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# INTERNATIONAL JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

## Volume 6 Issue 2 2014

### Review Articles

<a href="#">POTENTIAL MEDICINAL PROPERTIES OF <i>CARICA PAPAYA</i> LINN. - A MINI REVIEW</a> NATARAJAN SUDHAKAR, THEIVANAI @ VIDHYA RM.	1-4
<a href="#">IMPORTANCE OF MEDICATION ADHERENCE IN ENSURING EFFECTIVE AND COST-EFFICIENT HEALTHCARE</a> KAUSTUV BHATTACHARYA, KETOSETUO KUOTSU	5-8
<a href="#">IN VITRO EVALUATION OF HERB-DRUG INTERACTIONS: A REVIEW</a> LEENA PATIL, KIRTI KULKARNI, VINEETA KHANVILKAR, DR. VILASRAO KADAM	9-12
<a href="#">ROLE OF PIOGLITAZONE ON PROGRESSION OF ATHEROSCLEROSIS IN PREDIABETES: A MINI REVIEW</a> ROHIT KUMAR VERMA, POONAM TRIPATHI, AWANISH KUMAR PANDEY	13-15
<a href="#">A REVIEW ON LATEST GUIDELINES ON PROCESS VALIDATION OF EUROPEAN MEDICINES AGENCY</a> KOTHA ARUN KUMAR, N. VISHAL GUPTA, U NITIN KASHYAP, VEMURI PAVAN KUMAR	16-18
<a href="#">A COMPARISON BETWEEN OLD AND LATEST SYSTEMS IN DPCO</a> VEMURIPAVAN KUMAR, N. VISHAL GUPTA, KOTHA ARUN KUMAR	19-20
<a href="#">THE GENUS POLYGONUM (POLYGONACEAE): AN ETHNOPHARMACOLOGICAL AND PHYTOCHEMICAL PERSPECTIVES –REVIEW</a> GANAPATHI NARASIMHULU, KESIREDDY KATHYVEVELU REDDY, JAMALUDIN MOHAMED	21-45
<a href="#">ANTIMICROBIAL STEWARDSHIP - AN ALARMING CALL IN DENTISTRY</a> DARSHANA BENNADI	46-49
<a href="#">"IMMUNOMODULATORY AND ANTIOXIDANT ACTIONS OF DIETARY FLAVONOIDS"</a> DURGA.M, NATHIYA. S, DEVASENA.T	50-56

### Research Articles

<a href="#">PROTECTIVE ROLE, IN -VITRO FREE RADICAL SCAVENGING ACTIVITIES OF ALANGIUM SALVIFOLIUM (LINN.) AGAINST CCL4 INDUCED HEPATIC DAMAGE IN RATS</a> PABBA PARAMESHWAR, YELLU NARASIMHA REDDY	57-61
<a href="#">DEVELOPMENT AND EVALUATION OF BILAYER FLOATING TABLETS OF DILTIAZEM HCL</a> DR. SADHANA R SHAHI, VIDYA M. MADKAR, PRASHANT N KSHIRSAGAR, DR. S. S. KHADBADI	62-65
<a href="#">IN VIVO PHARMACOLOGICAL INVESTIGATION OF <i>MIMOSA PUDICA</i> L.</a> UDDIPON AZIZ, RUMANA AKTHER, MOHAMMAD SHAHRIAR, MOHIUDDIN AHMED BHUIYAN	66-69
<a href="#">DRUG UTILIZATION PATTERN AND PHARMACOECONOMIC STUDY IN PAEDIATRIC DENTISTRY AT A TERTIARY HOSPITAL</a> JAYANTHI M.K, SUSHMA NAIDU V	70-72
<a href="#">FORMULATION AND EVALUATION OF NANOSTRUCTURED LIPID CARRIER (NLC) OF LORNOXICAM</a> PANCHAXARI MALLAPPA DANDAGI, GAYATRI ANANT DESSAI, ANAND PANCHAKSHARI GADAD, VAIBHAV B. DESAI	73-77
<a href="#">INTRA-SPECIES COMPARISON OF <i>MARSILEA MINUTA</i> LINN. AND <i>MARSILEA QUADRIFOLIA</i> LINN. USING RAPD MARKERS TO ANALYZE THE GENETIC VARIATIONS</a> AMEY R. SHIRODKAR, SHRADDHA WADURKAR, GAJENDRA RAO, S. N. MURTHY, SHARAD D. PAWAR	78-80
<a href="#">FORMULATION AND EVALUATION OF ORODISPERSIBLE FILM OF SILDENAFILCITRATE</a> T. HASSANIEN SAGBAN, K. YEHIA ISMAIL	81-86
<a href="#">RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS QUANTITATIVE ESTIMATION OF EFVIRENZ, LAMIVUDINE AND ZIDOVUDINE IN TABLETS</a> B. RAJKUMAR, T. BHAVYA, S. KULSUM, A. ASHOK KUMAR	87-92
<a href="#">CHRONIC ANXIOLYTIC-LIKE ACTIVITY OF AQUEOUS EXTRACT OF CORIANDRUM SATIVUM SEEDS USING ELEVATED PLUS MAZE TEST IN SWISS ALBINO MICE</a>	93-95



