of all extracts ranging from2500 µg/ml, 1250 µg/ml, 625 µg/ml, 250 µg/ml, 125µg/mlfromstocksolution(10mg/ml).Thefinalmixtu re contained MEM, PMN cells, *C. albicans*cellsand extract. In the positive control assay unit, instead offruit extract, 100 µl of serum derived from the samehumanvolunteerwastaken.

Theassaytubeswereincubatedfor30minat37^{0}C. At the end of the incubation period, the tubeswerecentrifugedandsmallamountsoftheresidues were taken on separate microscopy slides. Smears wereprepared,airdriedandstainedwithGiemsa’sstain.N eutrophils were examined for the number of ingestedandassociated*C.albicans*witheachcell.[20,21]A verage

numberof*C.albicans*associatedPMNcellwasdeter mined foreachassayunitasshown inTable3.

Table3: Effectof*Cassia auriculata*extractsonphagocytosis of *candida albicans*by Human PMN *invitro*

Sr. No.	Concentration (µg)	CAMF	CAMR
1	2500	4.5±0.28	4.00±0.0
2	1250	4.2±0.25	3.75±0.25
3	625	2.5±0.28*	3.5±0.28
4	250	2.2±0.25**	3.5±0.28
5	125	2.75±0.25	2.75±0.25

Valuesareexpressedas Mean±SEM,n=4
* p<0.05 **p<0.01Control3.75±0.25
(CAMF*)Cassiaauriculata*methanolicflowersextract(CAMR)*Cassiaauriculata*methanolicrootsextract

Candidacidalassay: SameprocedureanddilutionswerefollowedasmentionedinsectionB-II(Phagocytosis) and the pellets of the assay tubes wereagain suspended in 100 µl MEM and further incubatedat 37^{0}C for 30

minutes. At the end of the incubationperiod, 0.25 mlsodium deoxycholate (2.5% in PBS)was added to each tube to lyse the leucocytes.Afterthis, 0.25 ml 0.01% methylene blue was added to eachtubeandmixedwell.Thetubeswerecentrifuged,supernatant was decanted and smears were prepared onmicroscopicslidesusingresultantpellets.Thepercentage of dead *candida* cells (stained) in each casewasdetermined.

The time schedule was adjusted in such a way thatafter addition of methylene blue to an assay tube, thereadings were taken within next 5 minutes as shown inTable4.[22]

Table 4: Effect of *Cassia auriculata*extracts on incellularkilling of*candidaalbicans*

Sr. No.	Concentration(µg)	CAMF	CAMR
1	2500	35.25±0.47**	33.50±0.28
2	1250	35.25±0.47**	31.25±0.47
3	625	30.25±.25**	31.75±0.25
4	250	28.00±0.40**	30.50±0.28
5	125	20.00±0.40**	21.75±0.62**

Valuesareexpressedas Mean±SEM,n=4
* p<0.05 ** p<0.01Control32.00±0.40
(CAMF*)Cassiaauriculata*methanolicflowersextract(CAMR)*Cassiaauriculata*methanolicrootsextract

Chemotaxis

Boyden'sChamber: Itwassimpleself-constructedapparatus in which the lower chamber is a 5 ml beakerand upper chamber is a tuberculin syringe with filter toitslowerend.Inupperchamberinwhichthecellsuspensionisplaced,thisisseparatedbyamicropore filter (5µm) (Millipore, Cat. No. SMWP-04700) fromthe lower chamber, in which the chemotactic factor wasplacedasshowninFig.1.[23]

The upper compartment of Boyden's chamberwas filled with cell suspension ensuring that the fluidlevel in the upper chamber was same as in the lowerchamber,otherwisethegradientwillbedisturbed(0.2mlsolutionwasusedinuppercompartmentofBoyden's chamber). Allowed the filters to wet from thetopbeforeputtingtheminthelower compartment.

When the upper compartment was placed in thelower compartment, the concentration of chemotacticfactor through the filter was zero and as soon as thefilterwasplacedinthechemotacticsolution,thegradientbeginstoformfromthe bottomoffilter.

