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	Home	About	Current	Archives	Submissions	
		Instr	uctions to A	uthors Cont	act Us	
					Search	

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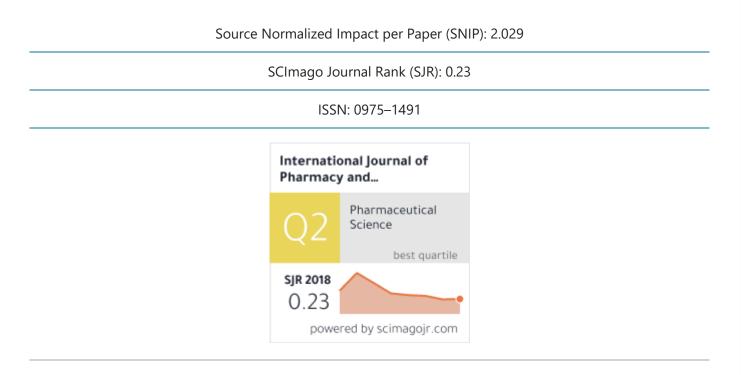


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1

# INTERNATIONAL JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

# Vol 6 Suppl 2, 2014

Review Articles	
APPLICATIONS OF IMPLANTABLE MEDICAL SENSORS FOR HEART FALIURE: A REVIEW	1-5
MURUGESH SHIVASHANKAR, VENKATA RAMANATHAN VINODINI, PUJA MISHRA, KALIAPPAN UMA	
IS MANGANESE INDUCED NEUROTOXICITY A POTENTIAL MODEL FOR PARKINSON'S DISEASE? AN OVERVIEW ON ITS COMPLICATIONS	6-15
MAHALAKSHMI A.M, RAMESH B. NIDAVANI, B. SURESH	
SIGNIFICANCE OF BENZOTHIAZOLE MOIETY IN THE FIELD OF CANCER	16-22
JITENDRA JENA	
2, 5-DIHYDROXY-3-UNDECYL-1, 4-BENZOQUINONE (EMBELIN)-A SECOND SOLID GOLD OF INDIA- A REVIEW	23-30
N. RADHAKRISHNAN, A. GNANAMANI.	
MICROPARTICULATE DRUG CARRIERS: A PROMISING APPROACH FOR THE DELIVERY OF ANTI HIV DRUGS	31-39
SELLAPPAN VELMURUGAN, MOHAMED ASHRAF ALI, PRAVEEN KUMAR	
HERBAL MEDICINES USED IN THE TRADITIONAL INDIAN MEDICINAL SYSTEM AS A THERAPEUTIC TREATMENT OPTION FOR OVERWEIGHT AND	40-47
OBESITY MANAGEMENT: A REVIEW	40-47
ROHIT KUMAR VERMA, THOMAS PARAIDATHATHU	
ENHYDRA FLUCTUANS: A REVIEW ON ITS PHARMACOLOGICAL IMPORTANCE AS A MEDICINAL PLANT AND PREVALENCE AND USE IN NORTH-EAST INDIA.	48-50
UPASANA SARMA, VEDANT V. BORAH, KANDARPA KR. SAIKIA, N. K. HAZARIKA	
RECENT ADVANCES IN BRAIN TARGETED DRUG DELIVERY SYSTEMS: A REVIEW	51-57
AASAVARI H. GUPTE, HARSHA T. KATHPALIA	
HEXOSOMES AS A NOVEL DRUG DELIVERY SYSTEM: A REVIEW	58-63
ARTICLE WAS REMOVED DUE TO PLAGIARISM AND COPYRIGHT VIOLENCE	
ALOE VERA IN ORAL DISEASES - A REVIEW	64 -66
B. DHEEPIKA, DR.T.N.UMA MAHESWARI	
<u>UNDERSTANDING OUR NATURAL NAIL – ANTIFUNGAL AGENTS</u>	67-73
FLOWERLET MATHEW, BINDUMOL K C, JIMSHA PAUL, ROCILIN P PATHADAN AND VINCY VARGHESE	
DIAGNOSTICS AND THERAPEUTIC APPLICATION OF GOLD NANOPARTICLES	74-87
"ARTICLE HAS BEEN REMOVED FROM PUBLICATION DUE TO PLAGIARISM AND COPYRIGHT VIOLENCE"	
BONE GRAFTS AND BONE SUBSTITUTES	88-91
TERESA MAO, KAMAKSHI V	
DEVELOPMENTS AND EMERGING ISSUES IN PUBLIC AND PRIVATE HEALTH CARE SYSTEMS OF KERALA	92-98
LEKSHMI S , G.P.MOHANTA, K.G.REVIKUMAR, P.K.MANNA	
TRENDS OF CLICK SYNTHESIS: A REVIEW	99-103
PRABODH SAPKALE, MEGHA SAHU, MAYUR CHAUDHARI DR. P. R. PATIL	
Reserch Articles	
A VALIDATED STABILITY-INDICATING RP-HPLC ASSAY METHOD FOR BOLDENONE UNDECYLENATE AND ITS RELATED SUBSTANCES	104-109
V. VENKATESWARLU AND K. HUSSAIN REDDY	
HEPATOPROTECTIVE AND ANTIPYRETIC EFFECT OF BARK OF NYCTANTHES ARBORTRISTIS LINN.	110-114
L. SHYAMALI SINGHA, MEENAKSHI BAWARI, MANABENDRA DUTTA CHOUDHURY	
ANALGESIC AND ANTHELMINTIC ACTIVITY OF VARIOUS EXTRACTS OF ANDROGRAPHIS PANACULATANEES. STEM	115-118
SATYAJIT DUTTA	
FORMULATION AND EVALUATION OF SR MATRIX TABLETS OF GLIPIZIDE USING ION EXCHANGE RESIN	119-125
P PRASHANT, J SAURABH, J POOJA	
ANTIOXIDANT ACTIVITY OF SELECTED PLANTS	126-128
KRISHNAVENI.M	
ANTI DIABETIC ACTIVITY OF POLY HERBAL FORMULATIONS	129-130
T. VISWANATH, A. SUVARCHALA KIRANMAI , K. HEMAMALINI, M. VIJUSHA, G. GIRISHA	

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# Vol 6 Suppl 2

1/0		
	SOLATION AND CHARACTERIZATION OF OLIGOSACCHARIDES COMPOSITION IN ORGANICALLY GROWN RED PITAYA, WHITE PITAYA AND PAPAYA	131-136
	X. MOHD ADZIM KHALILI, A. B. CHE ABDULLAH AND A. ABDUL MANAF ABLETS CONTAINING MICROSPHERES OF EUDRAGITE, POLY(3-HYDROXYBUTYRATE) AND SIMVASTATIN WITH IMPROVED DRUG DISSOLUTION	107 1 11
	<u>IATE</u>	137-141
	BIANCA R. PEZZINI, PAOLA A. A. BORBA, IZABEL C. CELESKI, MELISSA ZÉTOLA, MARCOS A. SEGATTO SILVA, GIOVANA C. BAZZO <u>YNTHESIS AND ANTI-MICROBIAL ACTIVITY OF SOME SUBSTITUTED BIS[2-((E)-2-(4-BENZYLIDENEAMINO)THIAZOL-4-YL)-4-METHYLPHENOL]</u> IETAL COMPLEXES	142-146
	JAY M. GHATOLE, KUSHAL R. LANJEWAR, MAHESH K. GAIDHANE	
	<u>'OTAL GLUCOSE AND CRUDE FIBER IN LOCAL RED SWEET POTATO [IPOMOEA BATATAS L. (LAM)] TUBER</u> AMDAN PANIGORO, DIAH DHIANAWATY	147-149
	URATIVE EFFECT OF WOODFORDIA FRUTICOSA KURZ FLOWERS ON N-NITROSODIETHYLAMINE INDUCED HEPATOCELLULAR CARCINOMA IN	150 155
	<u>IATS</u>	150-155
	I. NITHA, S. P PRABHA, P. N ANSIL, M. S LATHA MANAGEMENT OF TYPE 2 DIABETES MELLITUS: ASSESSMENT OF THE COMMUNITY PHARMACISTS' CONTRIBUTION IN SELECTED DISTRICTS OF	
	AMIL NADU STATE, INDIA	156-158
	AJA D, P.R. ANAND VIJAYAKUMAR, SATHYA SANTHY D, KRISHNA KUMAR M. S, JAYAKUMAR C, DR. P. VIJAYAN	
	HARMACOGNOSTIC EVALUATION OF CURCUMA NEILGHERRENSIS WT.	159-168
	I. YASODAMMA, D. CHAITHRA, C. ALEKHYA	
	IEW VALIDATED ISOCRATIC RP-HPLC METHOD FOR ASSAY OF FENOFIBRATE	169-172
	NILIP KUMAR SAHOO, PRAFULLA KUMAR SAHU,CHANDRA SEKHAR PATRO YSOSTAPHIN AS AN ALTERNATE THERAPY IN METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) INDUCED ENDOPHTHALMITIS: AN	
	XPERIMENTAL STUDY	173-175
	I.ASKARI, SHAMIM AHMAD, ABHISHEK, ABDUL WARIS, MOUSUMI MALAKAR	
	SURVEY OF UTILIZATION OF MEDICATIONS WITHOUT PRESCRIPTION AMONG IN DIFFERENT AGE GROUPS	176-178
	. RAGESH DRYOPTERIS COCHLEATA RHIZOME: A NUTRITIONAL SOURCE OF ESSENTIAL ELEMENTS, PHYTOCHEMICALS, ANTIOXIDANTS AND	
	INTIMICROBIALS.	179-188
	A. KATHIRVEL, A. K. RAI, G. S. MAURYA, V. SUJATHA	
	INTIMICROBIAL, ANTIOXIDANT AND CYTOTOXIC PROPERTIES OF STREPTOMYCESSP. (ERINLG-01) ISOLATED FROM SOUTHERN WESTERN GHATS	189-196
	. BALACHANDRAN, V. DURAIPANDIYAN, M. VALAN ARASU, S. IGNACIMUTHU PHYTOCHEMICAL SCREENING AND EVALUATION OF ( <i>INVITRO</i> ) ANTIOXIDANT ACTIVITY OF <i>ACHYRANTHES ASPERA LINN</i> ROOT EXTRACT	197-199
	1. ANAND, V. SELVARAJ, M. ALAGAR	
	FFECT OF NANO MOLAR CONCENTRATION OF METHYL PARATHION ON GOAT TESTIS	200-202
	HARMA R. K., GOYAL A. K., THAREJA K. AND BHAT R. A.	
	NSILICO DRUG DESIGN AND MOLECULAR DOCKING STUDIES OF SOME NOVEL BENZOTHIAZOLE DERIVATIVES AS ANTI-CANCER AND ANTI- NFLAMMATORY AGENTS	203-208
	DEEPTHY CHANDRAN, LEENA. K. PAPPACHEN, MANJU PRATHAP, JINSHA.M.J, JILSHA.G.	
	HYSICO-CHEMICAL SCREENING OF ALGERIAN LINSEED OIL AND CHARACTERIZATION OF THEIR FREE ACIDS METHYL ESTERS (FAMES)	209-215
	EENMEHDI HOUCINE, AMROUCHE ABDELILLAH, MEZIANE ABDELKADER, ZAABOUB IMENE, CHABANE SARI MERIEM, DAOUDI CHABANE SARI	
	<u>'REPARATION AND EVALUATION OF FLUOXEITINE HYDROCHLORIDE ORAL DISPERSIBLE TABLETS</u> DIVYA, V.RAVICHANDIRAN, V. LAVAKUMAR, C. SOWMYA, N.VENKATESHAN, M.NIRANJAN BABU	216-222
	DEPH FREE RADICAL SCAVENGING ACTIVITY OF TOMATO, CHERRY TOMATO AND WATERMELON: LYCOPENE EXTRACTION, PURIFICATION AND	
	UANTIFICATION	223-228
	'EHNIAT SHAHZAD, IJAZ AHMAD, SHAHNAZ CHOUDHRY, MUHAMMAD K SAEED, MUHAMMAD N KHAN Method development and validation of stadility indicating drupped for similitaneous estimation of dupata dine fumadate	
	IETHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC FOR SIMULTANEOUS ESTIMATION OF RUPATADINE FUMARATE IND MONTELUKAST SODIUM IN COMBINED TABLET DOSAGE FORM	229-233
	ASHKA JANI, JALDIP JASOLIYA, DIPESH VANSJALIA	
	STUDY TO EVALUATE THE EFFECT OF SPARFLOXACIN ON PENTOBARBITONE INDUCED SLEEP IN MICE	234-235
	IKSHYA ALVA, H N GOPALA KRISHNA, RAMYA KATEEL, CHARISHMA P R, MOHANDAS RAI, HARSHA S NAIK INTHELMINTIC ACTIVITY OF TRACHYSPERMUMAMMI(L)EXTRACT	236-238
	ISHWARYA K. APTE, V. S. KHOT, N. S. BIRADAR, S. B. PATIL	230-230
	TUDIES ON FORMULATION AND EVALUATION OF OSMOTICALLY CONTROLLED DRUG DELIVERY SYSTEM OF CARBAMAZEPINE	239-250
	ANCHAXARI MALLAPPA DANDAGI, CHIRAG PRAKASHBHAI PATEL, ROHIT SHARMA, ANAND PANCHAKSHARI GADAD, VINAYAK MASTIHOLIMATH	
	PREPARATION AND <i>IN VIVO</i> EVALUATION OF POORLY SOLUBLE DEFERASIROX DISPERSIBLE TABLETS BY HYDROXY PROPYL BETA CYCLODEXTRIN COMPLEXATION	251-256
	ANCHAXARI MALLAPPA DANDAGI, SURYASRI LAVANYA ADAVI, SEEPRARANI RATH, ANAND PANCHAKSHARI GADAD	
	REPARATION AND COMPARATIVE EVALUATIONOF LIQUISOLID COMPACT AND SOLID DISPERSION OF CANDESARTAN CILEXETIL	257-266
	HMED A. ABDUL ABBAS, ALAA A. ABDUL RASOOL, NAWAL A. RAJAB	
	FFECT OF CALCIUM, ALFACALCIDOL AND HEMODIALYSIS ON SECONDARY HYPERPARATHYROIDISM	267-272
	I. SHOPIT, ADNAN AL -ADHAL, ABDUL-KARIM SHEIBAN, M. AMOOD AL-KAMARANY IEPATOPROTECTIVE ACTIVITY OF KIRGANELIA RETICULATA POIR. (BAILL) ROOT AGAINST PARACETAMOL INDUCED HEPATO-TOXICITY IN	
	VISTAR RATS	273-278
	AJESH KUMAR SONI, RAGHUVEER IRCHHAIYA, VIHANGESH DIXIT, ZAHID AHMAD BHAT, HILAL AHMAD WANI, ASHIQ HUSSAIN NAJAR	
	ILLERGIC REACTION OF P-PHENYLENEDIAMINE ON SKIN INISHA BRIGIT SHAJAN	279-280