ARUN RAVINDRAN, MANOHAR V.R., MOHANDAS RAI, NIMISHA RAVEENDRAN, HARSHA NAIK	
<a href="#">SYNTHESIS AND BRINE SHRIMP LETHALITY TEST OF SOME BENZOXAZINE AND AMINOMETHYL DERIVATIVES OF EUGENOL</a>	96-98
MARCELLINO RUDYANTO,,, JUNI EKOWATI, TRI WIDIANDANI AND TOSHIO HONDA	
<a href="#">PRAFORMULATION STUDY OF P-METHOXYCINNAMIC ACID (PMCA) NANOEMULSION USING VEGETABLE OILS (SOYBEAN OIL, CORN OIL, VCO)</a>	99-101
TRISTIANA ERAWATI M., ESTI HENDRADI, WIDJI SOERATRI	
<a href="#">COMPARISON OF MAJOR SECONDARY METABOLITES QUANTIFIED IN ELICITED CELL CULTURES, NON-ELICITED CELL CULTURES, CALLUS CULTURES AND FIELD GROWN PLANTS OF <i>OCIMUM</i></a>	102-106
REBECCA MATHEW, P. DEEPA SANKAR	
<a href="#">MULTIPLE-UNIT CONTROLLED RELEASE PLATFORM FORMULATION BY WRUSTER PROCESS</a>	107-113
VIKRAM GHARGE, PEEYUSH SHARMA, INDRAJEET GONJARI, ANIL BHANDARI	
<a href="#">EFFECT OF SOLVENT TYPES ON PHENOLIC, FLAVONOID CONTENTS AND ANTIOXIDANT ACTIVITIES OF <i>SYZYGium GRATUM</i> (WIGHT) S.N.</a>	114-116
S. SETTHARAKSA, F. MADAKA,, L. SUEREE,, S. KITTIWISUT,, A. SAKUNPAK,, C. MOTON,, L. CHAROENCHAI,	
<a href="#">EFFECT OF CITRIC AND MALIC ACID ADDITIVES ON SHELF LIFE AND SENSORY CHARACTERISTICS OF ORANGE JUICE</a>	117-119
AHMED HUMAYUN, CHANDAN KUMAR GAUTAM, MUKUNDMADHAV, SUMEET SOURAV, C. RAMALINGAM	
<a href="#">COEXISTENCE OF AUTOIMMUNE DISEASE WITH TYPE I DIABETES MELLITUS IN LIBYAN PATIENTS</a>	120-124
EBTISAM A. ABOSMAHA, SAFA E. ALMSAHLI, SAMI G. ALSABRI, SOFIAN S. MOHAMED AND MUSTAFA GEBREIL	
<a href="#">O-PHTHALALDEHYDE BASED SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF SITAGLIPTIN IN TABLETS</a>	125-129
MAI A RAMADAN	
<a href="#">DIFFERENT CONCENTRATION OF ZINC TOLERANCE IN <i>CHLORELLA VULGARIS</i> &amp; THEIR EFFECT ON GROWTH AND BIOPIGMENT</a>	130-132
NAMITA SIKARWAR, G. P. SINGH	
<a href="#">MELATONIN AMELIORATES HYPERINSULINEMIA, GLUCOSE INTOLERANCE AND INSULIN RESISTANCE IN STZ-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS</a>	133-136
TARIQUE ANWER,	
<a href="#">SYNTHESIS AND CYTOTOXIC STUDIES OF 2, 3-DIMETHYLINDOLES AND TETRAHYDROCARBAZOLES</a>	137-140
T. O. SHRUNGESH KUMAR, K. M. MAHADEVAN, M. N. KUMARA	
<a href="#">SYNTHESIS OF NOVEL N-(ARYL) DIAZENYL THIAZOL-2-AMINES AND BEZYLIDENE-THIAZOLIDIN-4-ONES LINKED TO INDOLE NUCLEUS AS ANTIOXIDANT, ANTIMICROBIAL, ANTIMYCOBACTERIAL AND CYTOTOXIC AGENTS</a>	141-147
SAUNDANEANAND R., PRABHAKERWALMIK, KIRANKUMAR N. M., ANNAPURNA H.	