1. Filtersweretransferredfrommethanoltodistilled water then,
2. Distilled waterfor1min.
3. HarrisHaematoxylinfor30sec.to1min.
4. Distilled waterfor1min.
5. Tapwaterfor10min.
6. 6.70%ethanol for1min.
7. Theninamixture of:95 ethanol
   80ethanol
   20butanol for5min.
8. FinallyinXylolfor10 min.

And then filter was put it on slide taking care thatlower surface of filter which was in lower compartmentmustbe onupper side ontheslide.

The cover slip was put on slide and examined cellmigrationmicroscopically.Thecountofcellonthelowersurfacewas directly proportional to thenumberof cells placed on the top of the filter at the start of theexperiment.The observations were recorded as showninTable 5.

Table 5: Effect of Cassia *auriculata*extracts onhumanPMN cellschemotaxis.

Sr. No.	Concentration µg	CAMF	CAMR
1	2500	144.3±0.85**	68.00±0.40**
2	1250	130.8±0.47**	159.5±0.64**
3	625	131.3±0.47**	130.8±0.47**
4	250	118.8±0.47**	110.8±0.47**
5	125	110.5±0.28**	78.25±0.47**

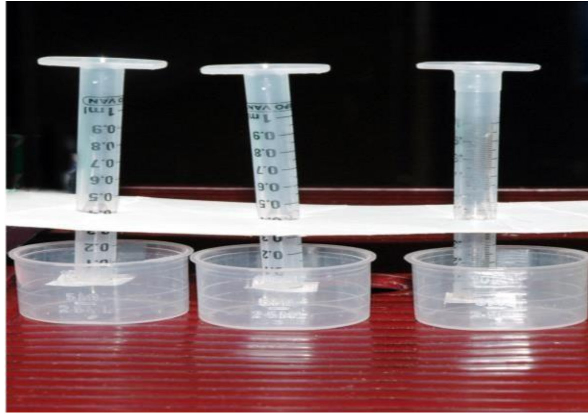

Fig.1:Boyden'sChamber

Procedure

Theuppercompartmentsoftheassayunitscontained PMNcellsuspensioninMEMatconcentration of about 1 X 106 cells/ ml. The lowercompartmentscontained2.5mlPBSconsistingdifferentconcentrationsofthemethanolicflowerextractsas2500µg/ml,1250µg/ml,625µg/ml,250µg/ml, 125µg/ml. In a positive control assay unit,lower compartment contained 2.5 ml PBS and 0.5 mlcasein solution (5 mg/ml). The negative control assayunitcontainedonly3mlofPBSinthelowercompartment.[24]

All the assay units were incubated at 37^0C for 75minutes,and thecells wereallowed tomigrate.

Attheendoftheincubationperiod,thefiltersattached to the tuberculin syringe in each assay unitwere picked off gently with forceps, fixed with 70%methanol and it was stained. After a few minutes in thealcohol, the glue was melt and the filter was becomeloose from the bottom of the syringe barrel. While carewas taken to touch only the rim and not the surface ofthefilter.[25]

Staining: Staining was the important step inchemotaxis.

Stainingprocedure wasasfollows:

Valuesareexpressedas Mean±SEM,n=4
**p<0.01 vsnegativecontrol
Negativecontrol20.75±0.47,positivecontrol136.00 ±0.40
(CAMF)*Cassiaauriculata*methanolicflowersextract(CAMR)*Cassiaauriculata*methanolicrootsextract

ResultandDiscussion

A recently produced anti-inflammatory drug's therapeutic modalities may be better assessed by studying the delayed type hypersensitivity response in rats' ear skin. Flowers and roots were both tested in oxazolone-induced immunological type of inflammation using methanolic extracts. The in-vivo model findings demonstrated that, in comparison to the methanolic root extract, the immunomodulatory effects of the floral extract were more powerful and dosage dependent.