Vol 6 Suppl 2

SINGLE NUCLEOTIDE POLYMORPHISM (SNPS) ANALYSIS OF MU-OPIOID RECEPTORS (OPRM1) USING DENATURING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (DHPLC) AMONG THE INTRAVENOUS DRUG USERS	281-287
TEH LK, BANNUR Z, ZAKARIA ZA, FAZLEEN HMH, SALLEH MZ,MOHD ZAKI SALLEH	
METABOLOMICS AND PHARMACOGENETICS BASED 5-FLUOROURACIL MONITORING IN COLORECTAL CANCER PATIENTS BANNUR Z, SALLEH MZ, HASHIM H, HENNESSY T, AZMI MN, ZAILANI MH, RAMASAMY P, ZAKARIA ZA, S SUNEET, H NGOW, G HENRY, TEH LK	288-295
PHYSICAL CHARACTERISTIC AND VIABILITY OF <i>LACTOBACILLUS ACIDOPHILLUS</i> MICROPARTICLE USING HPMC K100LV AND HPMC K4M AS MATRICES	296-298
 SUGIYARTONO,, DIKE BAGUS PAMUJI, AGIL ANTONO,,IDHA KUSUMAWATI , ISNAENI	
PRELIMINARY PHYTOCHEMICAL SCREENING AND INVITROANGIOTENSION ACTIVITY OF BIOACTIVE COMPOUND - STEROID ISOLATED FROM SARGASSUM ILICIFOLIUM	299-301
S. FAROOK BASHAC. MUTHUKUMAR	
DEVELOPMENT AND VALIDATION OF A GC/FID METHOD FOR IDENTIFICATION AND QUANTIFICATION OF MAIN COMPONENTS OF SATUREJA MONTANAL. ESSENTIAL OIL	302-306
ENTELA HALOCI,VILMA TOSKA, SILVIA VERTUANI, AGRON METO, ENKELEJDA GOCI, ENVER MUSTAFAJ, STEFANO MANFREDINI	
METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS QUANTITATIVE ESTIMATION OF CALCIUM DOBESILATE AND TROXERUTIN IN TABLETS BY REVERSE PHASE HPLC	307-311
N.J.R. HEPSEBAH, P. PADMA, A.ASHOK KUMARS	
DEVELOPMENT AND VALIDATION OF RP-HPLC AND HPTLC METHOD OF ANALYSIS FOR SIMULTANEOUS ESTIMATION OF AMBROXOL HCL, DEXTROMETHORPHAN HBR AND GUAIFENESIN IN PHARMACEUTICAL COUGH COLD PREPARATION AND STATISTICAL COMPARISON OF DEVELOPED METHODS	312-316
KRUNAL SAGATHIYA, HINA BAGADA	
ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METRONIDAZOLE AND AMOXICILLIN IN SYNTHETIC MIXTURE BY UV- VISIBLE SPECTROSCOPY	317-319
PARTH PATEL, PRIYA VARSHNEY, MINAL ROHIT	
AN EPIDEMIOLOGIACAL SURVEY ON PREVALENCE OF OBESITY AND DISEASES BURDEN IN COMMON PUBLIC G.LAKSHMI DURGA, CH.JHANSI, V.RAGHU RAM, RAMA RAO NADENDLA	320-322
ANTIOXIDANTAND ANTICANDIDAL ACTIVITY STUDIES ON PHYLLANTHIN COMPOUND FROM PHYLLANTHUS NIRURI	323-326
ANUSHA JR , REMYA RV, SASI PREMILA JM, ALBIN T FLEMING	
DETERMINATION OF BIOACTIVE COMPONENTS OF THE LEAVES OF COCCULUSHIRSUTUS(L.) DIELS USING GC-MS ANALYSIS	327-329
MUKESH KUMAR MEENA, NEELAM SINGH AND VIDYA PATNI MICROBIOLOGICAL ANALYSIS OF TOPICALS AVAILABLE IN BANGLADESH	330-332
JWEL RANA, TOHURA SULTANA, KAMAL KANTA DAS, RASHED NOOR	
CYTOTOXIC AND APOPTOTIC NATURE OF MIGRASTATIN, A SECONDARY METABOLITE FROM STREPTOMYCES EVALUATED ON HEPG2 CELL LINE	333-338
VINAYAGAM RAMBABU, S. SUBA, P.MANIKANDAN, SUBURAMANIYAN VIJAYAKUMAR	
EVALUATION OF ANTIULCER AND IN-VITRO ANTIOXIDANT ACTIVITIES OF IXORACOCCINEAFLOWERS AND POLYHERBAL EXTRACT IN WISTAR ALBINO RATS	339-344
PATIBANDLA NARESH BABU, NAGARAJU B, VINAY KUMARI	
TOXICITY PROFILE OF CINNAMON OIL BASED DRUG DELIVERY SYSTEM IN OREOCHROMISMOSSAMBICUS (TILAPIA)	345-350
M. JOYCE NIRMALA, JOHN THOMAS, ANDREW EBENAZER, SRIVATSAVA VISWANADHA, AMITAVA MUKHERJEE, N. CHANDRASEKARAN	
SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SOME NOVEL SUBSTITUTED L-ARGININE ANALOGUES S. K. ARIFA BEGUM, M. MADHURI, K. BHARATHI, KVSRG. PRASAD, A. RAJANI, K.HEMAMALINI, K. RUPA	351-354
5. N. ARIFA DEGUM, M. MADHURI, N. BHARATHI, NVSRG. PRASAD, A. KAJANI, N. HEMAMALINI, N. RUFA ANTI-PYRETIC ACTIVITY OF SOME SYNTHESIZED NOVEL L-ARGININE ANALOGUES (PEPTIDES)	355-356
SK. ARIFA BEGUM, M. MADHURI, A. RAJANI, K. HEMAMALINI	333-330
ANTIOXIDANT ACTIVITY AND QUANTIFICATION OF PHENOLIC COMPOUNDS OF EUPHORBIA ECHINUS	357-360
FATIMA AZZAHRA LAHLOU, FOUZIA HMIMID, MOHAMMED LOUTFI, NOUREDDINE BOURHIM	
INHIBITION OF CALCIUM OXALATE (CAOX) CRYSTALLIZATION IN VITRO BY THE EXTRACT OF BEET ROOT (BETA VULGAIS L.)	361-365
R. SARANYA, N. GEETHA	
FABRICATION OF MACHINERY FOR CONTINUOUS FORMATION OF THIN SHEET OF WOUND DRESSING MATERIAL VIJAYAN SUMATHI, THOTAPALLI PARVATHALESWARA SASTRY, RETHINAM SENTHIL,CHANDRABABU SHANTHI	366-368
FORMULATION DEVELOPMENT, STANDARDIZATION AND ANTIMICROBIAL ACTIVITY OF AGERATUMCONYZOIDES EXTRACTS AND THEIR	369-374
FORMULATION	309-374
RAJMANI PRAJAPATI, SUNITA ROY, SUDEEP MISHRA, S.K. RAZA AND L.K. THAKUR FORMULATION AND EVALUATION OF FLURBIPROFEN SOLID DISPERSION	375-384
AHMED LAITH DR. SHAIMAA N. ABD AL HAMMIDALAA A. ABD ALRASOOL	373-304
PURIFICATION OF STREPTOCOCCUS PNEUMONIAE CAPSULAR POLYSACCHARIDES USING ALUMINIUM PHOSPHATE AND ETHANOL	385-387
CHANDRASHEKAR MACHAARAVINDALAVANYARAMASWAMY NANNA	
EFFECTS OF CENTELLAASIATICA L., CURCUMA LONGA L., AND STROBILANTHESCRISPUS L. EXTRACTS ON 3 KIDNEY CELL LINES: IN VITRO CYTOTOXICITY ANALYSIS	388-392
H. HANISA, M.L. MOHDAZMI, M. SUHAILA, M.N.HAKIM	
EVALUATION OF DRUG CANDIDATURE OF SOME QUINAZOLINE- 4-(3H)-ONES AS INHIBITOR OF HUMAN DIHYDROFOLATE REDUCTASE ENZYME:	
	393-400
MOLECULAR DOCKING AND IN SILICOSTUDIES	393-400
<u>MOLECULAR DOCKING AND IN SILICOSTUDIES</u> BIPRANSH KUMAR TIWARY,RAVI KANT PATHAK, KIRAN PRADHAN, ASHIS KUMAR NANDA, ASIM KUMAR BOTHRA, RANADHIR CHAKRABORTY	
MOLECULAR DOCKING AND IN SILICOSTUDIES	393-400 401-405
<u>MOLECULAR DOCKING AND IN SILICOSTUDIES</u> BIPRANSH KUMAR TIWARY,RAVI KANT PATHAK, KIRAN PRADHAN, ASHIS KUMAR NANDA, ASIM KUMAR BOTHRA, RANADHIR CHAKRABORTY <u>FORMULATION AND EVALUATION OFRANITIDINE HYDROCHLORIDEAS FLOATING IN SITU GEL</u>	

Vol 6 Suppl 2	
ARINTORN RUKSIRIWANICH, JAKKAPAN SIRITHUNYALUG, KORAWINWICH BOONPISUTTINANT, PENSAK JANTRAWUT	
	413-4
SHIKA JAISHEE, USHA CHAKRABORTY EVELOPMENT AND VALIDATION OF LC-MS/MS METHOD FOR THE SIMULTANEOUS QUANTITATIVE ANALYSIS OF OXCARBAZEPINE AND ITS	
ETABOLITE 10-HYDROXYCARBAZEPINE IN K2EDTA PLASMA	422-4
ASHIF UL HAQ, NITESH KUMAR	
EVELOPMENT AND VALIDATION OF A ULTRA PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ASSAY OF CETIRIZINE HYDROCHLORIDE	430-4
RAVI KUMAR REDDY DR. V. KRISHNA REDDY, E. SASIKIRAN GOUD Y. RAMACHANDRA REDDY	
ORMULATION AND EVALUATION OF EXTENDED RELEASE METFORMIN HYDROCHLORIDE BEADS	433-4
OHAMED M. NAFADY, KHALEID M ATTALLA, MOHAMED A SAYED	
EVELOPMENT AND VALIDATION OF A ULTRA PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ASSAY OF MEBEVERINE YDROCHLORIDE	442-
RAVI KUMAR REDDY, DR. V. KRISHNA REDDY, E. SASIKIRAN GOUD, Y. RAMACHANDRA REDDY	
ABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF EPROSARTAN MESYLATE	446-
<u>ND HYDROCHLOROTHIAZIDE IN BULK AND TABLET DOSAGE FORM</u> IARANYA GUMULAPURAM, SRIDHAR THOTA, VENISETTY RAJ KUMAR, VIJAY KUMAR NAGABANDI	
MPARISON OFSUPPRESSIVE ACTIVITY OF THE CENTRAL NERVOUS SYSTEM	
ROM THE NEW DERIVATIVES N-BENZOYLPHENYLUREA	452-
AMBANGTRI PURWANTO	
EVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF NEUROLEPTIC DRUG ZOTEPINE IN JLK AND TABLET DOSAGE FORM	456
IRUTHI KOLAGANI, SRIDHAR THOTA, VENISETTY RAJ KUMAR, VENUMADHAV NEERATI	
ENTIFICATION OF MYCOLIC ACIDS OF MYCOBACTERIUMTUBERCULOSISBY GAS CHROMATOGRAPHY-FLAME IONIZATION DETECTOR	460
SRI DARMAWATI, DEBY KUSUMANINGRUM	
	465
ARCELLINO RUDYANTO, JUNI EKOWATI, TRI WIDIANDANI AND TOSHIO HONDA RUG-EXCIPIENTS COMPATIBILITY STUDIES OF NICORANDIL IN CONTROLLED RELEASE FLOATING TABLET	468
BOUL BAQUEE AHMED, LILA KANTA NATH	100
•	476
PETCHIAMMAL, WAHEETA HOPPER	
HE DEVELOPMENT AND VALIDATION OF A CHIRAL HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE IDENTIFICATION AND JANTIFICATION OF (R)-ENANTIOMER IN 7-ETHYL-10-HYDROXYCAMPTOTHECIN (SN-38)	480
RALA VENKATESHWARLU1,2, A. V. RAMA RAO, K. MUKKANTI AND S. V. SUBBA REDDY	
INCLEVING MACHESHIM ONDE NAND PARTICLES IN TWO ERESH WATER FISHES TH APIA (OREOCHROMIS MOSSAMRICUS) AND ZERRA FISH	487
<u>IANIO RERIO)</u>	107
HN THOMAS, S. VIJAYAKUMAR, S. THANIGAIVEL, AMITAVA MUKHERJEE, NATARAJAN CHANDRASEKARAN EPATOPROTECTIVE ACTIVITY OF METHANOL EXTRACT OF STEM BARK OF PROSOPISCINERARIA LINN AGAINST CARBON TETRACHLORIDE	
DUCED HEPATOTOXICITY	491
ELMURUGAN V, ARUNACHALAM G	
COMPARITIVE STUDY OF ANTIOXIDANT ACTIVITY OF BACCOPA MONNIERI (L.) PENNELL USING VARIOUS SOLVENT EXTRACTS AND ITS GC-MS VALYSIS	494
SUBASHRI, Y JUSTIN KOIL PILLAI	
	499
MAL S. M. ABU EL-ENIN	
	506
FEBINA BERNICE SHARON, RACHEL REGI DANIEL AND R. SHENBAGARATHAI	<b>F</b> 1 1
<u>EW QUINAZOLIN-2,4-DIONES FROM (2,4-DIOXO-1,4-DIHYDRO-2H- QUINAZOLIN -3-YLAMINO) ACETIC ACID HYDRAZIDE</u> AMDOUH ADLY HASSAN, AHMED MOHAMED MOSALLEM YOUNES, MOHAMED MOBARK TAHA,SAYED MOUSTAFA ABBOUDYAND ABOU-BAKR	511
AREDI ABDEL-MONSEF	
ORMULATION AND IN VITRO EVALUTION OF DIACEREIN LOADED NIOSOMES	515
ANDA M. ZAKI, ADEL A. ALI, SHAHIRA F. EL MENSHAWE AND AHMED ABDEL BARY	
<u>HARMACOLOGICAL SCREENING AND EVALUATION OF ANTI-PEPTIC ULCER PROPERTY OF LEAVES OF ARTOCARPUSINTEGRIFOLIA</u> EHA SHARMA, RAHUL P.K. MISHRA	522
	525
VITHIYA, RAJENDRAN KUMAR, SHAMPA SEN	0-0
	528
ONJUR AHMED LASKAR, MANABENDRA DUTTA CHOUDHURY, PANKAJ CHETIA	
	532
NNA BALAJI, MEER ISMAIL ALI	
NNA BALAJI, MEER ISMAIL ALI ENTIFICATION OF LEAD COMPOUNDS WITH COBRA VENOM NEUTRALISING ACTIVITY IN THREE INDIAN MEDICINAL PLANTS	536
NNA BALAJI, MEER ISMAIL ALI <u>ENTIFICATION OF LEAD COMPOUNDS WITH COBRA VENOM NEUTRALISING ACTIVITY IN THREE INDIAN MEDICINAL PLANTS</u> C. NISHA, S. SREEKUMAR, C.K. BIJU, P. N. KRISHNAN	
NNA BALAJI, MEER ISMAIL ALI <u>ENTIFICATION OF LEAD COMPOUNDS WITH COBRA VENOM NEUTRALISING ACTIVITY IN THREE INDIAN MEDICINAL PLANTS</u> C. NISHA, S. SREEKUMAR, C.K. BIJU, P. N. KRISHNAN	536 542

1/6/2020 Vol 6 Suppl 2	
VARALAKSHMI DURAIRAJ, GARIMA SHAKYA, RUKKUMANI RAJAGOPALAN	
DEVELOPMENT AND CHARACTERIZATION OF CO-GROUND MIXTURES AND SOLID DISPERSIONS OF ARIPIPRAZOLE WITH HYDROPHILIC CARRIERS MUNEERA BEGAM, D V GOWDA, VISHNU DATTA M, ARAVINDARAM S, SIDDARAMIAH H	552-557
PALLIATIVE EFFECT OF CURCUMIN ON STZ-INDUCED DIABETES IN RATS	558-563
REHABKAMEL, AMEL ABD ALLAH HASHIM, SAHAR ABD EL-MOHSEN ALI	
MEDICATION ADHERENCE TO ANTIDIABETIC THERAPY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS	564-570
MANJUSHA SAJITH, MADHU PANKAJ, ATMATAM PAWAR, AMIT MODI, RONAK SUMARIYA	571-574
BALANCING ANTI-AMYLOID AND ANTI-CHOLINESTERASE CAPACITY IN A SINGLE CHEMICAL ENTITY: IN-SILICODRUG DESIGN PAVADAI PARASURAMAN, RAMALINGAM SURESH, DHANARAJ PREMNATH	5/1-5/4
FORMULATION AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF A HERBAL OINTMENT PREPARED FROM CRUDE EXTRACTS OF AEGLE	
<u>MARMELOS, (BAEL)</u> JAYESH MHATRE, SMITA NAGARAL, SHRADDHA KULKARNI	575-579
A STUDY ON THE ADVERSE EFFECTS OF ANTICANCER DRUGS IN AN ONCOLOGY CENTER OF A TERTIARY CARE HOSPITAL	580-583
KIRTHI C, AZRA AFZAL, MOUNIKA REDDY, SYED AAMIR ALI, APARNA YERRAMILLI, SANJEEV SHARMA	000 000
STABILITY INDICATING CHIRAL HPLC METHOD FOR THE ESTIMATION OF ZALTOPROFEN ENANTIOMERS IN PHARMACEUTICAL FORMULATION	584-587
BYRAN GOWRAMMA, SUBRAMANIA NAINAR MEYYANATHAN, SUBRAMANIAN GOMATHY, BASAWAN BABU, NAGAPPAN KRISHNAVENI, BHOJRAJ	
SURESH	
PREPARATION, EVALUATION AND COMPARISON OF LIPID BASED DRUG DELIVERY SYSTEMS OF TACROLIMUS	588-591
PRANAV PATEL, TEJAL MEHTA#, SHITAL PANCHAL#	
PREPARATION AND EVALUATION OF NYSTATIN LOADED-SOLID-LIPID NANOPARTICLES FOR TOPICAL DELIVERY	592-597
LAITH HAMZA SAMEIN	
COMPARISION OF TOTAL PHENOLIC CONTENT OF SOME SELECTED INDIGENOUS GARCINIA SPECIES FOUND IN ASSAM	598-601
TARALI CHOWDHURY	
ANALGESIC ACTIVITY OF WITHANIACOAGULANS DUNAL FRUIT EXTRACTS IN EXPERIMENTAL ANIMAL MODELS	602-605
MS. ARCHANA K. SHENDKAR, MRS. SUGANDHA G. CHAUDHARI, DR. YOGESH K. SHENDKAR	
DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF QUERCETIN AND RUTIN IN AGANOSMADICHOTOMA [ROTH] K. SCHUM	606-608
GOMATHY SUBRAMANIAN, SUBRAMANIA NAINAR MEYYANATHAN, YAMJALA KARTHIK, ANJANA KARUNAKARANAIR AND DHANABAL S PALANISAMY	
IMPROVED BACTERICIDAL PROPERTY OF SILVER NANOPARTICLES FROM PENICILLIUM PINOPHILUM (MTCC 2192) IN A COMBINED FORM WITH	
CARBICILLIN AND MOXIFLOXACIN	609-612
ANIMA NANDA, SHAHNAZ MAJEED	
A PRELIMINARY ANTIHYPERGLYCEMIC AND ANTINOCICEPTIVE ACTIVITY EVALUATION OF AMORPHOPHALLUS CAMPANULATUS CORMS	613-616
MD MIZANUR RAHAMAN, MOHAMMED MEHDI HASAN, IMRUL HASAN BADAL, AUDITI SWARNA, SHAHNAZ RAHMAN, MOHAMMED RAHMATULLAH	
HEPATOPROTECTIVE ACTIVITY AND SUB ACUTE TOXICITY STUDY OF WHOLE PART OF THE PLANT ANOECTOCHILUSFORMOSANUSHAYATA	617-621
<u>(ORCHIDACEAE)</u> AMARESH PANDA, SEEMANCHALA RATH, DEBASHIS PRADHAN, ARPAN MAHANTY, BIJAN KUMAR GUPTA, NRIPENDRA NATH BALA	
AMARESH PANDA, SEEMANCHALA KAI H, DEDASHIS PRADHAN, ARPAN MAHANT I, DIJAN KOMAK GOPTA, NKIPENDKA NATH DALA ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF METHANOL EXTRACT OF VALLARIS SOLANACEA LEAVES	622-624
JOSHI PUNAM , PRIYA BHANU AND GAHLOT MANOJ	022-024
DEVELOPMENT AND IN-VITRO EVALUATION OF NICOTINE HARD CANDY LOZENGES FOR SMOKING CESSATION	625-629
PRENUKA, SHAYEDA, MADHUSUDAN RAO YAMSANI	025-027
EFFECT OF NATURAL SUNFLOWER OIL AND ITS COMPONENTS ON THE SKIN PERMEABILITY TO WATER AND SOME DRUGS	630-636
HASSAN M. GHONAIM, MASSIMO G. NORO AND JAMSHED ANWAR	000 000
SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL ACTIVITY AND CYTOTOXICITY STUDIES OF 2-((6-METHOXYBENZO[D] THIAZOL-2-YLIMINO)	
METHYL)-6-ETHOXYPHENOLAND ITS METAL COMPLEXES	637-643
MUDAVATH.RAVI, BATHINI USHAIAH, PALLIMONI SUJITHA, KARUNAKAR RAO KUDLE, CH.SARALA DEVI	
FACTOR INFLUENCE STUDY OF IVABRADINE HCL OSMOTIC PUSH PULL TABLETS USING FRACTIONAL FACTORIAL DESIGN	644-651
SONA.P.S, C. MUTHULINGAM, DR. G.GEETHA , DR. R VEKATA NARAYANAN	
DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF SULFAQUINOXALINE SODIUM IN WATER SOLUBLE POWDER FORMULATION	652-657
MASHHOUR GHANEM, SALEH ABU-LAFI, DIYAA MOHAMMAD	
VALIDATION OF IN VITRO ANALYTICAL METHOD TO MEASURE PAPAIN ACTIVITY IN PHARMACEUTICAL FORMULATIONS	658-661
CAROLINE C. FERRAZ, GUSTAVO HC VARCA, MARTA MDC VILA, PATRICIA S LOPES	
SMEDDS FORMULATION: DEMONSTRATION OF ENHANCED BIOAVAILABILITY OF PIOGLITAZONE IN RATS	662-665
HYMA.P, ABBULU.K.SUNIL S JALALPURE	
STATISTICAL DESIGNING OF ENRICHED PECTIN EXTRACT MEDIUM FOR THE ENHANCED PRODUCTION OF PECTINASE BY ASPERGILLUSNIGER NARAYANANMAHESH, RANGARAJANVIVEK, MANI ARUNKUMAR, SRINIVASAN BALAKUMAR	666-672
IN-VITRO AND IN-VIVO RELATIONSHIP AND INFLUENCE OF COVARIATESON PHARMACOKINETICS OF URAPIDIL SUSTAINED RELEASE CAPSULES	673-678
M. SUNDARAMOORTHI NAINAR, RAVISEKHAR KASIBHATTA, D.PRABAKARAN, V. PRAVEEN KUMAR AND ASHISH SAXENA	
HPLC DETERMINATION OF PHENOLICS AND FREE RADICAL SCAVENGING ACTIVITY OF ETHANOLIC EXTRACTS OF TWO POLYPORE MUSHROOMS	679-684
IMTIYAZ AHMAD SHEIKH, DEEPAK VYAS, MOHD ANIS GANAIE, KEERTI DEHARIYA, VINITA SINGH	
IMTIYAZ AHMAD SHEIKH, DEEPAK VYAS, MOHD ANIS GANAIE, KEERTI DEHARIYA, VINITA SINGH <u>OPTIMIZATION AND CHARACTERIZATION OF BIODEGRADABLE POLYMERIC NANOCAPSULES OF A CAMPTOTHECIN DERIVATIVE</u>	685-689
	685-689
OPTIMIZATION AND CHARACTERIZATION OF BIODEGRADABLE POLYMERIC NANOCAPSULES OF A CAMPTOTHECIN DERIVATIVE	