<a href="#">TOWARDS A SIMPLIFIED MODEL MEMBRANE OF SKIN LIPIDS: PREPARATION AND CHARACTERISATION OF A TERNARY LIPID MIXTURE</a>	148-152
,HASSAN M. GHONAIM, NAGARAJAN PERIASAMY, MASSIMO G. NORO,,JAMSHED ANWAR	
<a href="#">SYNTHESIS AND FORMULATIONS OF LIPID AMINOGLYCOSIDE CONJUGATES: NANOPARTICLES FOR EFFICIENT GENE AND SIRNA DELIVERY</a>	153-157
HASSAN M. GHONAIM,, IAN S. BLAGBROUGH	
<a href="#">PREVALENCE AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CANDIDA ALBICANS FROM LOW SOCIO-ECONOMIC GROUP</a>	158-162
SELVAM SARANYA. KANNAIYAN MOORTHY. SIVA SAKTHIVEL ARUL SHEEBA MALAR THAMBIDURAI PUNITHA.RAJA VINODHINI. MURUGESAN BHUVANESHWARI. CHINASAMY KANIMOZHI.	
<a href="#">THEORETICAL MODELING AND DOCKING STUDIES OF SILKWORM OCTOPAMINE RECEPTORS</a>	163-166
R. SUMATHY,, S. K. ASHWATH, B. B. BINDROOAND V. K. GOPALAKRISHNAN	
<a href="#">ANTIBACTERIAL ACTIVITY OF EXTRACTS FROM SALVIA OFFICINALIS AND ROSMARINUS OFFICINALIS OBTAINED BY SONICATION AND MACERATION METHODS</a>	167-170
MOUNYR BALOUIRI,, MOULAY SADIKI,, WESSAL OUEDRHIRI,ABDELLAH FARAH, SOUMYA EL ABED, SAAD IBNSOUDA KORAICHI	
<a href="#">MICROTUBULE DISRUPTING N-PHENYL-N'-(2-CHLOROETHYL) UREAS DISPLAY ANTICANCER ACTIVITY ON CELL ADHESION, P-GLYCOPROTEIN AND BCL-2-MEDIATED DRUG RESISTANCE</a>	171-179
JESSICA S. FORTIN, MARIE-FRANCE CÔTÉ, RÉNA G. DESCHESNES, ALEXANDRE PATENAUDE., JACQUES LACROIX, MARIE-ODILE BENOIT-BIANCAMANO AND RENÉ C.-GAUDREAU	
<a href="#">DEVELOPMENT, CHARACTERIZATION AND EVALUATION OF SOLID DISPERSIONS OF ARTEMETHER AND LUMEFANTRINE BY SOLVENT EVAPORATION METHOD USING HYDROPHILIC POLYMERS</a>	180-185
ANNA BALAJI, HARISHA KUMARI M, UDAY KUMAR G.	
<a href="#">DEVELOPMENT OF FAST DISSOLVING ORAL FILMS AND TABLETS OF CINNARIZINE: EFFECT OF SUPERDISINTEGRANTS</a>	186-191
DEEPAK HEER, GEETA AGGARWAL AND S.L. HARI KUMAR	
<a href="#">A STUDY ON ASSESSMENT OF CLINICAL PHARMACY SERVICES TO CARDIOLOGY DEPARTMENT IN TERTIARY CARE TEACHING HOSPITAL</a>	192-195
RAGESH G., SINDHUBHARATHI A, USHASRI M, SRINIVASULU A.	
<a href="#">THE EFFECT OF FISH OIL ON OXIDANT /ANTIOXIDANT STATUS IN DIABETIC RATS THROUGH THE REDUCTION OF ARACHIDONIC ACID IN THE CELL MEMBRANE</a>	196-199
JIHAN SEID HUSSEIN, ZAKARIA EL-KHAYAT, SAFAA MORSY, FATMA ORABY, GAMAL SINGER	
<a href="#">NUTRITIONAL AND NUTRACEUTICAL POTENTIAL OF WILD EDIBLE MACROLEPIOTOID MUSHROOMS OF NORTH INDIA</a>	200-204
BABITA KUMARIANDNARENDER SINGH ATRI	



ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF DIFFERENT FRACTIONS OF HIPTAGE BENGHALENSIS (LINN) BABU RAO BHUKYA	205-210
ISOLATION AND IDENTIFICATION OF SUBSTANCES WITH ANTI-HEPATITIS C VIRUS ACTIVITIES FROM <i>KALANCHOE PINNATA</i> CHIE AOKI,, SRI HARTATI, MEI RIA SANTI, LYDWINA, RININTA FIRDAUS, MUHAMMAD HANAFI, LEONARDUS B S KARDONO, YOHKO SHIMIZU,, PRATIWI SUDARMONO AND HAK HOTTA	211-215
INSILICO ANALYSIS OF PROTEINS OF <i>CURCUMA CAESIA</i> ROXB S. VIJAYA BHARATHI	216-220
PRESCRIBING PATTERN OF ANTIBIOTICS IN THE GENERAL MEDICINE AND PEDIATRICS DEPARTMENTS OF A TERTIARY CARE TEACHING HOSPITAL VENU GOPAL D, RAMA KRISHNA T, SIVA KUMAR A, VENKATA SUBBAIAH MEDA, RAVINDRA REDDY K	221-224
MOLECULAR DOCKING STUDIES OF PHENYLAMINOPYRIMIDINE AND PYRAZOLYLAMINOPYRIMIDINE DERIVATIVES AS JANUS KINASE 2 (JAK2) INHIBITORS RAJASEKHAR CHEKKARA,, NARESH KANDAKATLA, VENKATA REDDY GORLA,, SOBHA RANI TENKAYALA,, SUSITHRA ETHIRAJ	225-230
NANOEMULSION LOADED WITH MARIGOLD FLOWER EXTRACT ( <i>TAGETES ERECTA</i> LINN) IN GEL PREPARATION AS ANTI-WRINKLES COSMECEUTICAL PIMPORN LEELAPORNPISID, KANOKWAN KIATTISIN, PENSACK JANTRAWUT AND AMPAI PHRUTIVORAPONGKUL	231-236
FORMULATION AND EVALUATION OF GASTRORETENTIVE BEADS OF RANITIDINE HYDROCHLORIDE RAJKUMAR PATEL, RITESH S. BATHE, DEEPAK KHOBRAGADE, SACHIN JADHAV	237-242
EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF <i>CURCUMA CAESIA</i> ROXB. RHIZOMES IN LABORATORY ANIMALS SAMPADA BHOSALE SAWANT, GOPAL BIHANI, SMEETA MOHOD, SUBHASH BODHANKAR	243-247
FORMULATION AND <i>IN VITRO</i> STUDY OF KETOPROFEN PSEUDOLATEX GEL FOR TRANSDERMAL DRUG DELIVERY SYSTEMS JIRAPORNCHAI SUKSAEERE, CHAOWALIT MONTON, APIRAK SAKUNPAK, AND TOSSATON CHAROONRATANA	248-253
PHYTOCHEMICAL ANALYSIS OF THE LEAVES OF <i>CLERODENDRUM VISCOSSUM</i> VENT. PRIYANKAR DEY, SOMIT DUTTA, TAPAS KUMAR CHAUDHURI	254-258
FORMULATION AND <i>IN-VITRO</i> EVALUATION OF MODIFIED RELEASE TABLETS OF GLICLAZIDE (ANTIDIABETIC DRUG) SANJAY K. SHARMA, SHAILENDER MOHAN, MANISH JAIMINI	259-261
ISOLATION, CHARACTERIZATION AND INSILICO PHARMACOLOGICAL SCREENING OF MEDICINALLY IMPORTANT BIO-ACTIVE PHYTOCONSTITUENTS FROM THE LEAVES OF <i>IPOMOEA AQUATICA</i> FORSK. D. SIVARAMAN , P. PANNEERSELVAM AND P. MURALIDHARAN	262-267
EVALUATION OF ANTIOXIDANT CAPACITIES, FLAVONOID, PHENOLIC, CAROTENOID CONTENT FROM VARIOUS EXTRACTS OF FOUR KINDS BRASSICA HERBS IRDA FIDRIANNY, PUSPA UTARI, KOMAR RUSLAN W	268-272
ANTI-DIABETIC ACTIVITY AND ANTI-OXIDANT ACTIVITY OF NIDDWIN, A POLYHERBAL FORMULATION IN ALLOXAN INDUCED DIABETIC RATS T. SRUTHI, D. SATYAVATI, K. UPENDAR, C. PRADEEP KUMAR	273-277
NEW SPECTROPHOTOMETRIC ESTIMATION OF PRULIFLOXACIN USING 2,4- DINITROPHENYL HYDRAZINE REAGENT SWEETY S KANOLKAR, TEJA V WALKE	278-280
COST-COST ANALYSIS OF ANTI-DIABETIC THERAPY IN A TERTIARY HEALTHCARE INSTITUTION, NORTH-EASTERN NIGERIA GIWA ABDULGANIYU, TAYO FOLA	281-286
GENOMIC AND PROTEOMIC SIGNATURES OF RADIATION AND THERMOPHILIC ADAPTATION IN THE <i>DEINOCOCCUS-THERMUS</i> GENOMES RACHANA BANERJEE, AYAN ROY, SUBHASIS MUKHOPADHYAY	287-300
ANTI - INFLAMMATORY POTENTIAL OF AQUEOUS EXTRACT OF <i>SESBANIA SESBAN</i> (L.) MERR KATHIRAVAN SUBRAMANIAN , SHWETHA V KALAVA	301-304
EFFICACY OF INTRA-PERITONEAL ADMINISTRATION OF METFORMIN AND 5-FLUORURACIL IN PREVENTION OF INDUCED-COLORECTAL ABERRANT CRYPT FOCI IN MICE ABDULKAREEM H.ABD, ALAA GHANI HUSSEIN, AHMED SALIM MAHMOOD	305-308
DEVELOPMENT AND VALIDATION OF KNOWLEDGE, ATTITUDE, PRACTICE QUESTIONNAIRE FOR ASTHMA AND ASSESSMENT OF IMPACT OF PATIENT EDUCATION ON ASTHM PATIENTS RAJANANDHMG, NAGESWARIAD , ILANGOK	309-311
<i>IN VIVO</i> COMPARATIVE BIOAVAILABILITY STUDY OF TWO VALPROIC ACID SYRUP FORMULATIONS DISPENSED IN GAZA STRIP ISSAM ABUSHAMMALA, MAI RAMADAN, IBTHAL ELASTAL	312-314
SYNTHESIS AND ANTITUMOR ACTIVITY EVALUATION OF <i>N,N'</i> -DIBENZOYL- <i>N,N'</i> -DIETHYLUREA AGAINST HUMAN BREAST CANCER CELL LINE (MCF-7) NUZUL WAHYUNING DIYAH, JUNI EKOWATI, SISWANDONO	315-318
A COMPARATIVE EVALUATION OF ANTICANCER ACTIVITIES OF FLAVONOIDS ISOLATED FROM <i>MIMOSA PUDICA</i> , <i>ALOE VERA</i> AND <i>PHYLLANTHUS NIRURI</i> AGAINST HUMAN BREAST CARCINOMA CELL LINE (MCF-7) USING MTT ASSAY JOBY JOSE, SUDHEESH SUDHAKARAN, SUMESH KUMAR T.M, SONY JAYARAMAN, E. JAYADEVI VARIYAR	319-322
INCIDENCE OF ACUTE AND CHRONIC FORMS OF LEUKEMIA IN HARYANA RADHA RATHEE, MINAKSHI VASHIST, ASHOK KUMAR, SUNITA SINGH	323-325
EVALUATION OF ANALGESIC ACTIVITY OF BENZAPRIL IN ALBINO MICE	326-328