When it comes to diagnosing bacterial infections, the NBT test has seen extensive use. Research on immunodeficiency illnesses also makes use of NBT testing. The NBT test examined in vitro activities and found that endotoxin-treated neutrophils reduced Nitrobluetetrazolium (NBT) dye more effectively. Based on these results, it seems that bacterial endotoxins influence how neutrophils and lymphocytes in the human blood work. The fundamental idea behind the NBT test is to assess the percentage of neutrophils with blue deposits; only activated neutrophils can reduce the NBT dye. A healthy human volunteer's PMN cells were used in the NBT test. Both neutrophils and monocytes have shown a high rate of spontaneous NBT decrease. The number of NBT positive cells is significantly increased when E. coli endotoxin is used to stimulate monocytes and neutrophils. There was a correlation between the concentration of extracts utilized and the rise in NBT reduction by neutrophils following incubation. In the NBT test, methanolic flower extract was more active than methanolic root extract at all doses. Concentration determined the degree to which NBT was reduced. The methanolic floral extract significantly reduced the NBT compound at greater concentrations, much as the positive control. These results show that the methanolic floral extract increases neutrophil activation and, by extension, the decrease of NBT compound.

Human PMN cell phagocytosis assays show that PMN cell spans engulf Candida albicans after recognizing it as an alien particle. In this experiment, we counted the number of cells that had C. albicans attached to them after they were consumed. The PMN cell phagocytosis activity was enhanced by both extracts, although the methanolic flower extract was more effective than the

methanolic root extract.

Serum served as the gold standard in the candidacidal test. Using Candida albicans, researchers investigated how macrophages eliminate microbes that have been consumed. The findings show that the production of oxygen metabolites by the phagocytic cell is crucial for the effective candidacidal action of macrophages.

The efficacy of the methanolic Cassia auriculata flower extract as a candidacid was concentration dependant and statistically significant across all concentration levels. When compared to the positive control, the methanolic extract performed better at greater concentrations. While the methanolic flower extract was more effective against candida, the methanolic root extract was similarly effective.

Neutrophils and macrophages engage in chemotaxis when they migrate to the area around an antigenic agent and toward a chemotactic material. To prime and stimulate leukocytes, bacterial endotoxin is an effective agonist. There was a dose- and time-dependent response from neutrophils in suspension when exposed to the endotoxins (lipopolysaccharides, LPS). The neutrophils were activated with various endotoxins in varying amounts. The chemotaxis activity was maximum in the methanolic floral extract, while it was lowest in the methanolic root extract. In contrast to the 136.0 and 20.0 obtained by the positive and negative controls, respectively, the methanolic roots extract at a 5µg dosage had a maximal activity of 159.5. Nevertheless, at a greater dosage of 10µg, the activity was noticeably diminished. The activity of the methanolic floral extract increased cell migration in a concentration-dependent manner.

Conclusion

With a long history of traditional usage, Cassia auriculata Linn.has shown promise as a folk medicinal herb. The plant has recently been rationalized by screening it via several pharmacological activities. Research findings indicated that the herb has potential pharmacological effects. The plant is increasingly gaining relevance in the pharmaceutical field because to the large number of active phytoconstituentsthat have been extracted from it. This study compared the immunomodulatory activity of methanolic flower extract with that of methanolic root extract using a battery of in-vivo and in-vitro models, and the results showed that the former had much stronger effects. This means that flowers have a lot of room to grow in the realm of future therapeutic research, thanks to their many diverse applications.