# LOKRAJ SUBEDEE, RN SURESHA, MD. SIBGATULLAH, SIDDAMMA A., BRAHADEESH M

FORMULATION AND EVALUATION OF AN HERBAL CREAM FOR WOUND HEALING ACTIVITY	69	93-697
TRAILOKYA DAS, JIBAN DEBNATH, DR. BIPUL NATH, SUVAKANTA DASH		
RP-LC GRADIENT ELUTION METHOD FOR SIMULTANEOUS DETERMINATION OF RELATED SUBSTANCES OF ZALTOPROFE APPLICATION FOR DRUG EXCIPIENT COMPATIBILITY STUDY	<u>NAND PARACETAMOL AND</u> 69	98-703
PRADNYA A KARBHARI , SNEHA J JOSHI , SUVARNA I BHOIR		
ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF THE TISSUE EXTRACT OF PERNA VIRIDIS LINNAEUS, 1758 (MOLLUS	SCA: BIVALVIA) FROM	
VERSOVA COAST, MUMBAI	70	04-707
MADHU V. N&, P. SIVAPERUMAL, K. KAMALA, AJIT A. AMBEKAR AND B.G. KULKURNI		
MONONUCLEOTIDE PHOSPHATASE FROM GOAT LIVER: A POSSIBLE TARGET FOR DIVALENT HEAVY METAL CATIONS	70	08-714
SWAGATA MALLIK, MONALISA DEY, MOUSUMI DUTTA, ARNAB K. GHOSH, DEBASISH BANDYOPADHYAY EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF ALCOHOLIC EXTRACT OF LEAVES OF <i>LEUCAS ASPERAIN</i> ALBINO RA	ATS 71	15-719
NAVIN PATIL, SOMASHEKAR HS, SUNEEL KUMAR REDDY, VINOD NAYAK, NARENDRANATH S,KL BAIRY,AMRITA PARIDA,CI		13-719
RAHUL PKOTIAN.		
STATISTICAL CORRELATION AND QUANTIFICATION OF GLICLAZIDE BY SPECTROPHOTOMETRIC METHOD	72	20-722
LOPAMUDRA. ADHIKARI, P. N. MURTHY, 2UMA.SHANKAR MISHRA		
EFFECT OF NICORANDIL ON BASAL GLUCOSE LEVELS AND AFTER GLUCOSE CHALLENGE IN NORMAL EUGLYCEMIC ALBI		23-725
SURESHA.R.N, MOHAMMED SIBGATULLAH, JAYANTHI.M.K, KALABHARATHI.H.L, SATISH A.M, PUSHPA V.H, PRATHIMA C		. 730
PROTEIN BINDING INTERACTION STUDY OF OLMESARTAN MEDOXOMIL AND ITS METABOLITE OLMESARTAN BY FLUORI RITESH N. SHARMA, SHYAM S. PANCHOLI	<u>SCENCE SPECTROSCOPY</u> 72	26-729
FIRST ORDER DERIVATIVE AND DUAL WAVELENGTH SPECTROPHOTOMETRY METHODS DEVELOPMENT AND VALIDATION	IN FOR SIMILITANFOLIS	
ESTIMATION OF ALOGLIPTIN AND PIOGLITAZONE IN BULK AND DOSAGE FORM	73	30-738
RAVAL KASHYAP, U.SRINIVASA		
ANTI-OBESITY AND HYPOGLYCEMIC EFFECT OF ETHANOLIC EXTRACT OF CROTALARIA JUNCEA IN HIGH FAT DIET INDUC	ED HYPERLIPIDEMIC AND 73	39-742
HYPERGLYCEMIC RATS		
ORUGANTI RAJESH, VENISETTY RAJ KUMAR, PULIGILLA SHANKARAIAH	74	13-749
DISSOLUTION ENHANCEMENT OF TELMISARTANBYLIQUISOLID COMPACTS MAYS A. AL-SARRAF, AHMED A. HUSSEIN, AHMED S. ABDUL JABBAR	/4	13-749
EFFECT OF TYPE OF NON-VOLATILE SOLVENTS ON THE FORMULATION AND RELEASE OF VALSARTAN FROM LIQUID SOL	ID COMPACTS 75	50-754
CHELLA NAVEEN, RAMA RAO TADIKONDA	<u></u>	
GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF BIOACTIVE CONSTITUENTS IN THE ETHANOLIC EXTRACT	OF SACCHARUM	
<u>SPONTANEUM LINN.</u>	/ 5	55-759
J. AMUTHA ISWARYA DEVI, A. KOTTAI MUTHU		
DESIGN, SYNTHESIS, QSAR STUDIES AND IN VITRO EVALUATION OF NOVEL TRIAZOLOPIPERAZINE BASED B-AMINO AMII PEPTIDASE-IV (DPP-IV) INHIBITORS: PART-I	<u>IES AS DIPEPTIDYL</u> 76	60-765
SANJAY D.SAWANT, AMIT G.NERKAR, ARCHANA V. VELAPURE, NAYANA D.PAWAR		
INSILICO APPROACHES TOWARDS THE DRUG TARGET AURORKINASES USING THE ORTHO OR META SUBSTITUTED BEN	ZENE DERIVATIVES IN	
PYRAZOLES	76	66-770
SOBY DEVASIA, RANGADURAI.A		
SCREENING FOR POTENTIAL ANTIMCROBIAL COMPOUNDS FROM GANODERMABONINENSE AGAINST SELECTED FOOD BC PATHOGENS	RNE AND SKIN DISEASE 77	71-774
KHATIJAH ISMAIL, SYAHRIEL ABDULLAH, KHIMPHIN CHONG		
EVALUATION OF ACTIVE FRACTION FROM PLANT EXTRACTS OF ALSTONIA SCHOLARIS FOR ITS IN-VITRO AND IN-VIVO A	NTIVIRAL ACTIVITY 77	75-781
MOLLY ANTONY, CHANDRA SHEKHAR MISRA, THANKAMANI V		
HOMOLOGY MODELING FOR HUMAN ADAM12 USING PRIME, I-TASSER AND EASYMODELLER	78	32-786
P. RATHI SUGANYA, KABANI SUDEVAN, SUKESH KALVA, LILLY M. SALEENA		
SPECTROPHOTOMETRIC DETERMINATION OF PENEMS IN BULK AND INJECTION FORMULATIONS BY POTASSIUM FERRI (	<u>YANIDE AND FERRIC</u> 78	37-791
<u>CHLORIDE</u> V. DACHU DADU N. ADUNA VUMADI, A VASUNDI ADA		
K. RAGHU BABU, N. ARUNA KUMARI, A.VASUNDHARA HEPATO-PROTECTIVE EFFECTS OF <i>PIMPINELLA TIRUPATIENSIS</i> EXTRACT ON CYTOSOLIC AND MITOCHONDRIAL ENZYMI	ES ACAINST	
STREPTOZOTOCIN (STZ) -INJECTED PATHOGENIC DIABETIC RATS	<u>79</u>	92-797
GANAPATHI NARASIMHULU , SATHYAVELU REDDY KESIREDDYPASUPULETI VISWESWARA RAO, JAMALUDIN MOHAMED		
IN-VIVOANTI-INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITY OF HYDROALCOHOLIC EXTRACTOF PONGAMIA PINNATA	(L.) PIERRE SEED 79	98-803
DIVYA SINGH, RAHUL NAINWANI, AMIT GUPTA		
POLACRILIN RESIN AS MULTIFUNCTIONAL DIRECT COMPRESSION FILLER FOR PARACETAMOL TABLETS OPTIMIZED BY	BOX-BEHNKEN DESIGN 80	04-807
SIRAPRAPA CHANSATIDKOSOL, PRANEET OPANASOPIT, TANASAIT NGAWHIRUNPAT, PRASERT AKKARAMONGKOLPORN		
COMPARISION OF ANTIMICROBIAL EFFICACY OF TRIPHALA, WITHANIA SOMNIFERA AND SODIUM HYPOCHLORITE AGAI FAECALIS BIOFILM-AN INVITRO STUDY	<u>NST ENTEROCOCCUS</u> 80	08-811
SHIRUR KRISHNARAJ SOMAYAJI, NIDAMBUR VASUDEV BALLAL , SHOBHA KL, MOHANDAS RAO KG		
DESIGN, SYNTHESIS, QSAR STUDIES AND BIOLOGICAL EVALUATION OF NOVEL TRIAZOLOPIPERAZINE BASED B-AMINO A	MIDES AS DIPEPTIDYL	12-817
PEPTIDASE-IV (DPP-IV) INHIBITORS: PART-II	81	12-01/
SANJAY D. SAWANT, AMIT G. NERKAR, NAYANA D. PAWAR, ARCHANA V. VELAPURE		
METALLOTHIONEIN EXPRESSION IN MARINE CATFISH ARIUS ARIUS LIVER ON EXPOSURE TO CADMIUM USING IMMUNOH WESTERN BLOT	IST OCHEMISTRY AND 81	18-821
RAMALINGAM MANI, BOOMINATHAN MEENA, KARUPPIAH VALIVITTAN		
THE COST OF MANAGEMENT OF INTRACRANIAL ANEURYSMS BY EMBOLIZATION IN MOROCCO: ABOUT 48 CASES	82	22-826
CHEIKH AMINE EL ABBADI NAJIA, ISMAILI HATIM, ABABOU ADIL, CHERRAH YAHYA, EL QUESSAR ABDELJALIL		