SIDDAMMA.AMOGHIMATH, SURESHA R N, VAIBHAVI P S

<a href="#">DOCKING BASED PHARMACOPHORE MODEL FOR MYCOBACTERIUM TUBERCULOSIS PEPTIDE DEFORMYLASE INHIBITORS AND ITS APPLICATION IN DRUG DESIGNING</a>	329-331
PARVEEN PUNIA, SANDEEP SINGH, TILAK RAJ, R. RAGUVARAN, MEENU CHOPRA AND VIKAS DAHIYA	
<a href="#">FORMULATION AND EVALUTION OF MUCOADHESIVE BUCCAL FILMS OF ATORVASTATIN USING NATURAL PROTEIN</a>	332-337
DR. ANNA BALAJI, B. KRISHNAVENI, VISHNUVARDHAN GOUD	
<a href="#">ANTI HYPERLIPIDEMIC EFFECT OF AQUEOUS EXTRACT OF AEGLE MARMELLOS AND CAMELLIA SINENSIS IN OIL FED HYPERLIPIDEMIC RATS</a>	338-341
PRIYANGA SURIYAMOORTHY, MARGRET ROSALAND FATHIMA MARY, HEMMALAKSHMI SUBRHAMANIAN, DEVAKI KANAGASAPABATHY	
<a href="#">USE OF HERBAL NANO SILVER FOR FABRICATION OF ANTIMICROBIAL COTTON FABRICS AND TESTING ITS EFFICACY AGAINST MICROBES</a>	342-346
AYNUL RIFAYA, M. AND MEYYAPPAN, R.M.	
<a href="#">VALIDATED NORMAL PHASE HPTLC METHOD FOR SIMULTANEOUS QUANTIFICATION OF LEVOSULPIRIDE AND ESOMEPRAZOLE IN CAPSULE DOSAGE FORM</a>	347-350
PRAVIN D. PAWAR, SATISH Y. GABHE, SACHIN E. POTAWALE, KAKASAHEB R. MAHADIK	
<a href="#">THE ROLE OF PROTEIN DEFICIENCY IN THE HEALING OF MANDIBULAR FRACTURES IN RABBIT MODEL</a>	351-357
HAMID H. ENEZEI, AZLINA A, IGZEER Y. K	
<a href="#">QUORUM QUENCHING AND ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED FROM MEDICINAL PLANTS AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)</a>	358-362
M. SAFANA FARJEEN, S. K. DIVYA, T. JEYADOSS, M. ARUN KUMAR	
<a href="#">MANAGEMENT OF COMORBIDITIES IN CHRONIC KIDNEY DISEASE: A PROSPECTIVE OBSERVATIONAL STUDY</a>	363-367
PRANAVI DASARI, VENKATESHWARLU K, RAJ KUMAR VENISETTY	
<a href="#">BIO-PROSPECTING THE ANTIBACTERIAL AND ANTICANCER ACTIVITIES OF SILVER NANOPARTICLES SYNTHESIZED USING TERMINALIA CHEBULA SEED EXTRACT</a>	368-373
S. NANDAGOPAL, A. GANESH KUMAR, D.P. DHANALAKSHMI AND P. PRAKASH	
<a href="#">SYNTHESES, CHARACTERIZATIONS AND BIOLOGICAL SCREENING OF TITANIUM (IV) COMPLEXES DERIVED FROM ANTIBIOTIC DRUG(S)</a>	374-378
RAJ KAUSHAL, SHEETAL, RAJEEV KAUSHAL, KIRAN NEHRA	
<a href="#">IN SILICOSTUDY TO ELUCIDATE INHIBITORY EFFECT OF THIAZIDES ON PLASMEPSINS: IMPLICATIONS OF NEW ANTIMALARIAL DRUG DESIGN</a>	379-382
TARUN AGARWAL, SOMYA ASTHANA, PRERAK GUPTA, ASIF KHURSHEED	
<a href="#">ASSESSING THE BIOACTIVE CONSTITUENTS OF CADABA FRUTICOSA (L.) DRUCE THROUGH GC-MS</a>	383-385
MURUGESAN AMUDHA, SHANMUGAM RANI	
<a href="#">FORMULATION AND EVALUATION OF MULTIPARTICULATE SYSTEMS OF RIFAMPICIN AND ISONIAZID WITH IMPROVED RIFAMPICIN STABILITY</a>	386-388
VAMSHI KRISHNA T., M. SREENIVASA REDDY	
<a href="#">A STUDY ON THE ANTICONVULSANT AND ANTIANXIETY ACTIVITY OF ETHANOLIC EXTRACT OF PUNICA GRANATUM LINN.</a>	389-392
SWARNAMONI DAS, PHULEN SARMA	
<a href="#">FORMULATION OF CONTROLLED RELEASE OF LOXACIN TABLETS USING NATURAL POLYMERS</a>	393-396
S. R. SENTHIL KUMAR, V. GANESAN	
<a href="#">PHYTOCHEMICAL AND BIOLOGICAL EVALUATION OF LIATRIS SPICATA (L.) CORMS</a>	397-401
AHLAM M. EL FISHAWY, KADRIYA S. EL DEEB, SHAHIRA M. EZZAT, MARWA I. EZZAT AND AMANY A. SLEEM	
<a href="#">PHARMACOGNOSTIC EVALUATION AND ANTIBACTERIAL ACTIVITY OF DRY FRUITS OF PIPER ATTENUATUM BUCH-HAM</a>	402-406
RENU OHLYAN, AJIT KANDALE, AMARJEET YADAV	
<a href="#">AUTOMATION OF MACHINERY FOR COLLAGEN BASED THIN SHEET OF WOUND DRESSING MATERIAL</a>	407-409
VIJAYAN SUMATHI, THOTAPALLI PARVATHALESWARA SASTRY, CHANDRABABU SHANTHI	
<a href="#">HIGH DOSES OF UNCARIA TOMENTOSA (CAT'S CLAW) REDUCE BLOOD GLUCOSE LEVELS IN RATS</a>	410-415
PATRÍCIA FRANCISCONE MENDES, FERNANDO PONCE, DYANNE DUTRA FRAGA, FERNANDO PÍPOLE, FABIO FERREIRA PERAZZO AND ISIS MACHADO HUEZA	
<a href="#">PIPER LONGUM HEXANE FRACTION INDUCES INFERTILITY BY MODULATION OF INFLAMMATORY MEDIATORS AND GONADOTROPIN INSUFFICIENCY IN FEMALE RATS</a>	416-420
ABU HASNATH MD GOLAM SARWAR, SHIKHA SHARMA, MOHAMMED ARIF, SARJEET SINGH THAKUR, FAREEDA ATHAR, BEENA KHILLARE, SONU CHAND THAKUR	
<a href="#">EVALUATION OF INVITRO ANTI-OXIDANT AND ANTICANCER ACTIVITY OF CORIANDRUM SATIVUM AGAINST HUMAN COLON CANCER HT- 29 CELL LINES</a>	421-424
T. G. NITHYA, D. SUMALATHA	
<a href="#">ANTIBIOTIC RESISTANCE PROFILES OF BACTERIAL PATHOGENS FROM PRIVATE HOSPITALS IN DAR ES SALAAM, TANZANIA</a>	425-429
K. D. MWAMBETE AND B. NYAULINGO	
<a href="#">ASSESSMENT OF RISK FACTORS AND MEDICATION ADHERENCE OF HYPERTENSION PATIENTS IN A TERTIARY CARE TEACHING HOSPITAL</a>	430-433
V.HARISH KUMAR, M. MOUNIKA, G. TEJA REDDY, S. MAHESH KUMAR	
<a href="#">HYPOGLYCEMIC ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF MANILKARA ZAPOTA SEEDS IN STREPTOZOTOCIN INDUCED DIABETIC RATS</a>	434-437
SARADHA S, RUCKMANI A, CHOKKALINGAM M, MAIGNANAKUMAR R1, ARUNKUMAR R, MADHAVI E, LAKSHMIPATHY PRABHU R	