References

1. 1. Abuthan MO, Plants have anti-inflammatory properties, including several Cassia species. The field of fitness therapy. 1990; 4: 11.
2. 2. The Traditional Medicine of the Chittor District in Andhra Pradesh by Vedavathi, Mrudula, and Sudhakar. University of Tirupati, Herbal Folklore Research Centre, 1977;48–49.
3. 3. In Nadkarni KM, PapularPrakashan, Indian MateriaMedica, Volume 1, 1999, pages 284–285, pp.
4.
5. 4. Kirtikar KR, and Basu BD, Indian Medicinal Plants. Indian Book Distributor, Dehradun, India, 1935; 854-872. Second edition, Vol., reprint 1999.
6. 5. Reprinted by the Publication of Information Directorate, New Delhi, 2003;327-373. The Wealth of India, raw materials, updated version, Vol III, Council of Scientific and Industrial Research, New Delhi.
7. 6. The Impact of Cassia auriculata Linn, by Sabu MC and Sabburaju T. Influence on blood glucose levels, glucose uptake by the hemidiaphragm of isolated rats. Anthropolgy and Ethnopharmacology, 2002, 80(2), 203-204.
8. 7. The effects of Cassia auriculata L. flowers on preventing brain lipid peroxidation in rats treated with streptozotocin, by Latha M. and Pari L. Molecular and Cellular Biochemistry, 2003, vol. 243, no. 1, pp. 23–28.
9. 8. The impact of Cassia auriculata flowers on blood sugar, serum, and tissue lipids in rats with streptozotocin-induced diabetes, by Latha M. and Pari L. Singapore Medical Journal, 2002, 43(12), 617-621.
10. "Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India," by PerumalSamy and Ignacimuthu, 9. Citation: Journal of Ethnopharmacology, 2000, 69, 63–71.
11. The following publications were published by the National Science Council of Sri Lanka: 10. Screening of Indian plants for biological activity: I. Indian Journal of Experimental Biology.1968;6(4),232-247; 11. Jayaweera DMA. Medicinal plants utilized in Ceylon. Part I-IV. August 1982 in Colombo.
12. 12. Indian traditional medicines, by Chopra RN, New York: CBS Publications, 1999, 449.
13. Glossary of Indian Medicinal Plants with Active Principles, Part-I, New Delhi: National Institute of Science Communication and Information Resources, 1965–1981, pp. 177–178. 13. Asolkar LV, Kakkar KK, Chakre OJ.
14. 14. Cassia auriculata Constituents by Rao GN, Kumar PM, and Dhandapani VS. "Fitoterapia" (Medium 2000, 71(1), 82–83).
15. 15. 149–166 in Khandelwal KR, Practical Pharmacognosy, 13th edition, NiraliPrakashan, Pune, 2005. 12.
16. 16. The effects of FK506 (tacrolimus hydrate) on rats with chronic oxazolone-induced dermatitis were studied by Fujii Y, Takeuchi H, Tanaka K, Sakuma S, Ohkubo Y, and Mutoh S. 16. "European Journal of Pharmacology" (2002, vol. 456, no. 1, pp. 115-121).
17. In chronic granulomatous illness, the quantitative nitrobluetetrazolium test was developed by Baehner RL and Nathan DG. Journal of the New England Journal of Medicine, 1968;278(18),971-976.
18. 18. Endotoxins of Enteric Pathogens Can Affect Human Lymphocytes and Neutrophils, by Islam LN and Nurun-Nabi AH. The citation is from the Journal of Biochemistry and Molecular Biology, volume 36, issue 6, pages 565–571.
19. The immunomodulatory properties of Clausenaexcavata were studied by Manosroi, Saraphanchotiwitthaya, and Manosroi (1999).
20. Burm. of. wood extracts. The article is published in the Journal of Ethnopharmacology and can be found on page twenty-five.Lau, BH, Wan CP, and Park, CS A simple and quick microfluorometric phagocytosis

test. Publication date: 1993; volume 162, issue 1, pages 1–7. 21. Journal of Immunological Methods.Researchers Yamamura, Boler, and Valdimarsson When Candida albicans is unable to take up uridine, this is called phagocytosis. Article published in 1977 in the Journal of Immunological Methods, volume 14, issue 1, pages 19–24. 22.Resistance to systemic candidiasis and leukocyte candidacidal activity in cancer patients, by Lehrer RI and Cline MJ. Cancer, 1971, 27(5), 1211–1217, 23.Immunopotentiating and immunoprophylactic actions of lmmue 21, a polyherbal product, by Pallabi DE, Dasgupta SC, and Gomes A. Native American The Journal of Pharmaceuticals, 1998, 30(3), 163. 24.Boyden chamber test, Chen HC. Research on Cell Migration: Advances in Theory and Practice. 2005;15-22. 25.Kleinman HK, Albini A, Iwamoto Y, Aaronson SA, Kozlowski JM, Martin GR, McEwan RN, A quick in vitro method for measuring tumor cell invasiveness ability. Publication: 1987, in the journal Cancer Research, volume 47, issue 12, pages 3239–3245.