<ul> <li>TERMA CHARARDORT, KANTINYA EMATTACHARAY.</li> <li>STUDING CARANDARI, A CHILDRANDA EMANY, KORENA CHARANDA li></ul>	Vol 6 Suppl 2	
NYNTHES OF SOME NOW LA ATTAL ATTIKATION IN NOR PROVINCE INCORPORATED PERSON IN YOR SAA AND DAARETTS AGENTS AND HANNEN AND	QUALITY ASSESSMENT OF SOME INDIAN HONEYS IN STORAGE THROUGH HMF CONTENT AND INVERTASE ACTIVITY	827-830
EXUDDREE NUMAR. BANDHAAR REDUY. WAARMADHA BABU         SUBSET	TRINA CHAKRABORTI, KASHINATH BHATTACHARYA Synthesis of some novel 2. A thuatou dimedione incorror ated bydatou e dedinatives as anti-canced acents	021 024
EVALUATION OF THE FEPEL OF CONTRA SUMMINIANA READ HEADENDY NORMALL AND DIADETIC BATS USING BASING AVOIDANCE TASK MASINGKATH (HEATTS SUMAIL DARKS SUMMINIANA READ DIADEDDY CAMBORDENCTIVE FEPEL OF STHANOLIC ENTRACT OF MEDICAGO SATURA STEM ON ISOPROTERINOL INDUCED AVOCARDIAL INFARCTION IN SUMMINIAL INFU SUMAIL PHONLE CONTENTS SUMAIL DARKS SUMMINIANA READ AND LAS SUMMINIANA READ SUMMINIANA DIADETIC BATS (SUMMINIAL INFARCTION IN SUMMINIAL INFO THE SUMAIL DARKS SUMMINIANA READ ACTIVITY AND NUTRITIVE VALUE OF YOUNG TWES OF PSIMINI GUIGALI LINN, TAN UBBRUGAMI, ASSAM NA NUSCITI, ANT OUDAW, AND A.S. SUMAI. AN INSCITL INFO THE LIPPO ABRORMALTIVES IN TYPE 2 DUBRTES MELITUS PATIENTS IN VELLORE REGION. SOLTH INDIA NA NUSCITL INFO THE LIPPO ABRORMALTIVES IN TYPE 2 DUBRTES MELITUS PATIENTS IN VELLORE REGION. SOLTH INDIA NA NUSCITL INFO THE LIPPO ABRORMALTIVES IN TYPE 2 DUBRTES MELITUS PATIENTS IN VELLORE REGION. SOLTH INDIA NA NUSCITL INFO THE LIPPO ABRORMALTIVES IN TYPE 2 DUBRTES MELITUS PATIENTS IN VELLORE REGION. SOLTH INDIA NA NUSCITL INFO THE LIPPO ABRORMALTIVES IN TYPE 2 DUBRTES MELITUS PATIENTS IN VELLORE REGION. SOLTH INDIA NA NUSCITL INFO THE LIPPO ABRORMALTIVES INTO THE ACTIVITY OF VITAS 2 DUBRTES MELITUS PATIENTS IN VELLORE REGION. SOLTH INDIA NA NUSCITLATION FUNCTION FOR MELITARITY OF VITAS 2 DUBRTES MELITUS PATIENTS IN VELLORE REGION. SOLTH INDIA NA NUSCITLATION FUNCTION FOR THE ACTIVITY OF VITAS 2 DUBRTES STRATS 5 CTACATO PATIE ANTI HUNGLE CLAURING DUBRNESS IN MICE OF READ ACTIVITY OF VITAS 2 DUBRTES TRATS 5 CTACATOR WITH HUNGLE CLAURING ACTIVITY OF VITAS 2 DUBRTES SUMAIL SYNTE STRATS 5 COLORIDATION AND COMPLEX SUMAIL SYNTE WITH HUNGLE CLAURING DUBRNESS IN MICE SHAMUGARYAL, KUNNA (DUBRTES CONTENTS IN MATERIA SUMAIL ESTIMATES IN MICE SHAMUGARYAL, KUNNA (DUBRTES IN MICHAEN SCHWERE STRATS 5 COMPARISON AND CARGADY SHAMUGARYAL, KUNNA (DUBRTES IN MICE IN MICE IN ALLAY AND AND AND THE ANTI HUNGLES IN MICE IN MICE IN ALLAY AND AND AND AND THE ANTI HUNGLES IN MICE IN MICE INTERICATES OF LIPPOTENTIAL IN SUMAIL ESTIMATES IN		031-034
CANDERPORT CTUY EFFECT OF THANGLE ENTRACT OF MEDICAGE SATUR STEN ON ISOPROTERING. INDUCED INDUCABUIAL INFARCTION. IS SOMATHIN, VIJERINA M, KUSHA PHINGLE CONTINU, ANT OMDAY AND ANTINICROBIAL ACTIVITY AND NUTRITIVE VALUE OF YOUNG TWEE OF PSDRING GAGAN LINN. TAN DEBUGAINI, ASSAM OLETTAS, SUPROVENA, D.K. BORA AND LK. SAIKA AN INGENT INTO THE LIPP ALMOREMALTIES IN TYPE 7 DURIETS MELITIES INTERVIS IN VELORE REGION. SOUTH INDIA DIBUUGAINI, SAMA NUTRI THE LIPP ALMOREMALTIES IN TYPE 7 DURIETS MELITIES INTERVIS IN VELORE REGION. SOUTH INDIA DIBUUGAINI, SAMA NUTRI PHILANDE ALMOREMALTIES IN TYPE 7 DURIETS STRUCTURE OF THE AND NUTRI PHILANDE AND PRELIMINARY CLINICAL STUDY OF IL-LYNR ORDITECAL DIVERS CONTAINING RETORDIAC BEN ADARAMY IN A. MARGY AN. IL-SCTOWT DA A. ADD-ELMONERN K. A AMN N.C., DAYTE B. A. NUTRI PHILANDLE FLAVONUD. CAUCTERNOL CAUSE TRACTS OF LICURIUMTICAL DIVERS CONTAINING RETORDIAC EL-NARAMAVI N.A. MARGY AN. IL-SCTOWT DA A. ADD-ELMONERN K. A AMN N.C., DAYTE B. A. NUTRI PHILANDLE FLAVONUD. CAUGTERNOL CONTANT RED NOT BEAM AND AND RELIMINARY CLINICAL STUDY OF IL-LYNR ORDITECAL LEVYES ISING FRAP. DIPHI. ASSNS AND CORRELATION WITH PHILANDLE FLAVONUD. CAUCTERNOL CONTANT RED NOT BEAM AND AND RELIMINARY CLINICAL STUDY OF IL-LYNR ORDITECAL LEVYES ISING FRAP. DIPHI. ASSNS AND CORRELATION NUTRI PHILANDLE FLAVONUD. CAUCTERNOL CONTANT RED NOT ADDRESS AL-UDARAMY, SURGENSO SURVESSICAL CONTANT, DEFENSION IS DESCRIPTION OF THE STRUCTS OF CALOFROPIS GGAATEA AND CARCCA PHARA LATEX AGAINST NEW MITH PHILANDAL STATUS ON CONTANT. RED NOT ADDRESS AL-UDARAMIN, SURGENSO NUTRI STANDAR AND CARC PHILANDAR STATUS ON CONTANT, DEFENSION IS DESCRIPTION ON DO NOTATIVE DAMAGES IN NUCE SURVESSICAL CONTANT, DEPENDENT DIVERS AND CARCEA STATUS SURVESSICAL CONTANT, DEPENDENT DIVERS AND CARCEA STATUS	EVALUATION OF THE EFFECT OF COSTUS IGNEUS ON LEARNING AND MEMORY IN NORMAL AND DIABETIC RATS USING PASSIVE AVOIDANCE TASK	835-838
SNESS ALBROUND     GUINTIN S, UPRING MA, KUSSIA       PHENDER, CONTERT, ANT-COUNNET AND ANTHERGOBIAL ACTIVITY AND NUTRITIVE VALUE OF YOUNG TWIG OF PSDRIM GUIAGUE LINE, FROM       BARNARDAL CONTERT, ANT-COUNNET AND ANTHERGOBIAL ACTIVITY AND NUTRITIVE VALUE OF YOUNG TWIG OF PSDRIM GUIAGUE LINE, FROM       BARNARDAL CONTERT, ANT-COUNNET AND ANTHERGOBIAL ACTIVITY AND NUTRITIVE VALUE OF YOUNG TWIG OF PSDRIM GUIAGUE LINE, FROM       BARNARDAL CONTERT, ANT-COUNNET, AND AND LE, SAIKA.       NUNRA HARI, A, SOKAN P, RADDA SANASWATHY       SABRACATION, SANARDA SANASWATHY       REMARDATION, AND AND AND AND AND AND AND AND AND AND	SHASHIKANTH CHETTY, SHALINI ADIGA, SHIVKUMAR REDDY	
PHENGLAGUATES, ANT-CONNANT, AND ANTIMICROBIAL ACTIVITY AND NUTRITIVE VALUE OF YOUNG TWIG OF SEDIUM GUIAGUA LINK, FRAM (CHETA, S. UPADITVAY, DA CORA, AND L.K. SAIKA, AND CALLER, S. UPADITVAY, D. AND CALLER, SAIKA, AND CALLER, S. UPADITVAY, D. AND CALLER, SAIKA, AND CALLER, S. UPADITVAY, D. AND CALLER, SAIKA, AND CALLER, S. UPADITVAY, D. AND CALLER, SAIKA, AND CALLER, S. UPADITVAY, D. AND CALLER, SAIKA, AND CALLER, S. UPADITVAY, D. AND CALLER, SAIKA, AND CALLER, SAIKA, C. AND CALLER, SAIKA, SAIKA, AND CALLER, SAIKA, C. AND CALLER, SAIKA, SAIKA, AND CALLER, SAIKA, C. AND CALLER, SAIKA, SAIKA, AND CALLER, SAIKA, SAIKA, SAIKA, SAIKA, SAIKA, AND CALLER, SAIKA, S	<u>CARDIOPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF <i>MEDICAGO SATIVA</i> STEM ON ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN SWISS ALBINO RATS</u>	839-842
DBBUGGABL ASSAM     R4-30       DBBUGGABL ASSAM     R4-30       DBBUGGABL ASSAM     R4-30       L FINTA S. UNDAWAN, DK. ENDA AND L.R. SAIKIA.     R4-74       AN INSGET INTO THE LIFE ARROWNALTHES IN TYPE 2 DARBETS MILLITUS PATIENTS IN VELORE REGIOS. SOUTH INDIA     R4-74       SUBBLA AND A. SANKA P. RADIA SANAWATHY     R4-74       MURIE AND A. ASAN PERELIMMANY CLINICAL STUDY OF BELAYER ORDITICAL DEVICES CONTAINING KETOROLAC     R51-8       RANKAN M. A. MARKA A. M., EL-STROUTO D. A. ADD-ELMONEN C. D. CALUMAMATION     R51-8       REINDRINK CHILDRING CALUMATING ORDITAL AND THE STRACTS OF CICURBITACEAE LEAVES USING FRAP. DPPH ASSAYS ANDCORRELATION     R53-8       REINDRINK CHILDRING CALUMATING ORDITAL AND THE STRACTS OF CICURBITACEAE LEAVES USING FRAP. DPPH ASSAYS ANDCORRELATION     R53-8       MARTINE THEOLINE, LINDRING CALUMATING ORDITAL AND THE STRACTS OF CICURBITACEAE LEAVES USING FRAP. DPPH ASSAYS ANDCORRELATION     R54-8       MARTINE THEOLINE, ALMONG DAMATANANAN     R51-8     R53-8       SHANNUCARRIVA, K. JINU UNDRIVABANU, THA THAYUMANAVAN     R0-48     R4-48       COMPARATIVE STUDY ON THE EFFECT OF DIFFERENT SOLVENTS ENCODES OF CALUMATION AND CARICA PAPHER LATEX AGAINST NEW PHOLONOUS CONTANTIVE DAMAGES IN MICE     R9-49       SHANNUCARRIVA, K. JINU UNDRIVABANU, THA THAYUMANAVAN     R0-48     R0-48       COMPARATIVE STUDY ON THE EFFECT OF DIFFERENT SOLVENTS END COLOTION OF DATES OF CALUMATION AND AND AND AND AND AND AND AND AND AN		
AL INSTELL INTO THE LIPE ALROREMULTIES IN TYPE 2 DIAMETES MILLITUS INTERTS IN VIELDRE REGION, SOUTH INDIA ANDIA DAKA AS AND SONA PE ANDIA SARSNATHY INTO MILLIA DAKA AS AND PERLEMINARY CLINICAL STUDY OF BELAVER ORBUGGAL DEVICES CONTAINING KETOROLAG INTO MILLIA DAKA AND PERLEMINARY CLINICAL STUDY OF BELAVER ORBUGGAL DEVICES CONTAINING KETOROLAG INTO MILLIA DAKA AND PERLEMINARY CLINICAL STUDY OF BELAVER ORBUGGAL DEVICES CONTAINING KETOROLAG INTO MILLIA DAKA AND CHILDREN MILLIA DA MILLIA STUDY OF BELAVER OR BURGAN INTO MILLIA DAKA AND CHILDREN MILLIA DA MILLIA STUDY OF BELAVER OR DUGUED LEAVES LISING FRAP DPPH ASSAYS ANDCORRELATION INTO MILLIA DAKA AND CHILDREN MILLIA DA	DIBRUGARH, ASSAM	843-846
NUMBA ANA A, ASOKAN P, BANHA SARASWATIYU ABRICATION, KYULATIYON ADU PERLIMINANY CLINICAL STUDY OF BELAVER OROBUGCAL DEVICES CONTAINING KETOROLAG BASILACTION, KYULATIYON ADU PERLIMINANY CLINICAL STUDY OF BELAVER OROBUGCAL DEVICES CONTAINING KETOROLAG BASILACTION, KYULATIYON ADU PERLIMINANY CLINICAL STUDY OF BELAVER OROBUGCAL DEVICES CONTAINING KETOROLAG BASILACTION, KYULATIYON ADU PERLIMINANY CLINICAL STUDY OF BELAVER OROBUGCAL DEVICES CONTAINING KETOROLAG BASILACTION, CHILD STRUCTURY OF UTTER AGRICULTURY AT THACTS OF CLICIOBUTATICAL LAATS USING FRAP. DIPHI ASSAYS ANDCORRELATION BASILATI AND OCENIC ACTIVITY OF UTTER AGRICULTURY OF DEVICES CONTAINING KETOROLAG BASILATION OF ANTIONINANY ACTIVITY IN DIFFERENT SOLVENTS OF CALOTROPIS GRAVITA ALLAYS USING FRAP. DIPHI ASSAYS ANDCORRELATION BASILATION OF ANTIONINANY ACTIVITY IN DIFFERENT SOLVENTS OF CALOTROPIS GRAVITA AND CANCA PAPAVA LATEX AGAINST NEW BASILATION OF ANTIONINANY ACTIVITY IN DIFFERENT SOLVENTS OF CALOTROPIS GRAVITA AND CANCA PAPAVA LATEX AGAINST NEW BASILATION ON THE EFFECT OF DIFFERENT SOLVENT EXTRACTS OF CALOTROPIS GRAVITA AND CANCA PAPAVA LATEX AGAINST NEW BASILATION AND EVALUATION OT THE EFFECT OF DIFFERENT SOLVENT EXTRACTS OF CALOTROPIS GRAVITA AND CANCA PAPAVA LATEX AGAINST NEW BASILATION AND EVALUATION OF ENTERIC COATED MICROSPHERES OF KETOPHOFEN USING NATURAL POLYMERS FOR COLON DEBUG BASILATION AND EVALUATION OF ENTERIC COATED MICROSPHERES OF KETOPHOFEN USING NATURAL POLYMERS FOR COLON DEBUG BASILATION AND EVALUATION OF ENTERIC COATED MICROSPHERES OF KETOPHOFEN USING NATURAL POLYMERS FOR COLON DEBUG BASILATION AND EVALUATION OF ENTERIC COATED MICROSPHERES OF KETOPHOFEN USING NATURAL POLYMERS FOR COLON DEBUG BASILATION AND EVALUATION OF ENTERIC COATED MICROSPHERES OF KETOPHOFEN USING NATURAL POLYMERS FOR COLON DEBUG BASILATION AND EVALUATION OF ENTERIC COATED MICROSPHERES OF KETOPHOFEN USING NATURAL POLYMERS FOR COLON DEBUG BASILATION AND EVALUATION OF ENTERIC COATED MICROSPHERES OF KETOPHOFEN USING NATURAL POLYMERS FOR COLON DEBUG BASILATION AND E	J. CHETIA, S. UPADHYAYA, D.K. BORA AND L.R. SAIKIA.	
EABLECTION. EVALUATION AND PERLIMINARY CLINICAL STUDY OF BELAYER ORDINICAL DEVICES CONTAINING KETOROLAC B51-8 ENABARWI M. A, MAKYA M., EL-ESTOURY D. A, ABD-ELMONEDR R. A, AMIN M. G., IASTI B. A. ANTICHNING CLICARIESTORIE BCLI COR TRANSMINT OF CHALL INFORMATION AND INFORMATION AND INFORMATION	AN INSIGHT INTO THE LIPID ABNORMALITIES IN TYPE 2 DIABETES MELLITUS PATIENTS IN VELLORE REGION, SOUTH INDIA	847-850
REMETINAMEAND CHLORINERDINE ICL POR TREATMENT OF DEAL INFLAMMATION #51-8 IENABARAWI A, AMKKY A, M. ESETOWIT DA LESETUNIT A, A ADELINORE RE A, ANIN K, GI, STIE B. A ANTIONIMAT CAPACITIES FERD DIFFERENT POLARITIES EXTRACTS OF CICURBITACAE LEAVES USING FRAP. DIPH ASSAYS AND CORRELATION B53-8 IERA FIDRIANY, ACUNC DARMAWATT, SURVASIO IFE ANTI-ANDIGANIC ACTIVITY OF UTERS INTERACTS OF CICURBITACAE LEAVES USING FRAP. DIPH ASSAYS AND CORRELATION IFE ANTI-ANDIGANCI, ACTIVITY OF UTERS OF CALARYSIS INTERACTS B63-8 INADOR R. SAINBL ADEB A AL-ZODARDYL, SHALLA M HUSSAINZ, GHATTH ALI JASSIMS INVESTIGATION OF ANTIONIMAT ACTIVITY IN UTERBENTS SOLVANYS OF CALIFICATION POLICAULON SHANDUG APRIYA, K. HINU UDHYABANU, THA.THAATMAANAN COMPARITIES TURY ON THE FFERT OF DIFFERENT SOLVANY AND COMPARITIES TURY ON THE FFERT OF DIFFERENT SOLVANY AND COMPARITIES TURY ON THE FFERT OF DIFFERENT SOLVANY AND COMPARITIES AND THA THAATMAANANA COMPARIYA, K. HINU UDHYABANU, THA.THAATMAANANA COMPARIYA, K. HINU DOHYABANU, THA.THAATMAANANA COMPARIYA, K. HINU DOHYABANU, THA.THAATMAANANA COMPARIYA, K. HINU DOHYABANU, THA THAATMAANANA COMPARIYA, K. HINU DOHYABANU, THA THAATMAANANA COMPARIYA, SAINA AN ITHO STUDY SHANDUGAPRYA, K. HINU DOHYABANU, THA THAATMAANANA KANAN KANAN KANAN KANAN KAN COMPARIYA, KANNA KAN COMPARIYA, SAINAYA AN ITHO STUDY SHANDUGAPRYA, K. HINU DOHYABANU, THA THAATMAANANA KANAN KANAN KANAN KANAN KAN COMPARIYA, SAINTA KHARI, PARALABANA, K SETH PORMULATION AND DIVALIATION OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING NATURAL POLIVIEED TYPE 2 DIABETIC BATS PARIO COUNDING THE POTENDE IN COATED MICROSPHERES OF KETOPROFEN USING NATURAL POLIVIEED TYPE 2 DIABETIC BATS PARIO COUNDING CALUARY NA KANTHA, KANTHANAN K SETH FORMULATION OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING NATURAL POLIVIEED TYPE 2 DIABETIC BATS PARIO COUNDING THA KHARI, KANTHANAN K SETH FORMULATION OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING NATURAL POLIVIEED TYPE 2 DIABETIC BATS PARIO COUNDING KANANANANAN AND BOLEDAHANANA K SETH FORMULATION OF ENTERIC COATED MI		
ANTIONINAT CARACTERS FROM DEFERENT POLARITIES EXTRACTS OF CUCURBITACEA <sup>®</sup> LEAVES USING FRAP. DPPH ASSAYS ANDCORRELATION WITH PHENOLOGYNC ACTIVITY OF UTEX AGAINS CASTRIS LEAVES DETRACTS IRDA FIDRIANY, AGUNG DARMAWATT, SUKRASNO IREA ANTI ANDIOCENIC ACTIVITY OF UTEX AGAINS CASTRIS LEAVES DETRACTS IRDA FIDRIANY, AGUNG DARMAWATT, SUKRASNO IREA ANTI ANDIOCENIC ACTIVITY OF UTEX AGAINS CASTRIS LEAVES DETRACTS IRDA FIDRIANY, AGUNG DARMAWATT, SUKRASNO IREA ANTI ANDIOCENIC ACTIVITY OF UTEX AGAINS CASTRIS LEAVES DETRACTS IRDA FUNCTION OF ANTIORIANT, ACTIVITY IN DEFERENT SULVENTS OF CALOFTROPIS GEGATEA AND CARICA PLAVA LATEX AGAINST NEW IRUSES AGAINST ON OF THE FETCH OF DEFERENT SULVENTS OF CALOFTROPIS GEGATEA AND CARICA PLAVA LATEX AGAINST NEW IRUSSO A, ANTI, CPEETAWARE JE PANICKER SG PPHROLOQUINOLINE QUINONE HAS THE POTENTIAL TO AMELIORATE PTU INDUCED LIPID PEROXIDATION AND OXIDATIVE DAMAGES IN NICE IRUSSO A, ANTI, CPEETAWARE JE PANICKER SG PHROLOQUINOLINE QUINONE HAS THE POTENTIAL TO AMELIORATE PTU INDUCED LIPID PEROXIDATION AND OXIDATIVE DAMAGES IN NICE IRUSSO A, ANTIVITY OF DIROLIN A POLYHEIRAL FORMULATION JI IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS IRUSSA MARINDA KAR INTENIABETIC ACTIVITY OF DIROLIN A POLYHEIRAL FORMULATION JI IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS IRUSSA MARINDA S, LARIKUMAR INTENIABETIC ACTIVITY OF DIROLIN (A POLYHEIRAL FORMULATION JI IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS IRUSSA MARINDA INTENIAS THA MARINER, SALARAMAN, A K STRI INTENIAS THO AND INTERT RE ALARAMAN, A K STRI INTENIS INTENDE TO DIROLIN (A POLYHEIRAL FORMULATION JI IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS IRUSSA MARINDA INTENIS AT MUNAR K, KATHIRER BALARAMAN, A K STRI INTENIS INTENDE TO DIROLIN (A POLYHEIRAL FORMULATION JI IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIS INTENIS INTENDE OVER EXPRESSION IN BREAST CANCER INTENIS INTENDE OVER EXPRESSION IN BREAST CANCER INTENDE VIEW INTENDE INTENDE INTENDE INTENDE INTENDE INTENDE INTENDE INTENDE INTENDE	TROMETHAMINEAND CHLORHEXIDINE HCL FOR TREATMENT OF ORAL INFLAMMATION	851-85
WITH PIENOLIC. FLAVORODIC CARDITINOIC CONTENT     693-9       WITH PIENOLIC. FLAVORODIC CARDITINOIC CONTENT     693-9       MAR FIDRALMAY, CALING DARAMAY, SIKRASNO     863-8       THE ANTI ANGIGGENIC ACTIVITY OF UTEX AGAUS CASTUS LEAVES EXTRACTS     863-8       INADER B. SAHIBL, ADEED A AL-ZURADTI, SIKRASNO     870-8       SRAAMUGARRIYA, K. JINU UDHYABANU, THA THAYUMANAYAN     874-8       COMPARATIVE STUDY ON THE FFFECT OF DIFFERENT SOLVENT EXTRACTS OF CALOTROPIS GIGANTEA AND CARLCA PIPAYA LATEX AGAINST NEY       RATERIAL ISOLGT, A. NI, VITRO STUDY     860-8       SHARNDAG, AARTT C, PREETAMRAJ JP, PANCKER SG     860-8       PIEROLOQUINOLINE, QUINONE HAS THE POTENTIAL TO AMELIORATE PTU INDUCED LIPID PEROXIDATION AND OXIDATIVE DAMAGES IN MICE     860-8       NARENDRA KUMAR, ANAND KAH     874-8     893-9       KANENGALUMAR, ANAND KAH     874-8     893-9       NERA STANGAL, AND PIENTRIC COATED MICROSPHERES OF KEOPROFEN USING NATURAL POLYMERS FOR COLON PUENCH     893-9    <	EL- NABARAWI M. A, MAKKY A. M. , EL-SETOUHY D. A., ABD-ELMONIEM R. A, AMIN M. G., JASTI B. A.	