<a href="#">RUBIA CORDIFOLIA LINN MITIGATES EXPERIMENTAL HEPATOCARCINOGENESIS BY REGULATING MMP-2 AND SPARC</a> PN SHILPA,, SN DEVARAJ AND V SIVARAMAKRISHNAN	438-443
<a href="#">EVALUATION OF PHYTOCHEMICAL, ANTIBACTERIAL AND FREE RADICAL SCAVENGING PROPERTIES OF AZADIRACHTA INDICA (NEEM) LEAVES</a> GARIMA PANDEY, KK VERMA, MUNNA SINGH	444-447
<a href="#">A SIMPLE METHOD FOR DETECTION OF KRAS AND BRAF HOTSPOTS MUTATIONS IN PATIENTS WITH COLORECTAL CANCER</a> HAJAR JADDA, ELMOSTAFA EL FAHIME, FOUAD KETTANI, HICHAM BELLAOUI	448-452
<a href="#">STUDIES ON SYNTHESIS, CHARACTERIZATION AND APPLICATION OF SILVER NANOPARTICLES USING MIMOSA PUDICA LEAVES</a> SAMUEL AKASH RAJ. R, DIVYA. S, SINDHU. S, KASINATHAN. K AND ARUMUGAM P	453-455
<a href="#">FORMULATION AND IN-VITRO EVALUATION OF TRIMETAZIDINE DIHYDROCHLORIDE FLOATING BEADS</a> MOWAFAQ M GHAREEB, ZAINAB A RADHI	456-460
<a href="#">FORMULATION AND IN VITRO EVALUATION OF DICLOFENAC SODIUM SUSTAINED RELEASE MATRIX TABLETS USING CARNAUBA WAX AS A MATRIX FORMER</a> MOHAMMED K. AHMED AND YEHIA I. KHALIL	461-466
<a href="#">FORMULATION DEVELOPMENT, CHARACTERIZATION &amp; ESTIMATION OF ACID NEUTRALIZATION CAPACITY OF SHANKHA BHASMA TABLETS FOR THE TREATMENT OF DYSPEPSIA</a> ANKIT SETH, SANTOSH K. MAURYA, ASHISH SRIVASTAVA	467-469
<a href="#">EVALUATION OF PHYSICAL PROPERTIES OF JIT-TRA-ROM FAST DISINTEGRATING TABLETS PREPARED BY THE DIRECT COMPRESSION METHOD</a> CHAOWALIT MONTON, WORAWAN SAINGAM, JIRAPORNCHAI SUKSAEREE	470-472
<a href="#">DEVELOPMENT AND VALIDATION OF GC METHOD FOR THE ESTIMATION OF EUGENOL IN CLOVE EXTRACT</a> B. Y. K. SRUTHI, B.M. GURUPADAYYA, VENKATA SAIRAM.K, T. NARENDRA KUMAR	473-476
<a href="#">ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF SEED KERNEL EXTRACTS OF ENTADA PHASEOLOIDES MERRILL.</a> PARAG KADAM, SUBHASH L. BODHANKAR	477-481
<a href="#">DEVELOPMENT AND VALIDATION OF RANITIDINE HYDROCHLORIDE IN HUMAN PLASMA BY LIQUID CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY AND ITS APPLICATION</a> SENTHIL KUMAR K. R., J.S.K.NAGARAJAN, S.N. MEYYANATHAN	482-485
<a href="#">SYNTHESIS OF NOVEL ANTIMICROBIAL DERIVATIVES OF 3-SUBSTITUTED PYRROLIDINE2, 5-DIONES USING PHARMACOPHORE HYBRID APPROACH: PART-I</a> SMITA PAWAR, SANJAY SAWANT, AMIT NERKAR, ASHOK	486-490
<a href="#">ANTICRYPTOCOCCAL ACTIVITY OF ALKALOID RICH FRACTION OF LEAVES OF PROSOPIS JULIFLORA - A FUTURE PROMISING SUPPLEMENTARY THERAPY FOR CRYPTOCOCCOSIS AND CRYPTOCOCCAL MENINGITIS?</a> VALLI S, GOKULSHANKAR S, MOHANTY BK, RANJITH MS, ASHUTOSH SR, REMYA V	491-495
<a href="#">FABRICATION AND IN VITRO EVALUATION OF POROUS OSMOTIC PUMP BASED CONTROLLED DRUG DELIVERY OF METOPROLOL SUCCINATE</a> A. PRASHANTH KUMAR	496-500
<a href="#">IN VITRO EVALUATION OF XANTHINE OXIDASE INHIBITORY ACTIVITY OF SONCHUS ARVENSIS LEAVES</a> RINI HENDRIANI, ELIN YULINAH SUKANDAR, KUSNANDARANGGADIREDJA, SUKRASNO	501-503
<a href="#">IN VITRO ANTHELMINTIC ACTIVITY OF HYDROETHANOLIC AND AQUEOUS SESBANIA SESBAN, PERS. LEAF EXTRACT AGAINST MONEIZIA EXPANSA AND PARAMPHISTOMES</a> LIMSAY R. P., JANGDE C. R., AFROZ JAHAN AND SWATI UMA P.	504-505
<a href="#">INFLUENCE OF LARGED AILY DOSES OF METFORMIN ON THE EXPRESSION OF INTERLEUKIN-1B AND C-REACTIVE PROTEIN IN TYPE 2 DIABETES PATIENTS</a> BAN HOSHI KHALAF, MANAL KHALID ABDULRIDHA, KADHIM ALI KADHIM, SAADABDUL RAHMAN HUSSAIN	505-509
<a href="#">EVALUATION OF BACILLUS CEREUS AND BACILLUS PUMILUS METABOLITES FOR CNS DEPRESSANT AND ANTICONVULSANT ACTIVITIES</a> VIJAYA KUMAR M. L, THIPPESWAMY B AND KUPPUSI I. J	510-514
<a href="#">RHEIN INDUCED CELL DEATH AND APOPTOSIS THROUGH CASPASE DEPENDENT AND ASSOCIATED WITH MODULATION OF P53, BCL-2/BAX RATIO IN HUMAN CELL LINES</a> ATHEER A. AL-FATLAWI, ANEES A. AL-FATLAWI, MD. ZAFARYAB, MD. IRSHAD, IRFAN AHMAD, ZAKIA KAZIM, AYAZ AHMAD, M. MOSHAHID A. RIZVI	515-519
<a href="#">STABLE COLLOIDAL CHITOSAN/ALGINATE NANOCOMPLEXES: FABRICATION, FORMULATION OPTIMIZATION AND REPAGLINIDE LOADING</a> ENAS ELMOWAFY, RIHAB OSMAN, ABD EL-HAMEED A. EL-SHAMY, GEHANNE AS AWAD	520-525
<a href="#">IN VITROANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF WHOLE PLANT OF CALYCOPTERIS FLORIBUNDA (LAM.)</a> BHUVANESWARI SANTHARAM, GANESH P AND SORANAM R	526-529
<a href="#">PREPARATION AND EVALUATION OF RICE BRAN OIL MASK</a> JIRAPORNCHAI SUKSAEREE, CHAOWALIT MONTON, LAKSANA CHAROENCHAI, PATHAMAPORN PATHOMPAK	530-533
<a href="#">BIPHASIC DOSE RESPONSE EFFECT OF STACHYS OCYMASTRUM ON THE RETICULOENDOTHELIAL SYSTEM PHAGOCYTTIC ACTIVITY</a> BENMEBAREK A, ZERIZER S, LAKHAL H, KABOUICHE Z	534-537
<a href="#">BIOCHEMICAL EFFECT OF INDUSTRIAL EFFLUENCE ON GERMINATING SEEDS OF CICER ARIENTUM</a> DIVYAPRIYA.S, DIMI DIVAKARAN, DEEPTHI K. P	538-542