IRRA FURANEY, ACINE DARMAWATI, SURANOU THE ANTI ANGIOGENI, ACEMANI, THEXA GAUSS CASUES LEAVES DETRACTS IN ANDRER B. SANIHA, DAEERA AL-ZURADUT, SHALLAL M HUSANR2, GHATTH ALI JASSM3 INVESTIGATION OF ANTIONIAMT ACTIVITY IN DIFFERENT SOLVENTS OF <i>GAMPHALUM POLICAULON</i> 866-38 INVESTIGATION OF ANTIONIAMT ACTIVITY IN DIFFERENT SOLVENTS OF <i>GAMPHALUM POLICAULON</i> 870-85 SHARMUGARRIVA, K. JINU UDHYABANU, THA THAVUMANAVAN COMMARTUTY STUTY IN ON THE FEFTOR OF DIFFERENT SOLVENTS OF <i>GAMPHALUM POLICAULON</i> 870-85 SHARMUGARRIVA, K. JINU UDHYABANU, THA THAVUMANAVAN COMMARTUTY STUTY IN ON THE FEFTOR OF DIFFERENT SOLVENT EXTRACTS OF <i>CALOTROPIS GIGANTEA</i> AND <i>CARICA PLAVAI</i> LATEX AGAINST NEW RATTERIAL, SOLATTS - AN <i>IN VITRO STUDY</i> 871-86 NARENDRA KUMAR, IN ONE HAS THE POTENTIAL TO AMELIORATE PTIL INDUCED LIPID PERONDATION AND OXIDATIVE DAMAGES IN MICE PURBOLOUINOLINE OULINONE HAS THE POTENTIAL TO AMELIORATE PTIL INDUCED LIPID PERONDATION AND OXIDATIVE DAMAGES IN MICE 1947-80	ANTIOXIDANT CAPACITIES FROM DIFFERENT POLARITIES EXTRACTS OF CUCURBITACEAE LEAVES USING FRAP, DPPH ASSAYS ANDCORRELATION WITH PHENOLIC ELAVONOID CAROTENOID CONTENT	858-862
ITHE ANTI ANGIOGENIC ACTIVITY OF <i>UTEX AGRUES CASTUS LEAVES INTRACTS</i> 863-9       INNDER IS SAIRED, ADEED A AL-ZUBARDYL, SHALLAL M RUSSAINZ, GHATT HALIJASSIN3     870-9       SHANDUGAPRIVA, K. JINU UDH VABANU, THA THAYUMANAVAN     874-8       COMPARTIVE STUDY ON THE FFECT OF DIFFERENT SOLVENT EXTRACTS OF CALIFORDPS GIGANTEA AND CARICA PAPAPIA LATEX AGAINST NK     874-8       MATCERIAL ISOLATS - ANN AUTOR STUDY     880-9       MARTIDRA KUMAR, ANAND KAR     880-9       MARNDRA KUMAR, ANAND STUDY OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING AATURAL POLYMERS FOR COLON DRUG     880-9       PERCOLOQUINOLINE, QUIRONE HAS THE FOTENTIAL TO AMELIORATE PTU INDUCED LIPID PERCUBARTION AND ONDATIVE DAMAGES IN MICE     893-9       ROMULATION AND EVALUATION OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING AATURAL POLYMERS FOR COLON DRUG     896-9       ROMULATION AND EVALUATION OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING AATURAL POLYMERS FOR COLON DRUG     993-9       ROMORTHI, C. STORTINI, KUMAR     894-9       KANAGATHARAN, KAUTHARA     804-9       KANAGATHARAN, KAUTHARA     804-9       SYNEBISSTIC ANTI-CANCER ACTIVITY OF CURCUMINA AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES     90-9       VILLUTION OF HERESCURIC AUTOR AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES     90-9       VILLUTION OF HERESCURIC AUTOR AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES     90-9       VILLUTION OF HERESCURACUTICAL AND BIAST CANCERS     901-9		
INVESTIGATION OF ANTIONIDANT ACTIVITY IN DIFFERENT SOLVENTS OF GAAPHALUM POLYCAULON 970-95 SHANMUGAPRIVA, K. JINU UDHYABANU, THA, THAYUMANAVAN COMMARTIVE STUDY ON THE FFECT OF DUFFERENT SOLVENT EXTRACTS OF CALOTROPIS GEANTEA AND CARLO PAPHAL LATEX AGAINST INF 74-87 ARTERNAL SOLVENTS - AN ANTIONID STUDY RNUNKO A, ANKT I, PRESTAMBAI JP, PNINCKER SC PYROLOQUINOUR, GUINOSE HAND STUDY COMPARIANT EXTRACTS OF CALOTROPIS GEANTEA AND CARLO PAPHAL LATEX AGAINST INF 74-87 ARTERNAL SOLVENTS - AN ANTION STUDY PROLOQUINOUR, GUINOSE HAND STUDY COMPARIANT E THI INDUCED LIPID PERONIDATION AND CONDITIVE DAMAGES IN MICE PRONILATION AND EVALUATION OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING NATURAL POLYMERS FOR COLON DRUG PELIVERY NERA SHARMA, S.L.HARIKUMAR NANARAMA KAN FORMULATION OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING NATURAL POLYMERS FOR COLON DRUG PELIVERY NERA SHARMA, S.L.HARIKUMAR NANDA KAN FORMULATION OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING NATURAL POLYMERS FOR COLON DRUG PELIVERY NERA SHARMA, S.L.HARIKUMAR NANDA KAN FORMULATION OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING NATURAL POLYMERS FOR COLON DRUG PELIVERY NERA SHARMA, S.L.HARIKUMAR NATURAHETIC ACTIVITY OF DUBOLIN (A POLYMERBAL FORMULATION) IN STREFT0ZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATE PELIVENTY NERA SHARMA, SANTHA KANAR NITUMAETIC ACTIVITY OF DUBOLIN (A POLYMERBAL FORMULATION) IN STREFT0ZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATE PELIVENTY NITUMAETIC ACTIVITY OF DUBOLIN (A POLYMERBAL FORMULATION) AGAINST VARIOUS CANCER CELL LINES COMORTIL (CS STUDY BOLON CARUNA SHART NA BALABANAN, AK SETH VALUATION OF MERZ/NEUVICHALY AK SHARTHRAN NY HELES/NEU DOLON CARUTHE EXPRESSION IN BREAST CANCER COMORTIL (CS DUBOLINA, KATHIRESAN NY HELES/NEU OF MALTERY DOLON COLLONNIN AND BOLON THAND FORMAMIDINE DERIVATIVES FROM 3-AMINO- HILL HALAMANE DUBORDER AND AND FOLMEDHALY MADDE BOUNDALY COMORTIVE DATABAL SHARTHAN NY HELES/NEU OF MALTERY DUBOLINA (CHICUMAINTA MAD THOM MULBERENY (MORUSALER AND MAD THOM NY HILE CANTING CANDE BOUNDAL SH	THE ANTI ANGIOGENIC ACTIVITY OF VITEX AGNUS CASTUS LEAVES EXTRACTS	863-86
SHAMUGAPRIVA, K. JINU UDHVABANI, THA THAVUMANAVAN COMPARATIVE STUDY ON THE EFFECT OF DIFFERENT SOLVENT EXTRACTS OF <i>CALOTROPIS GIGANTEA</i> AND <i>CARICA PAPAPA</i> LATEX AGAINST NEW R74-8 KUSRO A, ART (C, PRETMARAUP) P. MNCKER SG PYRROJOQUINOLINE QUINONE HAS THE POTENTIAL TO AMELIORATE PTU INDUCED LIPID PERXIDATION AND OXIDATIVE DAMAGES IN MICE R60-8 WARENDRA KUMARA, ANAN DE ALTAE TO TENTIAL TO AMELIORATE PTU INDUCED LIPID PERXIDATION AND OXIDATIVE DAMAGES IN MICE R60-8 WARENDRA KUMARA, ANAN DE ALTAE TO TENTIAL TO AMELIORATE PTU INDUCED LIPID PERXIDATION AND OXIDATIVE DAMAGES IN MICE R60-8 WARENDRA KUMARA, ANAN DE ALTAE THE POTENTIAL TO AMELIORATE PTU INDUCED LIPID PERXIDATION AND OXIDATIVE DAMAGES IN MICE R60-8 WARENDRA KUMARA, ANAN DEAL POTENTIAL TO AMELIORATE PTU INDUCED LIPID PERXIDATION AND OXIDATIVE DAMAGES IN MICE R60-8 WARENDRA KUMARA, ANAN DEA PARAMANA A STEPH EVALUATION AND EVALUATION OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING NATURAL POLYMERS FOR COLON DRUG R61-8 WARENDRA KUMARINA, S.L.HARIKUMAR ANTIDIABETIC ACTIVITY OF DIBOLIN (A POLYMERBAL FORMULATION) IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS R93-8 RANACIATABRA, S.L.HARIKUMAR NATIDIABETIC ACTIVITY OF DIBOLIN (A POLYMERBAL FORMULATION) IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS R94-9 SWREGISTIC ANTICA ACCER ACTIVITY OF CURCUMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES R01-9 SWREGISTIC ANTICAANCER ACTIVITY OF CURCUMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES R01-9 SWREGISTIC ANTICAANCER ACTIVITY OF CURCUMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES R01-9 SWREGISTIC ANTICAANCER ACTIVITY OF CURCUMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES R01-9 SWREGISTIC ANTICAANCE ACTIVITY OF CURCUMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES R02-1000CICALLY AND PHARMACUTTICALLY CARCUT E PYEINDINE AND FORMAMIDINE DERIVATIVES FROM 3-AMINO- H1H-2H-4H-4H-2H-2H-2H AND ANDER MARA R ARABA AND AND SUDOPHANCER AND AND CHARACTE	HAYDER B. SAHIB1, ADEEB A AL-ZUBAIDY1, SHALLAL M HUSSAIN2, GHAITH ALI JASSIM3	
COMPARATIVE STUDY ON THE EFFECT OF DIFFERENT SOLVENT EXTRACTS OF CALOTROPIS GIGANTEA AND CARGA PAPAYA LATEX AGAINST NEW BACTERIAL ISOLATES - AN IN VITRO STUDY RUISBO A, ARATI C, PRETAMRAJ JP, PANICKER SG PYROLOQUINOLINE QUINONE HAS THE POTENTIAL TO AMELIORATE PTU INDUCED LIPID PEROXIDATION AND OXIDATIVE DAMAGES IN NICE NARRNDRA KUMAR, ANAD KAR NERNDRA KUMAR, ANAD KAR NARRNDRA KUMAR, ANAD KAR NARRNDRA KUMAR, SL. HARIKUMAR ANTIDIABETIC ACTIVITY OF DIROLIN (A POLYHERRAL FORMULATION) IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS 893-8 RAJESH A MAHESHWARL SONYA KHATRI, R BALARAMAN, A K SETH ANTIDIABETIC ANTI-CANCER ACTIVITY OF CIRCIMMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES SYNERGISTIC ANTI-CANCER ACTIVITY OF CIRCIMMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES SYNERGISTIC, ANTI-CANCER ACTIVITY OF CIRCIMMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES SYNERGISTIC, ANTI-CANCER ACTIVITY OF CIRCIMMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES SYNERGISTIC, ANTI-CANCER ACTIVITY OF CIRCIMMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES SYNERGISTIC, ANTI-CANCER ACTIVITY OF CIRCIMMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES SYNERGISTIC, AND PHARMACEUTICALLY ACTIVE PYRIMIDINE AND FORMAMIDINE DERIVATIVES FROM 3-MINO- HEWMAT MOHAMMED DARDEER AND ABOU-BAKR HAREOL ABDEL-MONSEF ASSESSION DARDEER AND ABOU-BAKR HAREOL ABDEL-MONSEF ASSESSION ANALASIS BUNLEU SUNG THONG, CHRAPHA BUTIMAN, KUSUMA JITSAEN PHETACUCIC INTERPENSIS ROM MATERIA DARDER IOWALVER CONNITIONS ANAHWSH FEROZ, NICHAT RAZVI,SANA CHAVAS, FARKISHEENA ANUM, LUBBAR CHAZAL, SAED AHMAD SIDDIQUI OPTIMZED ULTRASONC-ASSISTED EXTRACTION OF ANTIONADAT FROM MULBERRY (MORUS ALBAL L.) LEAVES USING MULTIPLE LINEAR ERGRESSION ANALASIS SYNTHESIS AND MOLECULAR DOCKING STUDY OF NALKYL/ARYL-2-ARYL INDOL-3-YL GUYOXYLAMIDES AS NOVEL ANTICANCER AGENTS SYNT	INVESTIGATION OF ANTIOXIDANT ACTIVITY IN DIFFERENT SOLVENTS OF GNAPHALIUM POLYCAULON	870-87
COMPARATIVE STUDY ON THE EFFECT OF DIFFERENT SOLVENT EXTRACTS OF CALOTROPIS GIGANTEA AND CARGA PAPAYA LATEX AGAINST NEW BACTERIAL ISOLATES - AN IN VITRO STUDY RUISBO A, ARATI C, PRETAMRAJ JP, PANICKER SG PYROLOQUINOLINE QUINONE HAS THE POTENTIAL TO AMELIORATE PTU INDUCED LIPID PEROXIDATION AND OXIDATIVE DAMAGES IN NICE NARRNDRA KUMAR, ANAD KAR NERNDRA KUMAR, ANAD KAR NARRNDRA KUMAR, ANAD KAR NARRNDRA KUMAR, SL. HARIKUMAR ANTIDIABETIC ACTIVITY OF DIROLIN (A POLYHERRAL FORMULATION) IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS 893-8 RAJESH A MAHESHWARL SONYA KHATRI, R BALARAMAN, A K SETH ANTIDIABETIC ANTI-CANCER ACTIVITY OF CIRCIMMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES SYNERGISTIC ANTI-CANCER ACTIVITY OF CIRCIMMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES SYNERGISTIC, ANTI-CANCER ACTIVITY OF CIRCIMMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES SYNERGISTIC, ANTI-CANCER ACTIVITY OF CIRCIMMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES SYNERGISTIC, ANTI-CANCER ACTIVITY OF CIRCIMMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES SYNERGISTIC, ANTI-CANCER ACTIVITY OF CIRCIMMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES SYNERGISTIC, AND PHARMACEUTICALLY ACTIVE PYRIMIDINE AND FORMAMIDINE DERIVATIVES FROM 3-MINO- HEWMAT MOHAMMED DARDEER AND ABOU-BAKR HAREOL ABDEL-MONSEF ASSESSION DARDEER AND ABOU-BAKR HAREOL ABDEL-MONSEF ASSESSION ANALASIS BUNLEU SUNG THONG, CHRAPHA BUTIMAN, KUSUMA JITSAEN PHETACUCIC INTERPENSIS ROM MATERIA DARDER IOWALVER CONNITIONS ANAHWSH FEROZ, NICHAT RAZVI,SANA CHAVAS, FARKISHEENA ANUM, LUBBAR CHAZAL, SAED AHMAD SIDDIQUI OPTIMZED ULTRASONC-ASSISTED EXTRACTION OF ANTIONADAT FROM MULBERRY (MORUS ALBAL L.) LEAVES USING MULTIPLE LINEAR ERGRESSION ANALASIS SYNTHESIS AND MOLECULAR DOCKING STUDY OF NALKYL/ARYL-2-ARYL INDOL-3-YL GUYOXYLAMIDES AS NOVEL ANTICANCER AGENTS SYNT	SHANMIIGAPRIYA, K. JINII JIDHYABANII. THA THAYIMANAVAN	
HALTERIAL INDUATES - AN IN UTION OF ENTERIC CONTENTIAL TO AMELIORATE PTU INDUCED LIPID PEROXIDATION AND OXIDATIVE DAMAGES IN MICE PYRBOLOQUINOLINE QUINOME HAS THE POTENTIAL TO AMELIORATE PTU INDUCED LIPID PEROXIDATION AND OXIDATIVE DAMAGES IN MICE BORNEA KUMAR, ANAND KAR ORONULATION OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING NATURAL POLYMERS FOR COLON DRUG DELIVERY NERA SHARMA, S.L.HARIKUMAR ANTIDIABETIC ACTIVITY OF DIBOLIN (A POLYHERBAL FORMULATION) IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS BORNA KUMAR, AND I VALUATION OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING NATURAL POLYMERS FOR COLON DRUG DELIVERY NERA SHARMA, S.L.HARIKUMAR ANTIDIABETIC ACTIVITY OF DIBOLIN (A POLYHERBAL FORMULATION) IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS BORS BAGISHA MARESIWARI, SONIXA RHATRI, R BALARAMAN, A K SETH VALUATION OF HERZ/NEU OYER EXPRESSION IN BREAST CANCER KANAGATHARA.N, KAVITHA.K SYNERGISTIC ANTI-CANCER ACTIVITY OF CURCUMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES OLOGITHI, C. SENTHIL KUMAR, K KATHIRESAN SYNTHESISOF BIOLOGICALUY AND PHARMAGEUTICALLY ACTIVE PYRIMIDINE AND FORMAMIDINE DERIVATIVES FROM 3-AMINO- HERMAT MOHAMMED DARDEER AND ABOU-BAKR HAREDI ABDEL-MONSEF INSERSISTERI OF PHARMACEUTICAL QUALITY CONTIGU, AND EQUIVALINCE OF VARIOUS BRANDS OF AMILODIPINE BESYLATE (5 MG) TABLETS 909-9 WALHAGEL BIT HE PAKISTAM MARKET UNDER BIOWAVER CONDITIONS MAHWISH FERCZ, NIGHAT RAZVI,SANA GHAYAS, FAKHSHEENA ANJUM, LUBNA GHAZAL, SAEED AHMAD SIDDIQUI OPTIMZED ULTRE SONG ASSISTED FATRACTION OF ANTIONIDANT FROM MULBERRY (MOUS ALBAL ) LEAVES USING MULTIPLE LINEAR BUOLEU SUNGTHONG, CHIRAPHA BUTIMAN, KUSUMA JITSAENG FERTACULIC TRUTCERPISES FROM AMATEMIC GENUS AS ACCETYLCHOLINESTERASE INHIBITORS YATHESIS AND MOLECULAR DOCKING STUDY OF N-ALKYL/ARYL-2-ARYL INDOL-3-YL GIYONYLAMIDES AS NOVEL ANTICACRE AGENTS 918-9 EVALUEUS UNGTHONG, CHIRAPHA BUTIMAN, KUSUMA JITSAENG CHARACTERISATION AND IN-YTRO FOLA CHARACTERISATION AND DIVATION OF TERMINALI	COMPARATIVE STUDY ON THE EFFECT OF DIFFERENT SOLVENT EXTRACTS OF CALOTROPIS GIGANTEA AND CARICA PAPAYA LATEX AGAINST NEW	074 07
PYRROLOQUINOLINE QUINONE HAS THE POTENTIAL TO AMELIORATE PTU INDUCED LIPID PEROXIDATION AND OXIDATIVE DAMAGES IN MICE SAGAREDRA KUMAR, ANAND KAR (CONTROL AND CONTROL CONTROL AND CONTROL	BACTERIAL ISOLATES - AN IN VITRO STUDY	8/4-8/
NARENDRA KUMAR, ANAND KAR " CORNULATION AND EVALUATION OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING NATURAL POLYMERS FOR COLON DRUG DELIVERY " REMA SHARMA, S.L.HARIKUMAR " ANTIDIABETIC ACTIVITY OF DIBOLIN (A POLYHERBAL FORMULATION) IN STREFTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS SP3-8 RAPESH A MARESHWARI, SONYA KHATRI, B RALARAMAN, A K SETH EVALUATION OF HERZ/NEU OVER EXPRESSION IN BREAST CANCER B96-9 KANAGATHARA, KAVITHA.K SVERGISTIC ANTI-CANCER ACTIVITY OF CURCUMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES 901-9 C. MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN SYNTHESISTOF BIOLOGICALLY AND PHARMACRUTICALLY ACTIVE PYRIMIDINE AND FORMAMIDINE DERIVATIVES FROM 3-AMINO- ILIVES FORM J-ALIVITY OF CURCUMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES 901-9 C. MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN SYNTHESISTOF BIOLOGICALLY AND PHARMACRUTICALLY ACTIVE PYRIMIDINE AND FORMAMIDINE DERIVATIVES FROM 3-AMINO- ILIVE JAHI AJA JI, DIN JOHI-S, ILO-DBENZOPYRIMIDINO 14, 5-BINAPTHALIN-2, 4-DIONE HEMMAT MOHAMMED DARDEER AND BADU-BARR HAREDI ABDEL-MONSEF ASSESSMENT OF PHARMACCIVICOL OUNTOL, AND FOUVALENCE OF VARIOUS BRANDS OF AMLODIPINE BESYLATE (S MG) TABLETS 900-9 PHARMACUTICAL QUALTY CONTROL AND FOUVALENCE OF VARIOUS BRANDS OF AMLODIPINE BESYLATE (S MG) TABLETS 901-9 PHARMONAMED DARDEER AND ABOU-BARR HAREDI ABDEL-MONSEF SUNTA SUSSISSMENT OF PHARMACCIVICAL QUARTINE C ONDITIONS MAHWISH FEROZ, NIGHAT RAZVI,SANA GHAYAS, FAKHSHEENA ANJUM, LUBNA GHAZAL, SAEED AHMAD SIDDIQUI 909-9 PHATAZO LITRASONIC-ASSISTED EXTRACTION OF ANTINXIDANT FROM MULBERRY ( <i>MORUS ALEA L</i> ) LEAVES USING MULTIPLE LINEAR 914-9 SUNLEU SUNCHONG, CHRAPHA BUTIMAN, KUSUMA JITSAENG PENTACYCLIC TRITERPENES FROM <i>MATTENUS</i> GENUS AS ACETYLCHOLINESTERASE INHIBITORS 914-9 SUNLEU SUNCHONG, CHRAPHA BUTIMAN, KUSUMA JITSAENG 927-9 SUNAU BADUGANU, TAYO FOLA. 914-9 SUNLEU SUNCHONG, CHRAPHA BUTIMAN, KUSUMA JITSAENG 927-9 SUNAU SUNCH SUNCH SUNDA V. SUNAA, N. PRASHANTHA 914-15 STHLE COST OF LANTICAS STUDY OF MALKYI, ARYL-2, ARYL		000.00