<a href="#">A NEW METHOD OF EXTRACTION, ISOLATION AND DETERMINATION OF SOLANESOL FROM TOBACCO WASTE (<i>NICOTIANA TOBACUM</i> L.) BY NON-AQUEOUS RP-HPLC</a>	543-546
SRINIVASA RAO ATLA, BHAVANI RAJA, BASAVA RAJU DONTAMSETTI	
<a href="#">CHEMICAL COMPOSITION AND LOCOMOTOR ACTIVITY OF <i>ANDALIMAN</i> FRUITS (<i>ZANTHOXYLUM ACANTHOPODIUM</i> DC.) ESSENTIAL OIL ON MICE</a>	547-550
MOELYONO MOEKTIWARDoyo, MUCHTARIDI MUCHTARIDI, ELI HALIMAH	
<a href="#">EVALUATION OF IN VIVOE-IN VITRO RELEASE OF THEOPHYLLINE FROM NOVEL CONTROLLED RELEASE MICROSPHERES</a>	551-555
CUI MEI CHEN, GUIYIN LI, PING DING	
<a href="#">UTILIZATION OF CO-CRYSTALLIZATION FOR SOLUBILITY ENHANCEMENT OF A POORLY SOLUBLE ANTIRETROVIRAL DRUG - RITONAVIR</a>	556-558
KUSHAL SHAH, SHEETAL BORHADE, VAISHALI LONDHE	
<a href="#">DEVELOPMENT OF TLC AUTOGRAPHICALLY GUIDED GC-MS PROFILE OF MAJOR PHYTOCHEMICAL CONSTITUENTS FOR COMPARATIVE ASSESSMENT IN DIFFERENT EXTRACTS OF <i>AGERATUM CONYZOIDES</i> L.</a>	559-561
S. MISHRA, B. DWIVEDI, A. SINGH, L.K.THAKUR, R.RANI, R.PARJAPATI	
<b>Letter to editor</b>	
<a href="#">MISTY ROLE OF AMYGDALA IN FEMALE REPRODUCTIVE BEHAVIOR</a>	562-563
PRASENJIT CHAUDHURI, KOUSHIK BHATTACHARYA, PALLAV SENGUPTA	

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## PRAFORMULATION STUDY OF *P*-METHOXYCINNAMIC ACID (PMCA) NANOEMULSION USING VEGETABLE OILS (SOYBEAN OIL, CORN OIL, VCO)

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### ABSTRACT

**Objective:** This study aims were to obtain the praformulation data of *p*-methoxycinnamic acid (PMCA) (solubility in buffer; apparent partition coefficient between buffer and vegetable oils), to determine the fatty acids content and screening HLB needs of each oils as well as to determine the composition of nanoemulsion using these oils, surfactants (Tween 80 and Span 80) and ethanol 96% as cosurfactant.

**Methods:** Solubility test of PMCA in acetate buffer pH 4.2±0.2 were conducted with shakes and followed by measuring PMCA amount dissolved by spectrophotometer. To determine the PMCA apparent partition coefficients between buffer pH 4.2±0.2 and soybean oil, corn oil, VCO, respectively, PMCA dissolved in buffer then oil that has been saturated with buffer were added. Firstly by shaking followed by centrifugations than PMCA remained amount in the buffer was measured by spectrophotometer. Determination of fatty acid contents in the oils was used GC-MS while screening HLB was carried by various compositions of surfactants and cosurfactant to obtain a clear emulsion (nanoemulsion).

**Results:** From the results of this study, the solubility of PMCA in acetate buffer pH 4.2±0.2 was 70.04±0.66 mg/L. PMCA apparent partition coefficient in buffer pH 4.2±0.2 and soybean oil, corn oil, and VCO, respectively were 2.39; 2.38 and 2.41. The main contents of soybean oil fatty acids were 19.57% palmitic acid, 45.2% linoleic acid, 25.36% elaidic acid, and 7.07% stearic acid. The main contents of corn oil fatty acids were 26.86% palmitic acid, 31.52% linoleic acid, 31.30% elaidic acid and 4.68% stearic acid. The main contents of VCO fatty acids were 32.41% lauric acid, 24.15% myristic acid, 15.68% palmitic acid, 2.29% linoleic acid, 11.06% elaidic acid and 5.22% stearic acid. These oils HLB needs was 14 and the ratio of surfactant - cosurfactant which can formed a clear emulsion (nanoemulsion) was 6:1

**Conclusion:** 1) To produce nanoemulsion with soybean oil, corn oil, and VCO as oil phase HLB needs was 14 and the ratio of surfactant and cosurfactant was 6:1. 2) Nanomulsions with soybean oil and corn oil more turbid than nanoemulsion with VCO.