BORNULATION AND EVALUATION OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING NATURAL POLYMERS FOR COLON DRUG     886-8       VEHA SHARMA, S.L.HARIKUMAR     886-8       ANTIDABETIC ACTIVITY OF DIBOLIN (A POLYHERBAL FORMULATION) IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS     893-8       RAJESH A MAHESHWARI, SONYA KHATR, R BALARAMAN, A K SETH     886-9       VALUATION OF HEXZ/NEU OVER EXPRESSION IN BREAST CANCER     898-9       KANAGATHARA,N, KAVITHA.K     801-9       SYNTHESISOF BIOLOGICALLY AND PHARMACEUTICALLY ACTIVE PYRIMIDINE AND FORMAMIDINE DERIVATIVES FROM 3-AMINO- IN-2U ADMARKET UNDER AND REDUXOPYRIMIDINO 14,5-BINATTIALIN-2-DIONE     904-9       HIRMAT MOHAMMED DARDER AND ABOU-BAKE HAREDI ABDEL-MONSEF     895-9       XALLABLE OF PHARMACEUTICAL, QUALITY CONTROL, AND EQUIVALENCE OF VARIOUS BRANDS OF AMLODIPINE BESYLATE (S MG) TABLETS     909-9       MALHABLE AND ABOULAKER HAREDI ABDEL-MONSEF     845-83       SENSENSENT OF PHARMACEUTICAL, QUALITY CONTROL, AND EQUIVALENCE OF VARIOUS BRANDS OF AMLODIPINE BESYLATE (S MG) TABLETS     909-9       MALHABLE AND ABACE AND ABOUL-BAKE HAREDI ABDEL-MONSEF     914-9       MALHABLE AND ARKET UNDER BLOWATER CONDITIONS     914-9       MALHABLE VALUEANE DARGE AND ABOULAKER HAREDI ABDEL-MONSEF     914-9       MALHABLE ANA ANAYSIS     914-9       BUNLEU SUNGTHONG, CHIRAPHA BUTTMAN, KUSUMA JITSAENG     914-9       SUNESSA C. BORGIGUES, FERNANDO C. SILVA, GENIUS AS ACCTVLCHOLINESTERASE INHIBITORS     914-9       SUNSSA G. RONGI		880-88
DELIVERY     865-3       VEHA SHARMA, S.L.HARIKUMAR     865-3       VEHA SHARMA, SL.HARIKUMAR     893-3       VEHA SHARMA, SL.HARIKUMAR     893-3       VEHA SHARMA, SONIYA KHATRI, R BALARAMAN, A K SETH     894-3       VALLATION OF HERZ/NEU OVER EXPRESSION IN BREAST CANCER     894-3       VANCATHARA, KAUTHALA     894-3       SYNERGISTIC ANTI-CANCER ACTIVITY OF CURCUMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES     901-9       MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN     904-9       MONGTHI, C. SENTHIL KUMAR, K. KATHIRESAN     904-9       I.H.H.H.J.SH. JOH JOAN'S, JO-D BENZOPYRIMIDINO (4,5-B)NAPTHALIN-2,4-DIONE     904-9       HEMMART MOHAMMED DARDER AND BADU-BAKR HARDI ABDEL-MONSEF     904-9       VAILABLE IN THE PARKISTANI MARKET UNDER BIOWAIVER CONDITIONS     909-9       VAILABLE IN THE PARKISTANI MARKET UNDER BIOWAIVER CONDITIONS     909-9       VAILABLE IN THE PARKISTANI MARKET UNDER BIOWAIVER CONDITIONS     914-9       VAILABLE IN THE PARKISTANI MARKET UNDER BIOWAIVER CONDITIONS     914-9       VAILABLE IN THE PARKISTANI MARKET UNDER BIOWAIVER CONDITIONS     914-9       VAILABLE IN THE PARKISTANI MARKET UNDER BIOWAIVER CONDITIONS     914-9       VAILABLE IN THE PARKISTANI MARKET UNDER BIOWAIVER CONDITIONS     914-9       VAILABLE IN THE PARKISTANI MARKET UNDER BIOWAIVER CONDITIONS     914-9       VILLABLE IN THE PARKISTANI MARKET UNDER BIOWAIVER CONDITIONS		
INTIDIABETIC ACTIVITY OF DIBOLIN (A POLYHERBAL FORMULATION) IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS 893-8 KAJESH A MAHESHWARI, SONIYA KHATRI, R BALARMAN, A K SETH SYALUATION OF HERZ/NEU OVER EXPRESSION IN BREAST CANCER 898-9 ANAGATHARAN, KAUTHAK 899-9 ANAGATHARAN, KAUTHAK 990-9 ANAGATHARAN, KAUTHAK 990-9 ANAGATHARAN, KAUTHAK 900-9 2. MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN 910-9 2. MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN 910-9 1. MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN 910-9 1. MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN 910-9 1. MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN 900-9 1. MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN 910-9 1. MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN 900-9 1. MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN 900-9 1. MOORTHI, C. SENTHIL KUMAR, K. SATHIREDI ABDEL-MONSEF SESSINGTO, MAINTAN, MARKET UNDER BIOWAIVER CONDITIONS 4. MINISH FEROZ, NIGHAT RAZVI, SANA GHAYAS, FAKHSHEENA ANJUM, LUBAR GHAZAL, SAEED AHMAD SIDDIQUI 909-9 1. MILEU SUNGTHONG, CHIRAPHA BUTIMAN, KUSUMA JITSAENG ***TACYCLIC TRITERPENES FROM MATEAUXIS CENUS AS ACETYLCHOLINESTERASE INHIBITORS 911-9 3. UNLEU SUNGTHONG, CHIRAPHA BUTIMAN, KUSUMA JITSAENG ***TACYCLIC TRITERPENES FROM MATEAUXIS CENUS AS ACETYLCHOLINESTERASE INHIBITORS 911-9 3. MAEDISA C. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE, JACQUELINE A. TAKAHASHL, BIBANE L. G. MATILDES, GRÁCIA D. E. 1. MARDETHIK, R. SILVA, SINDEY A. VIERRAF LIHD 914-9 3. MAEDISA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE, JACQUELINE A. TAKAHASHL, BIBANE L. G. MATILDES, GRÁCIA D. E. 1. MATHINANORTHY, C. THILAGAVATHI 914-9 3. MAEDISA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE, JACQUELINE A. TAKAHASHL, BIDARE L. G. MATILDES, GRÁCIA D. E. 1. MARACTERIZATION AND IN-VITRO EVALUATION OF		886-89
RAJESH A MAHESHWARI, SONIYA KHATRI, R BALARAMAN, A K SETH VALUATION OF HERZ/NEU OVER EXPRESSION IN BREAST CANCER 8898-9 (ANAGATHARA.N, KAVITHA.K VYALUEALYNEU OVER EXPRESSION IN BREAST CANCER 8091-9 2. MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN SYNTHESSOF BIOLOGICALLY AND PHARMACCUTICALLY ACTIVE PYRIMIDINE AND FORMAMIDINE DERIVATIVES FROM 3-AMINO- 1. MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN SYNTHESSOF BIOLOGICALLY AND PHARMACCUTICALLY ACTIVE PYRIMIDINE AND FORMAMIDINE DERIVATIVES FROM 3-AMINO- 1. MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN SYNTHESSOF BIOLOGICALLY AND PHARMACCUTICALLY ACTIVE PYRIMIDINE AND FORMAMIDINE DERIVATIVES FROM 3-AMINO- 1. H. 21, H4, H4, H5, 11, 10, 11, AH-5, 10-O- BERUOPYRIMIDINO-(H, 5- SINATHALIN-2, 4-DIONE HEMMAT MOHAMMED DARDEER AND ABOU-BAKR HAREDI ABDEL-MONSEF SSSESSMENT OF PHARMACEUTICAL QUALITY CONTROL AND EQUIVALENCE OF VARIOUS BRANDS OF AMLODIPINE BESYLATE (5 MG) TABLETS WALHWISH FEROZ, NIGHAT RAZVI,SANA GHAYAS, FAKHSHEENA ANJUM, LUBNA GHAZAL, SAEED AHMAD SIDDIQUI PTIMIZED ULTRASONIC-ASSISTED EXTRACTION OF ANTIOXIDANT FROM MULBERRY ( <i>MORUS ALBA</i> L.) LEAVES USING MULTIPLE LINEAR TEXTACYCLIC TRITERPENES FROM MAYTENUS GENUS AS ACETYLCHOLINESTERASE INHIBITORS 3UNLEU SUNGTHONG, CHIRAPHA BUTIMAN, KUSUMA JITSAENG TEXTACYCLIC TRITERPENES FROM MAYTENUS GENUS AS ACETYLCHOLINESTERASE INHIBITORS 3UNLEU SUNGTHONG, CHIRAPHA BUTIMAN, KUSUMA JITSAENG TEXTACYCLIC TRITERPENES FROM MAYTENUS GENUS AS ACETYLCHOLINESTERASE INHIBITORS 3UNLEU SUNGTHONG, CHIRAPHA BUTIMAN, NUSUMA JITSAENG TEXTACYCLIC TRITERPENES FROM MAYTENUS GENUS AS ACETYLCHOLINESTERASE INHIBITORS 3UNLEU SUNGTHONG, CHIRAPHA BUTIMAN, NUSUMA JITSAENG TEXTACYCLIC TRITERPENES FROM MAYTENUS GENUS AS ACETYLCHOLINESTERASE INHIBITORS 3UNLEU SUNGTHONG, CHIRAPHA BUTIMAN, NUSUMA JITSAENG TEXTACYCLIC TRITERPENES FROM AMAYTENUS GENUS AS ACETYLCHOLINESTERASE INHIBITORS 3UNLEU SUNGTHONG, CHIRAPHA BUTIMAN, NUSUMA JITSAENG TEXTACYCLIC TRITERPENES FROM AMAYTENUS GENUS AS ACETYLCHOLINESTERASE INHIBITORS 3UNLEU SUNGTHONG, CHIRAPHA DO		
NALUATION OF HER2/NEU OVER EXPRESSION IN BREAST CANCER     898-9       CANAGATHARAN, KAUTTHA.K     91-9       YNERGISTIC ANTI-CANCER ACTIVITY OF CURCUMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES     91-9       MOORTHIL, C. SENTHIL KUMAR, K. KATHIRESAN     904-9       HI 2H, 4H, AAH, 5H, JOH, JOAH-S, IO-O BENZOPYRIMIDINC-[4,5-BINAPTHALIN-2,4-DIONE     90-9       HI 2H, 4H, AAH, SH, JOH, JOAH-S, IO-O BENZOPYRIMIDINC-[4,5-BINAPTHALIN-2,4-DIONE     90-9       NAILABLE IN THE PAKINTAN MARKET UNDER BIOWAIVER CONDITIONS     90-9       NAILABLE IN THE PAKINTAN MARKET UNDER BIOWAIVER CONDITIONS     91-9       NAILABLE IN THE PAKINTAN MARKET UNDER BIOWAIVER CONDITIONS     91-9       YNTHESISOF BIOLOGICALLY AND PHARMACEUTICAL UNDATTE CONDITIONS     91-9       NAILABLE IN THE PAKINTAN MARKET UNDER BIOWAIVER CONDITIONS     91-9       YNTHESISOF MONG-SSISTED EXTRACTION OF ANTIOXIDANT FROM MULBERRY (MORUS ALBA L.) LEAVES USING MULTIPLE LINEAR     91-9       YNTHESIS ON DAUSTIONS, CHRAPHA BUTIMAN, KUSUMA JITSAENG     91-9       YNTHESIS AG NOBRIGUES, FERNANDO C. SULVA, LUCENIR P. DUARTE-JACQUELINE A. TAKAHASHI,BIBIANE L. G. MATILDES, GRÁCIA D. F.     91-9       YNTHESIS AG NOBRIGUES, STOPT PUT II DIABETES MELLITUS IN A DEVELOPING ECONOMYZ     92-9       YNTHESIS AG NOBRIGUES, FERNANDA C. SULVA, LUCENIR P. DUARTE-JACQUELINE A. TAKAHASHI,BIBIANE L. G. MATILDES, GRÁCIA D. F.     92-9       YNTHESIS AG NOBLECULAR DOCKING STUDY OF A-ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS		893-89
ANAGATHARA.N, KAVITHA.K		898-90
C. MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN SYNTHESISOF BIOLOGICALLY AND PHARMACEUTICALLY ACTIVE PYRIMIDINE AND FORMAMIDINE DERIVATIVES FROM 3-AMINO- [H,2H,4H,5H,5H,10H,10AH-5,10-0-BENZOPYRIMIDINO.[4,5-B]NAPTHALIN-2,4-DIONE HERMAT MOHAMMED DARDEER AND ABOU-BAKR HAREDI ABDEL-MONSEF SSSESSMENT OF PHARMACEUTICAL QUALITY CONTROL AND EQUIVALENCE OF VARIOUS BRANDS OF AMLODIPINE BESYLATE (5 MG) TABLETS NAALABLE IN THE PAKISTANI MARKET UNDER BIOWAIVER CONDITIONS MAHWISH FERKZ, NIGHAT RAZVI,SANA CHAYAS, FAKHSHEENA ANJUM, LUBNA GHAZAL, SAEED AHMAD SIDDIQUI OPTIMIZED ULTRASONIC-ASSISTED EXTRACTION OF ANTIOXIDANT FROM MULBERRY ( <i>MORUS ALBA</i> L.) LEAVES USING MULTIPLE LINEAR PRIACYCLIC TRITERPENES ROM <i>MAIYTENUS</i> GENUS AS ACETYLCHOLINESTERASE INHIBITORS 3UNLEU SUNGTHONG, CHIRAPHA BUTIMAN, KUSUMA JITSAENG PETACYCLIC TRITERPENES FROM <i>MAIYTENUS</i> GENUS AS ACETYLCHOLINESTERASE INHIBITORS ANESSA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE, JACQUELINE A. TAKAHASHI, BIBIANE L. G. MATILDES, GRÁCIA D. F. SILVA, ROQUELINE R. SILVA, SIDNEY A. VIERA-FILHO YNTHESIS AD MOLECULAR DOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS YNTHESIS AD MOLECULAR DOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS YNTHESIS AD MOLECULAR DOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS YNTHESIS AD MOLECULAR DOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS YNTHESIS AD MOLECULAR DOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS YNTHESIS AD MOLECULAR TOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS YNTHESIS AD MOLECULAR TOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS YNTHESIS AD MOLECULAR TOCKING STUDY OF MALTIRA HOLYNC (TAGUACULAR ACTERISTICS OF SOLID LIPID YNTHESIS AND MOLECULAR TOCKING STUDY OF MALTIRA HOLYNC (TAGUACULAR AND GLYCERYL MONOSTE		
SYNTHESISOF BIOLOGICALLY AND PHARMACEUTICALLY ACTIVE PYRIMIDINE AND FORMAMIDINE DERIVATIVES FROM 3-AMINO: 1H.2H.4H.4AH.SH.10H.10AH-5.10-0-BENXOPYRIMIDINO-14.5-BINAPTHALIN-2.4-DIONE HEMMAT MOHAMMED DARDEER AND ABOU-BAKR HAREDI ABDEL-MONSEF ASSESSMENT OF PHARMACEUTICAL QUALITY CONTROL AND EQUIVALENCE OF VARIOUS BRANDS OF AMLODIPINE BESYLATE (5 MG) TABLETS AVAILABLE IN THE PAKISTANI MARKET UNDER BIOWAIVER CONDITIONS WAHWISH FEROZ, NICHAT RAZVI,SANA GHAVAS, FAKHSHEENA ANJUM, LUBNA GHAZAL, SAEED AHMAD SIDDIQUI OPTIMIZED ULTRASONIC-ASSISTED EXTRACTION OF ANTIOXIDANT FROM MULBERRY ( <i>MORUS ALBA</i> L.) LEAVES USING MULTIPLE LINEAR BUNLEU SUNGTHONG, CHIRAPHA BUTIMAN, KUSUMA JITSAENG PENTACYCLIC TRITERPENES FROM <i>MAYTENUS</i> GENUS AS ACETYLCHOLINESTERASE INHIBITORS UNAESSA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE, JACQUELINE A. TAKAHASH, BIBIANE L. G. MATILDES, GRÁCIA D. F. SIVAROQUELINE R. SILVA, SIDNEY A. VIEIRA-FILHO SYNTHESIS AND MOLECULAR DOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS SYNTHESIS AND MOLECULAR DOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS SYNTHESIS AND MOLECULAR DOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS SYNTHESIS AND MOLECULAR DOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS SYNTHESIS AND MOLECULAR DOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS SYNTHESIS AND MOLECULAR DOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS SYNTHESIS AND MOLECULAR DOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS SYNTHESIS AND MOLECULAR DOCKING STUDY OF TERMINALIA CHEBULA EXTRACT FOR ANTIBACTERIAL POTENTIAL CHARACTERIZATION AND IN-VITRO EVALUATION OF TERMINALIA CHEBULA EXTRACT FOR ANTIBACTERIAL POTENTIAL CHARACTERIZATION AND IN-VITRO FUALUATION OF TERMINALING CAMON NORMAR ROSITA, DWI SETVAWAN, WIDI	SYNERGISTIC ANTI-CANCER ACTIVITY OF CURCUMIN AND BIO-ENHANCERS COMBINATION AGAINST VARIOUS CANCER CELL LINES	901-90
1H, 2H, 4H, 4AH, 5H, 10H, 10AH-5, 10-0-BENZOPYRIMIDINO-[4, 5-B]NAPTHALIN-2, 4-DIONE       904-93         HERMART MOHAMMED DARDEER AND ABOU-BARR HAREDI ABDEL-MONSEF       ASSESSMENT OF PHARMACEUTICAL QUALITY CONTROL AND EQUIVALENCE OF VARIOUS BRANDS OF AMLODIPINE BESYLATE (5 MG) TABLETS       909-9         ASSESSMENT OF PHARMACEUTICAL QUALITY CONTROL AND EQUIVALENCE OF VARIOUS BRANDS OF AMLODIPINE BESYLATE (5 MG) TABLETS       901-9         MAILABLE IN THE PARISTANI MARKET UNDER BIOWAIVER CONDITIONS       904-94         MARWISH FEROZ, NIGHAT RAZVI,SANA GHAVAS, FAKHSHEENA ANJUM, LUBNA GHAZAL, SAEED AHMAD SIDDIQUI       914-94         POTIMIZED ULTRASONIC-ASSISTED EXTRACTION OF ANTIOXIDANT FROM MULBERRY ( <i>MORUS ALEAL</i> L) LEAVES USING MULTIPLE LINEAR       914-95         BUNLEU SUNGTHONG, CHIRAPHA BUTIMAN, KUSUMA JITSAENG       918-90         VANESSA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE, JACQUELINE A. TAKAHASHLBIBIANE L. G. MATILDES, GRÁCIA D. F.       918-90         SUVAROULINE R. SILVA, SIDNEY A. VIERAFILHO       918-90         SYNTHESIS AND MOLECULAR DOCKING STUDY OF N-ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS       921-91         N. M. JAGADEESH, K. M. MAHADEVAN, M. N KUMARA, N. PRASHANTHA       927-92         WHAT IS THE COST OF ILLNESS OF TYPE II DIABETES MELLITUS IN A DEVELOPING ECONOMY?       927-93         GIWA ABDULGANIYU, TAYO FOLA       927-94         CHARACTERISATION AND IN-VITRO EVALUATION OF TERMINALIA CHEBULA EXTRACT FOR ANTIBACTERIAL POTENTIAL      932-93	C. MOORTHI, C. SENTHIL KUMAR, K. KATHIRESAN	
HERMAT MOHAMMED DARDEER AND ABOU-BAKR HAREDI ABDEL-MONSEF ASSESSMENT OF PHARMACEUTICAL QUALITY CONTROL AND EQUIVALENCE OF VARIOUS BRANDS OF AMLODIPINE BESYLATE (5 MG) TABLETS AVAILABLE IN THE PARISTANI MARKET UNDER BIOWAIVER CONDITIONS MAHWISH FEROZ, NIGHAT RAZVI,SANA GHAYAS, FAKHSHEENA ANJUM, LUBNA GHAZAL, SAEED AHMAD SIDDIQUI OPTIMIZED ULTRASONIC-ASSISTED EXTRACTION OF ANTIOXIDANT FROM MULBERRY ( <i>MORUS ALBA</i> L.) LEAVES USING MULTIPLE LINEAR PREGRESSION ANALYSIS BUNLEU SUNGTHONG, CHIRAPHA BUTIMAN, KUSUMA JITSAENG PENTACYCLIC TRITERPENES FROM <i>MAYTENUS</i> GENUS AS ACETYLCHOLINESTERASE INHIBITORS VARESSA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE,JACQUELINE A. TAKAHASHI,BIBIANE L. G. MATILDES,GRÁCIA D. F. SILVA,ROQUELINE R. SILVA, SIDNEY A. VIEIRA-FILHO SYNTHESIS AND MOLECULAR DOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS 921-92 GWA ABDULGANIYU, TAYO FOLA CHARACTERISATION AND IN-VITRO EVALUATION OF TERMINALIA CHEBULA EXTRACT FOR ANTIBACTERIAL POTENTIAL R. RATHINAMOORTHY, G. THILAGAVATHI PHYSICAL CHARACTERIZATION OF BESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID PANDORATICE (SLN) LOADED PARA METHOXY CINNAMIC ACID (PMCA) NOORMA ROSTA, DWI SETYAWAN, WIDJI SOERATRI, SUWALDI MRTODIHARDJO COMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION PAULASIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION 930-93 PORMULATION AND EVALUATION OF ONCE DAILY OSMOTIC TABLET OF KETOPROFEN POEHAN MHG, KAZI MS, ANSARI MA.		904-90
ASSESSMENT OF PHARMACEUTICAL QUALITY CONTROL AND EQUIVALENCE OF VARIOUS BRANDS OF AMLODIPINE BESYLATE (5 MG) TABLETS AVAILABLE IN THE PARISTANI MARKET UNDER BIOWAIVER CONDITIONS WAHWISH FEROZ, NIGHAT RAZVI,SANA GHAYAS, FAKHSHEENA ANJUM, LUBNA GHAZAL, SAEED AHMAD SIDDIQUI OPTIMIZED ULTRASONIC-ASSISTED EXTRACTION OF ANTIOXIDANT FROM MULBERRY ( <i>MORUS ALBA</i> L.) LEAVES USING MULTIPLE LINEAR POPTIMIZED ULTRASONIC-ASSISTED EXTRACTION OF ANTIOXIDANT FROM MULBERRY ( <i>MORUS ALBA</i> L.) LEAVES USING MULTIPLE LINEAR POPTIMIZED ULTRASONIC-ASSISTED EXTRACTION OF ANTIOXIDANT FROM MULBERRY ( <i>MORUS ALBA</i> L.) LEAVES USING MULTIPLE LINEAR PENTACYCLIC TRITERPENES FROM <i>MATTENUS</i> GENUS AS ACETYLCHOLINESTERASE INHIBITORS BUNLEU SUNGTHONG, CHIRAPHA BUTIMAN, KUSUMA JITSAENG PENTACYCLIC TRITERPENES FROM <i>MATTENUS</i> GENUS AS ACETYLCHOLINESTERASE INHIBITORS VANESSA G. RODRIQUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE, JACQUELINE A. TAKAHASHI, BIBIANE L. G. MATILDES, GRÁCIA D. F. SILVA, ROQUELINE R. SILVA, SIDNEY A. VIEIRA-FILHO SYNTHESIS AND MOLECULAR DOCKING STUDY OF N-ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS SYNTHESIS AND MOLECULAR DOCKING STUDY OF N-ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS SYNTHESIS AND MOLECULAR TOFILLNESS OF TYPE II DIABETES MELLITUS IN A DEVELOPING ECONOMY? GIVA ABDULGANIYU, TAYO FOLA CHARACTERIZATION OF EVALUATION OF TERMINALIA CHEBULA EXTRACT FOR ANTIBACTERIAL POTENTIAL CHARACTERIZATION OF BEESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID NANOPARTICLE (SLN) LOADED PARA METHOXY CINNAMIC ACID (PMCA). NOORMA ROSITA, DWI SETYAWAN, WIDJI SOERATRI, SUWALDI MRTODIHARDJO COMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION STUDIES AKILAN-C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL C.S, SUNDARA MAHALINGAM.MA FORMULATION AND EVALUATION OF ONCE DAILY OSMOTIC TABLET OF KETOPROFEN DEHGHAN MHG, KAZI MS, ANSARI MA.		
available in The Parkstani Marker Under Blowalver Conditions         wahwish FEROZ, NIGHAT RAZVI,SANA GHAYAS, FAKHSHEENA ANJUM, LUBNA GHAZAL, SAEED AHMAD SIDDIQUI         OPTIMIZED ULTRASONIC-ASSISTED EXTRACTION OF ANTIOXIDANT FROM MULBERRY (MORUS ALBA L.) LEAVES USING MULTIPLE LINEAR       914-9         BUNLEU SUNGTHONG, CHIRAPHA BUTIMAN, KUSUMA JITSAENG       918-9         VANESSA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE,JACQUELINE A. TAKAHASHI,BIBIANE L. G. MATILDES,GRÁCIA D. F.       918-9         VANESSA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE,JACQUELINE A. TAKAHASHI,BIBIANE L. G. MATILDES,GRÁCIA D. F.       921-9         N. M. JAGADEESH, K. M. MAHADEVAN, M. N KUMARA, N. PRASHANTHA       927-9         SYNTHESIS AND MOLECULAR DOCKING STUDY OF N-ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS       921-9         N. M. JAGADEESH, K. M. MAHADEVAN, M. N KUMARA, N. PRASHANTHA       927-9         GIWA ABDULGANIYU, TAYO FOLA       927-9         GIWA ABDULGANIYU, TAYO FOLA       932-9         R. RATHINAMOORTHY, G. THILAGAVATHI       932-9         NANOPARTICLE (SLN) LOADED PARA METHOXY CINNAMIC ACID (PMCA).       939-9         NOORMA ROSITA, DWI SETYAWAN, WIDJI SOERATRI, SUWALDI MRTODIHARDJO       939-9         COMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION       946-9         YUDIES       AKILAN.C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL.C.S, SUNDARA MAHALINGAM.M	ASSESSMENT OF PHARMACEUTICAL QUALITY CONTROL AND EQUIVALENCE OF VARIOUS BRANDS OF AMLODIPINE BESYLATE (5 MG) TABLETS	000-01
OPTIMIZED ULTRASONIC-ASSISTED EXTRACTION OF ANTIOXIDANT FROM MULBERRY (MORUS ALBA L.) LEAVES USING MULTIPLE LINEAR       914-9         REGRESSION ANALYSIS       914-9         BUNLEU SUNGTHONG, CHIRAPHA BUTIMAN, KUSUMA JITSAENG       918-9         YANESSA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE, JACQUELINE A. TAKAHASHLBIBIANE L. G. MATILDES, GRÁCIA D. F.       918-9         YANESSA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE, JACQUELINE A. TAKAHASHLBIBIANE L. G. MATILDES, GRÁCIA D. F.       921-9         VANESSA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE, JACQUELINE A. TAKAHASHLBIBIANE L. G. MATILDES, GRÁCIA D. F.       921-9         V.M. JAGADEESH, K. M. MAHADEVAN, M. N KUMARA, N. PRASHANTHA       921-9         WHAT IS THE COST OF ILLNESS OF TYPE II DIABETES MELLITUS IN A DEVELOPING ECONOMY?       927-9         GIWA ABDULGANIYU, TAYO FOLA       932-9         CHARACTERISATION AND IN-VITRO EVALUATION OF TERMINALIA CHEBULA EXTRACT FOR ANTIBACTERIAL POTENTIAL       932-9         VANOPARTICLE (SLN) LOADED PARA METHOXY CINNAMIC ACID (PMCA).       930-9         VOORMA ROSITA, DWI SETYAWAN, WIDJI SOERATRI, SUWALDI MRTODIHARDJO       930-9         OMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION STUDIES       946-9         AKILAN.C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL.C.S, SUNDARA MAHALINGAM.M.A       951-9         ODEHGHAN MHG, KAZI MS, ANSARI MA.       951-9		505-51
914-9         914-9         914-9         914-9         914-9         914-9         914-9         914-9         914-9         914-9         914-9         914-9         914-9         914-9         920-00         920-01         920-01         920-01         920-01         920-01         921-01         921-01         921-01         921-01         921-01         921-02         921-02         921-03         921-03         921-04         921-05         921-07         921-07         921-08         921-09         921-09         921-01         921-02         921-03         921-04         921-05         921-05         921-01         921-02         921-02         921-03         921-04         921-04         921-05         921-04 <td></td> <td></td>		
PETTACYCLIC TRITERPENES FROM MAYTENUS GENUS AS ACETYLCHOLINESTERASE INHIBITORS       918-92         VANESSA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE, JACQUELINE A. TAKAHASHI, BIBIANE L. G. MATILDES, GRÁCIA D. F.       921-92         VANESSA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE, JACQUELINE A. TAKAHASHI, BIBIANE L. G. MATILDES, GRÁCIA D. F.       921-92         VANESSA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE, JACQUELINE A. TAKAHASHI, BIBIANE L. G. MATILDES, GRÁCIA D. F.       921-92         VANESSA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE, JACQUELINE A. TAKAHASHI, BIBIANE L. G. MATILDES, GRÁCIA D. F.       921-92         VAN JAGADEESH, K. M. MAHADEVAN, M. N. KUMARA, N. PRASHANTHA       927-92         GIWA ABDULGANIYU, TAYO FOLA       927-92         GIWA ABDULGANIYU, TAYO FOLA       932-92         VA. RARCTERIZATION AND IN-VITRO EVALUATION OF TERMINALIA CHEBULA EXTRACT FOR ANTIBACTERIAL POTENTIAL       932-92         X. RATHINAMOORTHY, G. THILAGAVATHI       939-92         PHYSICAL CHARACTERIZATION OF BEESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID       939-92         VOORMA ROSITA, DWI SETYAWAN, WIDJI SOERATRI, SUWALDI MRTODIHARDJO       946-92         COMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION STUDIES       946-92         AKILAN.C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL.C.S, SUNDARA MAHALINGAM.M.A       951-92         ODEHGHAN MHG, KAZI		914-91
VANESSA G. RODRIGUES, FERNANDO C. SILVA, LUCIENIR P. DUARTE, JACQUELINE A. TAKAHASHI, BIBIANE L. G. MATILDES, GRÁCIA D. F. SILVA, ROQUELINE R. SILVA, SIDNEY A. VIEIRA-FILHO SYNTHESIS AND MOLECULAR DOCKING STUDY OF <i>N</i> -ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS YN M. JAGADEESH, K. M. MAHADEVAN, M. N KUMARA, N. PRASHANTHA WHAT IS THE COST OF ILLNESS OF TYPE II DIABETES MELLITUS IN A DEVELOPING ECONOMY? GIWA ABDULGANIYU, TAYO FOLA CHARACTERISATION AND IN-VITRO EVALUATION OF TERMINALIA CHEBULA EXTRACT FOR ANTIBACTERIAL POTENTIAL. R. RATHINAMOORTHY, G. THILAGAVATHI PHYSICAL CHARACTERIZATION OF BEESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID NANOPARTICLE (SLN) LOADED PARA METHOXY CINNAMIC ACID (PMCA) NOORMA ROSITA, DWI SETYAWAN, WIDJI SOERATRI, SUWALDI MRTODIHARDJO COMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION STUDIES AKLAN.C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL.C.S, SUNDARA MAHALINGAM.M.A FORMULATION AND EVALUATION OF ONCE DAILY OSMOTIC TABLET OF KETOPROFEN DEHGHAN MHG, KAZI MS, ANSARI MA.	BUNLEU SUNGTHONG, CHIRAPHA BUTIMAN, KUSUMA JITSAENG	
SILVA,ROQUELINE R. SILVA, SIDNEY A. VIEIRA-FILHO SYNTHESIS AND MOLECULAR DOCKING STUDY OF N-ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS SYNTHESIS AND MOLECULAR DOCKING STUDY OF N-ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS SYNTHESIS AND MOLECULAR DOCKING STUDY OF N-ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS SYNTHESIS AND MOLECULAR DOCKING STUDY OF N-ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS SYNTHESIS AND MOLECULAR DOCKING STUDY OF N-ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS WHAT IS THE COST OF ILLNESS OF TYPE II DIABETES MELLITUS IN A DEVELOPING ECONOMY? SIVA ABDULGANIYU, TAYO FOLA CHARACTERISATION AND IN-VITRO EVALUATION OF TERMINALIA CHEBULA EXTRACT FOR ANTIBACTERIAL POTENTIAL PHYSICAL CHARACTERIZATION OF BEESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID NANOPARTICLE (SLN) LOADED PARA METHOXY CINNAMIC ACID (PMCA) NOORMA ROSITA, DWI SETYAWAN, WIDJI SOERATRI, SUWALDI MRTODIHARDJO COMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION STUDIES AKILAN.C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL.C.S, SUNDARA MAHALINGAM.M.A FORMULATION AND EVALUATION OF ONCE DAILY OSMOTIC TABLET OF KETOPROFEN DEHGHAN MHG, KAZI MS, ANSARI MA.		918-92
SYNTHESIS AND MOLECULAR DOCKING STUDY OF N-ALKYL/ARYL-2-ARYL INDOL-3-YL GLYOXYLAMIDES AS NOVEL ANTICANCER AGENTS 921-9. N. M. JAGADEESH, K. M. MAHADEVAN, M. N KUMARA, N. PRASHANTHA 927-9. GIWA ABDULGANIYU, TAYO FOLA 927-9. GIWA ABDULGANIYU, TAYO FOLA 932-9. R. RATHINAMOORTHY, G. THILAGAVATHI 932-9. R. RATHINAMOORTHY, G. THILAGAVATHI 932-9. NOORMA ROSITA, DWI SETYAWAN, WIDJI SOERATRI, SUWALDI MRTODIHARDJO 939-9. NOORMA ROSITA, DWI SETYAWAN, WIDJI SOERATRI, SUWALDI MRTODIHARDJO 934-9. AKILAN.C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL.C.S, SUNDARA MAHALINGAM.M.A FORMULATION AND EVALUATION OF ONCE DAILY OSMOTIC TABLET OF KETOPROFEN 951-9. DEHGHAN MHG, KAZI MS, ANSARI MA.		
WHAT IS THE COST OF ILLNESS OF TYPE II DIABETES MELLITUS IN A DEVELOPING ECONOMY?       927-93         GIWA ABDULGANIYU, TAYO FOLA       932-91         CHARACTERISATION AND IN-VITRO EVALUATION OF TERMINALIA CHEBULA EXTRACT FOR ANTIBACTERIAL POTENTIAL       932-91         R. RATHINAMOORTHY, G. THILAGAVATHI       939-91         PHYSICAL CHARACTERIZATION OF BEESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID       939-91         NANOPARTICLE (SLN) LOADED PARA METHOXY CINNAMIC ACID (PMCA).       939-92         NOORMA ROSITA, DWI SETYAWAN, WIDJI SOERATRI, SUWALDI MRTODIHARDJO       946-91         COMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION TO 946-91       946-92         STUDIES       946-92       946-92         AKILAN.C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL.C.S, SUNDARA MAHALINGAM.M.A       951-92         CORMULATION AND EVALUATION OF ONCE DAILY OSMOTIC TABLET OF KETOPROFEN       951-92         DEHGHAN MHG, KAZI MS, ANSARI MA.       951-93		921-92
GIWA ABDULGANIYU, TAYO FOLA CHARACTERISATION AND IN-VITRO EVALUATION OF TERMINALIA CHEBULA EXTRACT FOR ANTIBACTERIAL POTENTIAL CHARACTERISATION AND IN-VITRO EVALUATION OF TERMINALIA CHEBULA EXTRACT FOR ANTIBACTERIAL POTENTIAL PHYSICAL CHARACTERIZATION OF BEESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID PHYSICAL CHARACTERIZATION OF BEESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID PHYSICAL CHARACTERIZATION OF BEESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID PHYSICAL CHARACTERIZATION OF BEESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID PHYSICAL CHARACTERIZATION OF BEESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID PHYSICAL CHARACTERIZATION OF BEESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID PHYSICAL CHARACTERIZATION OF BEESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID PHYSICAL CAS NOT SETYAWAN, WIDJI SOERATRI, SUWALDI MRTODIHARDJO COMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION STUDIES AKILAN.C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL.C.S, SUNDARA MAHALINGAM.M.A FORMULATION AND EVALUATION OF ONCE DAILY OSMOTIC TABLET OF KETOPROFEN DEHGHAN MHG, KAZI MS, ANSARI MA.	N. M. JAGADEESH, K. M. MAHADEVAN, M. N KUMARA, N. PRASHANTHA	
CHARACTERISATION AND IN-VITRO EVALUATION OF TERMINALIA CHEBULA EXTRACT FOR ANTIBACTERIAL POTENTIAL       932-92         R. RATHINAMOORTHY, G. THILAGAVATHI       939-92         PHYSICAL CHARACTERIZATION OF BEESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID NANOPARTICLE (SLN) LOADED PARA METHOXY CINNAMIC ACID (PMCA).       939-92         NOORMA ROSITA, DWI SETYAWAN, WIDJI SOERATRI, SUWALDI MRTODIHARDJO       946-92         COMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION STUDIES       946-92         AKILAN.C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL.C.S, SUNDARA MAHALINGAM.M.A       951-92         FORMULATION AND EVALUATION OF ONCE DAILY OSMOTIC TABLET OF KETOPROFEN       951-92         DEHGHAN MHG, KAZI MS, ANSARI MA.       951-92	WHAT IS THE COST OF ILLNESS OF TYPE II DIABETES MELLITUS IN A DEVELOPING ECONOMY?	927-93
R. RATHINAMOORTHY, G. THILAGAVATHI PHYSICAL CHARACTERIZATION OF BEESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID NANOPARTICLE (SLN) LOADED PARA METHOXY CINNAMIC ACID (PMCA). NOORMA ROSITA, DWI SETYAWAN, WIDJI SOERATRI, SUWALDI MRTODIHARDJO COMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION STUDIES AKILAN.C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL.C.S, SUNDARA MAHALINGAM.M.A FORMULATION AND EVALUATION OF ONCE DAILY OSMOTIC TABLET OF KETOPROFEN DEHGHAN MHG, KAZI MS, ANSARI MA.		
PHYSICAL CHARACTERIZATION OF BEESWAX AND GLYCERYL MONOSTEARAT BINARY SYSTEM TO PREDICT CHARACTERISTICS OF SOLID LIPID       939-9.         NANOPARTICLE (SLN) LOADED PARA METHOXY CINNAMIC ACID (PMCA).       939-9.         NOORMA ROSITA, DWI SETYAWAN, WIDJI SOERATRI, SUWALDI MRTODIHARDJO       946-9.         COMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION       946-9.         STUDIES       946-9.         AKILAN.C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL.C.S, SUNDARA MAHALINGAM.M.A       951-9.         CORMULATION AND EVALUATION OF ONCE DAILY OSMOTIC TABLET OF KETOPROFEN       951-9.         DEHGHAN MHG, KAZI MS, ANSARI MA.       951-9.		932-93
NANOPARTICLE (SLN) LOADED PARA METHOXY CINNAMIC ACID (PMCA).       933-9.         NOORMA ROSITA, DWI SETYAWAN, WIDJI SOERATRI, SUWALDI MRTODIHARDJO       946-9.         COMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION STUDIES       946-9.         AKILAN.C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL.C.S, SUNDARA MAHALINGAM.M.A       951-9.         FORMULATION AND EVALUATION OF ONCE DAILY OSMOTIC TABLET OF KETOPROFEN       951-9.         DEHGHAN MHG, KAZI MS, ANSARI MA.       951-9.		
COMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION       946-9.         STUDIES       AKILAN.C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL.C.S, SUNDARA MAHALINGAM.M.A       946-9.         FORMULATION AND EVALUATION OF ONCE DAILY OSMOTIC TABLET OF KETOPROFEN       951-9.         DEHGHAN MHG, KAZI MS, ANSARI MA.       951-9.		939-94
STUDIES       946-9.         AKILAN.C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL.C.S, SUNDARA MAHALINGAM.M.A       946-9.         FORMULATION AND EVALUATION OF ONCE DAILY OSMOTIC TABLET OF KETOPROFEN       951-9.         DEHGHAN MHG, KAZI MS, ANSARI MA.       951-9.		
AKILAN.C.A, SRIVIDHYA.M, MOHANA PRIYA.C, JEBA SAMUEL.C.S, SUNDARA MAHALINGAM.M.A <u>FORMULATION AND EVALUATION OF ONCE DAILY OSMOTIC TABLET OF KETOPROFEN</u> 951-9- DEHGHAN MHG, KAZI MS, ANSARI MA.		946-95
FORMULATION AND EVALUATION OF ONCE DAILY OSMOTIC TABLET OF KETOPROFEN       951-9.         DEHGHAN MHG, KAZI MS, ANSARI MA.       951-9.		
		951-95
INSIGHTS INTO THE STRUCTURAL AND FUNCTIONAL ASPECTS OF RELA BY MOLECULAR MODELING AND DOCKING CALCULATIONS 958-9	DEHGHAN MHG, KAZI MS, ANSARI MA.	
	INSIGHTS INTO THE STRUCTURAL AND FUNCTIONAL ASPECTS OF RELA BY MOLECULAR MODELING AND DOCKING CALCULATIONS	958-96

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AMBILY NATH I.V, LOKA BHARATHI P. A, DEEPTI D. DEOBAGKAR\*

Vol 6 Suppl 2

 Corrigendum

 ANTIDIABETIC AND HYPOLIPIDEMIC ACTIVITY IN STEM OF JATROPHA GOSSYPIFOLIA L. (ORIGINAL ARTICLE)
 968

 NEHA RAHUJA, AKANSHA MISHRA, RAKESH MAURYA, MAHENDRA NATH SRIVASTAVA, AKHILESH KUMAR TAMRAKAR, SWATANTRA KUMAR JAIN, ARVIND KUMAR SRIVASTAVA

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**Research Article** 

# PHYSICAL CHARACTERISTIC AND VIABILITY OF *LACTOBACILLUS ACIDOPHILLUS* MICROPARTICLE USING HPMC K100LV AND HPMC K4M AS MATRICES

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#### ABSTRACT

Objective: Lactobacillus Acidophillus is widely used in food supplement that requires viability in the range of  $10^6 - 10^{12}$  cfu /per gram. This microbe is not stable in acidic conditions and has been reported that the number of their colonies in fermented milk products decreased by 5 logs in acidic solution. In the other side,, the probiotic microbe is required to survive GI tract passage and remains viable. In order to maintain their viability and increase their stability in such environments, Lactobacillus Acidophillus, were entrapped in HPMC K100LV as a protectant using microencapsulation technique.

Methods: The *Lactobacillus acidophillus* cells were inoculated in MRS broth media at 37 °C for 48 hours. A number of cell (more than 10°cfu/ml) wassuspended in 10% milk solution. The cultures were then cooled at 20°C for about 12 hours, and subsequently mixed with suspension of HPMC K4M 0%, 0,3%, 0,5%, 0,8% and HPMC K100LV 0%, 0,5%, 1% and 1,5%. The mixtures were then dried using spray drying method.. The Physical characteristics of the microparticles and the viability of the microbe in the microcapsules were evaluated, including the microparticle morphology, size and moisture content. The viability was measured by Total Plate Count method.

Results: showed that all microparticles exhibited spherical shape with the size in range of 6,0 - 8,0 um. The moisture content was in range of 8,0 - 10,0 %. The highest viability was obtained by formulation of HPMC K100LV 1% and HPMC K4M 0,3%.

Conclusion: of this study is that HPMC K100LV and HPMC K100M are good matrices for probiotic microcapsule based on the physical characteristic and the microbe viability (probiotic viability > 10<sup>7</sup> cfu/g)

Keywords: Lactobacillus acidophillus, HPMC K100LV, HPMC K4M, Physical characteristic, Viability

#### INTRODUCTION

Most of the comensal bacteria, which are more than 2000 species live in human intestinal area and provides health benefits to the host, because of the improved microbial balance in the intestine. They are from two procaryotic microorganisms, i.e. lactobacillus and bifidobacterium with their various different species. The intestinal microbial colonization starts at birth and continues during the subsequent phases of life that form an individual intestinal microbiota. Such microorganisms are known as probiotics [1,2,3,4]. It has been generally accepted that the probiotics exerts health benefits if their minimum count is about 10<sup>6</sup> cfu per g of food per consumption (5). They should survive GI tract passage and adhere to the intestinal mucosa or other target sites in GIT [4]. It has been known that there were significant reduction of the number of living microorganisms in GIT. The reduction process in gastric juice was a pH dependent where the pH of gastric juice is ranging from 1 to 4. It was reported that pH plays an important role in cells death. There were 3,5 and 2,2 log reduction on the viability after lactobacillus acidophillus were inoculated for 90' in simulated gastric juice at pH 1,5 and 2,5 respectively [6,7]. Another research reported that lactobacillus and bifidobacterium sp. lost more than 90% after have been exposed to simulated gastric juice at pH 2 [6,7,8]

Lactobacillus acidophillus is widely used regarding to the health benefiting effect. Lactobacillus are extensively incorporated into yoghurts, cultured milk drinks, cheese or as dietary supplements in the form of dried dosage forms. [10,11]. Entrapment of living cells in a matrix called microencapsulation is a method used for protection of the immobilized materials as well as for a controlled release in intestinal mucosa. The outer layer or the wall of the microcapsule will protect the cell against moisture, heat, strong acidic and other extreme conditions during storage, manufacturing as well as during digestion Microencapsulation method has a lot of advantages, including the protection effect from misture, heath or other extreme conditions. However, it still causing significant cell death, since most common encapsulation method uses spray-drying that involves heating [11,12,13]. The microencapsulation method, allow the incorporation of Lactobacillus acidophillus and another probiotic microorganism. in which a polymer acts as outer layer or protectant [12,13]. A number of microencapsulation techniques including: spray drying, inclusion complication, extrusion, co-crystallization and gel entrapment (extrusion, emulsification, coacervation). Spraydrving (dehydration method) has been oftenly used in industries among other cell preservation methods, because it is cheaper, cost effective and suitable for a large-scale production. The cost effectiveness of spray drying was estimated to be six time cheaper per kilogram compared to freeze drying [14]. The disadvantage of this method is that many microorganisms can not tolerate the drying process due to the high heat involved. Other factors that may affect the survival during spray-drying process are the type of the strain, growth phase, protective medium used, outlet temperature of spraydrier and pre- treatment of the culture. [13,14] This study investigated the use of HPMC K100LV (0%, 0,5%, 1% and 1,5%) and HPMC K4M (0%, 0,3%, 0,5% and 0,8% ) as protectant. The used of HPMC to enhance probiotic surival through heat treatment has been studied previously. HPMC is safe, nonionic polymer that minimize interaction problems when they are used in acidic, basic, or other electrolytic system. They can be used for preparing formulations with water soluble or insoluble drugs and at high or low doses. [15]. The polymer is therefore useful for the cell because it assist the adaption of microbe to the environment. Furthermore the polymer reduces the osmotic differences between the cellular internal compartment and the environment. In this study, a dried probiotic powder was produced using spray drying methods with temperature of 60° C.

#### MATERIALS AND METHODS

#### Materials

Lactobacillus acidophillus was obtained from Microbioliogy Laboratorium-Brawidjaja University, Indonesia.

HPMC K4 M and HPMC K100LV (Pharmaceutical Grade) were purchased from PT. Lawzim Zecha. *de Man Ragosa Shorpe* (MRS) broth media was purchased from

#### Methods

#### 1. Preparation of Spray Dried Milk-Probiotic -HPMC K4M

The *Lactobacillus acidophillus* cell in amount of 1 ose were inoculated in MRS broth media at 37  $^{\circ}$ C for 48 hours. The number of cell should be more than 10 $^{\circ}$ cfu/ml and suspended in 10% milk solution.

The cultures were than cooled at  $20^{\rm o}$  C for about 12 hours, and subsequently mixed with suspension of HPMC K4M 0%, 0,3%, 0,5%, 0,8% and HPMC K100LV 0%, 0,5%, 1% and 1,5%.

The mixtures were then dried using spray dryer (Lab-Plant SD-Basic Spray Dryer)

#### Physical Characterizations

#### 1. pH measurement

The pH of the milk and milk-probiotic was measured using a pH meter SCHOTT glass mainz, CG 842 type.

#### Table 1: Composition of Probiotic Microparticle with HPMC K4M and PMC K100LV as a matrix

Composition	HPMC K100LV			HPMC K4M				
	F1	F2	F3	F4	F5	F6	F7	F8
Milk-Probiotic	400 ml	400 ml	400 ml	400 ml	400 ml	400 ml	400 ml	400 ml
HPMC K100LV	-	2,5 g	5 g	7,5 g	-	-	-	-
HPMC K100M	-	-	-	-	-	0,15 g	0,25 g	0.40 g
Aquadest	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

#### 2. Viscosity Measurement

The viscosity of the milk and milk probiotic was measured using VT-04 viscometer

#### 3. Moisture content determination

The moisture content of the microparticles was determined using Moisture Analyzer HB43-S Metler Toledo

#### 4. Size and morphology of probiotic microparticles

The size of microparticles was measured using optical microscope. The morphology of the particles was visualized using SEM (FEItype:inspect-S50)

#### Viability measurement

Viability of lactobacili in milk (before spray drying) and in microparticle (after spray drying) was assessed using MRS media. 1 ml probiotic milk or 1 gram microparticel was mixed with 9 ml sterile Phosphate Buffer Salin (PBS) solution. A serial dilution ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ ,  $10^{-8}$ ,  $10^{-9}$  and  $10^{-10}$ ) of this suspension was made and then spread on the MARS agar. and incubated at 37 oC for 48 hours. The viability of the probiotic was reported as TPC (cfu/ml or cfu/g) and survivability (N/No x 100%).

#### Statistical analysis

One Way ANOVA with Honestly Significant Differenc (HSD) Tukey was used to analyze the results. Confidence limits of 95% (  $\alpha$  = 0,05 ) were used to determine statistical significance.

#### **RESULT AND DISCUSSION**

Table 2: The pH and viscosity of milk and milk-probiotic after24 hours

Material	рН	Viscosity	
Milk	6.41 ±0,01	0,4±0,01	
Milk-Probiotic	4,33±0,00	0,7±0,00	

The results showed that the fermented milk has a lower pH compared to the non-fermented milk (p<0.05) (Table 2). The decrease of pH indicated that there were accumulation of lactic acid and acetic acid as a result of lactosa metabolism. *Lactobacillus acidophillus* has an ability to metabolize lactose to lactic acid, acetic acid and CO2. The viscocity data showed that milk- probiotic had a greater viscosity than normal milk. It indicated that microorganisms in the milk contribute the solid component and resulted in increasing viscosity. Spray dried powders of lactobacillus acidophillus -containing microparticle with HPMC has a mean size higher than microparticle without HPMC, and increasing HPMC concentration resulting in increasing particle mean size.

#### Table 3: The Mean Particle size and moisture content measurement

Measure ment	F 1	F II	F III	F IV	FV	F VI	F VII	F VII
Mean	6,7	7,2	7,1	8,4	7,6	7,3	8,1	8,0
Diameter (µm)	25	83	42	92	5	9	1	7
Moisture	10,	7,4	7,7	8,7	10,	10,	9,5	8,9
Content (%)	54	1	9	0	45	09	1	6

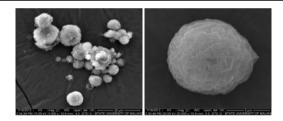


Fig. 1: Morphology of Probiotic Microparticle without a HPMC (5000x and 20.000x)

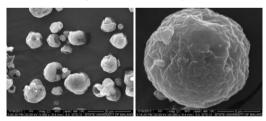


Fig. 2: Morphology of Probiotic Microparticle with HPMC K100LV (5000x and 20.000x)

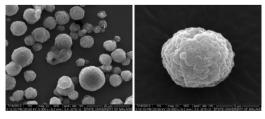


Fig. 3: Morphology of Probiotic Microparticle with HPMC K4M (5000x and 20.000x)

It might be explained that increasing HPMC concentration will increase the composition of wall microparticle. The microparticle morphology is spherical with rough surface. It might be explained that rate of evaporation was not proportional to the rate of film formation.

The data obtained in these study showed that all microparticle had moisture content greater than recommended range, i.e. 2,80% - 5,60% [13] and the moisture contents increased with the greater matrix concentration (Table 3). This can be explained that the matrix used (milk and HPMC) exhibited a higroscopic properties. The drying temperatur, 60 °C was too low and was not necessary in regards to fulfill the recommended moisture content.

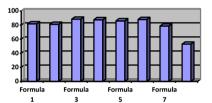
The morphology of microparticle (SEM image, Fig. 1) showed that the mcroparticles was spherical and have a rough surface.

#### Table 4: TPC value ( cfu/ml or cfu/g) of *Lactobacillus* acidophilus in the probiotic microparticle with HPMC K100LV and HPMC K4M as a matrix

Group	HPMC K100LV	HPMC K4M
Probiotic milk	306,3x10 <sup>7</sup> ± 10,9x10 <sup>7</sup>	$162 \ge 10^{12} \pm 9,5 \ge 10^{12}$
Formula I	5,7x10 <sup>7</sup> ± 1,5x10 <sup>7</sup>	$157 \ge 10^{10} \pm 5,29 \ge 10^{10}$
Formula II	4,8x10 <sup>7</sup> ± 3,2x10 <sup>7</sup>	$295 \text{ x} 10^{10} \pm 1,53 \text{ x} 10^{10}$
Formula III	24,4x10 <sup>7</sup> ± 6,3x10 <sup>7</sup>	139 x 10 <sup>9</sup> ± 2,53 x 10 <sup>9</sup>
Formula IV	20,5x10 <sup>7</sup> ± 5,1x10 <sup>7</sup>	3,30 x 10 <sup>7</sup> ± 2,64 x 10 <sup>7</sup>

Table 5: Viability (%)of <i>Lactobacillus</i> in the probiotic
mikroparticle with HPMC K100LV and HPMC K4M as a matrix

Group	HPMC K100LV	HPMC K4M
Probiotic milk	100,00 ± 0,00	100,00 ± 0,00
Formula I	81,69 ± 1,29	85,99 ± 0,074
Formula II	81,03 ± 0,21	87,93 ± 0,153
Formula III	88,34 ± 1,01	78,58 ± 0,125
Formula IV	87,54 ± 1,01	53,01 ± 0,285



# Fig. 4: Viability (%) of *lactobacillus acidophillus* in the microparticle using HPMC K100LV and HPMC K4M as a matrix

Table 4 and 5 showed the viability ( cfu ) and percentage survival (%) of *lactobacillus acdophillus* probiotic. The data indicated that viability of microparticles was lower than probiotic milk (before spray dried). There was a viability reduction by approximately 20%. The viability reduction of microparticle compared with probiotic milk can be explained as a result of heating process during spray drying.

No significant difference ( p > 0,05 ) were obtained between *Lactobacillus Acidophillus* viability of HPMC K100LV 0% and 0,5%, HPMC K4M 0% and 0,3%. It showed that both polymers have an equal protective effect when using HPMC K100LV 0,5% and HPMC K4M. The profile of *lactobacillus acidophillus* viability in HPMC K100LV group was different from HPMC K4M group (Statistic?). In HPMC K4M group, the greater the concentration, the greater the viability. It can be explained: that increasing HPMC concentration will result in increased viscosity and increasing microbe entrapment. During the viability test process, part of the cell still entrapped. However, it was still higher than  $10^7 \, {\rm cfu/g} \, (3,30x10^7 \, {\rm cfu/g} \, )$ 

#### CONCLUSION

- LactobacillusAcidophillus microparticle using HPMC K100LV and K4M as a matrices has a spheric and rough, and the particle size ranging from 6,0 to 8,0 μm.
- **2.** *LactobacillusAcidophillus* microparticle using HPMC K100LV and K4M as a matrices has moisture content 7,0 10,0 %, out of recommended range, 2,80 5,60
- Lactobacillus Acidophillus microparticle using HPMC K100LV and K4M as matrices, result a probiotic viability > 10<sup>7</sup> cfu/g

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