**Keywords:** PMCA, Solubility, Apparent partition coefficient, Fatty acids, Soybean oil, Corn oil, VCO, HLB needs.

### INTRODUCTION

One of the drug delivery system that has been known to increase the solubility and penetration of the drug is nanoemulsion system. Nanoemulsion system consists of a water phase, oil phase, surfactant and cosurfactant [1]. Increased penetration of the system is due to the increasing amount of material in the form of molecular medicine so that differences in the concentration of the drug outside and inside skin which can be greater as the driver of the penetration process, in addition to the amount of surfactant and cosurfactant high enough to serve as an enhancer [2]. Another advantage of this system is that the spontaneous creation without the need for heating and vigorous stirring. To increase the use of natural resources and improve the products of traditional medicine into modern medicine, the nanoemulsion delivery system is applied to the *p*-methoxycinnamate acid (PMCA). In order to attempt the development of PMCA as topical anti-inflammatory preparations on nanoemulsion system utilizing plant oils (soybean oil, corn oil, VCO). PMCA is a component of the *Kaempferia galangal* rhizome, widely used as a traditional medicine (Jamu) [3]. *Kaempferia galangal* rhizome by Javanese traditional herb used in a formula named "bobok" blend with *oryza sativa*. The formula function removes the body pain due to sprains, exercise or heavy work. To formulate a topical preparation is needed praformulation data, then in this research study involves determining solubility and partition coefficient of PMCA, determine the fatty acid content and screening HLB need of vegetable oil and to get a comparison of surfactant-cosurfactant which can produce a clear (translucent) emulsion.

### MATERIALS AND METHODS

These research started by qualitative analysis of PMCA by IR JASCO FT/IR-5300 Instrument and Differential Thermal Analysis (DTA), than determination of  $\lambda$  maximum and PMCA standard curve in acetate buffer pH 4.2±0.2 followed by solubility test of PMCA in acetate buffer pH 4.2±0.2. Solubility test of PMCA in acetate buffer pH

4.2±0.2 were conducted by shaking for 4 hours at 150 rpm followed by measuring PMCA amount dissolved by spectrophotometer. To

determine the PMCA apparent partition coefficients between buffer pH 4.2±0.2 and soybean oil, corn oil, VCO, respectively, PMCA (50 ppm and 60 ppm) was dissolved in 10.0 mL acetate buffer pH 4.2±0.2 then oil (0.5; 1.0 and 2.0 mL) that has been saturated with acetate buffer pH 4.2±0.2 were added. Firstly by shaking for 2 hours at 150 rpm, followed by centrifugations for 15 minutes at 2200 rpm than PMCA remained amount in the buffer was measured by spectrophotometer.

Determination of fatty acid contents in the oils was used GC-MS while screening HLB was carried by various combinations of surfactants (Tween 80 and Span 80 to produce HLB 10,11,12,13 and 14). To obtain a clear emulsion one portion of oil added with nine portion of combination of surfactant and cosurfactant (1:1 ; 2:1; 3:1 etc. .... 9:1), than added slowly with acetate buffer pH 4.2±0.2.

### RESULTS AND DISCUSSION

The result of PMCA qualitative test by IR JASCO FT/IR-5300 Instrument between wave's numbers 450 - 4000 cm<sup>-1</sup> that found the infrared spectra as showed Figure 1 and PMCA melting temperature (174.4°C) by Differential Thermal Analysis (DTA) showed in Figure 2.

Table 1: The PMCA solubility in acetate buffer pH 4.2±0.2

Solubility of PMCA in ACETATE buffer pH 4.2 ±0.2	
Replicate	Concentration (mg/L)
1	69.69
2	69.44
3	70.54
4	68.99
5	71.10
6	70.45
mean ± SD	70.04 ± 0.66

Results of the determination of the maximum wavelength of PMCA in acetate buffer solution of pH 4.2±0.2; the PMCA maximum wavelength obtained in acetate buffer pH 4.2±0.2 was 301 nm.

Regression equation of the relationship curve between the absorbance vs. PMCA concentration obtained  $y = 0.1063x - 0.0014$  with a correlation coefficient  $r = 0.9999$ .

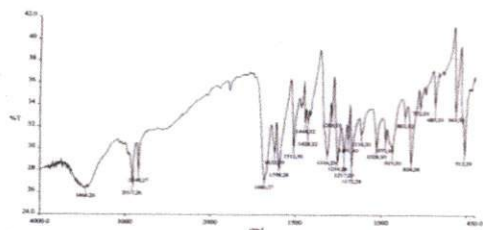


Fig. 1: PMCA infrared spectra between waves number 450 - 4000  $\text{cm}^{-1}$  by IR JASCO FT/IR-5300 Instrument.

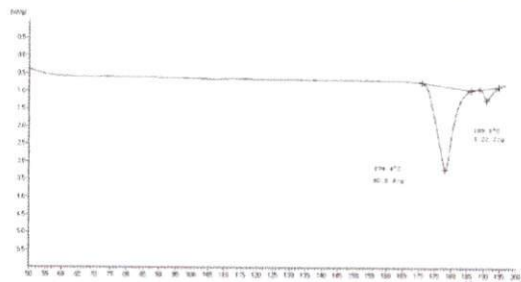


Fig. 2: PMCA melting temperature ( $174.4^\circ\text{C}$ ) by Differential Thermal Analysis (DTA)

PMCA solubility determination in acetate buffer solution of pH  $4.2 \pm 0.2$  begins with the timing of PMCA saturated solubility in acetate buffer solution of pH  $4.2 \pm 0.2$  can be seen in Figure 3, and the results of ANOVA test is known all PMCA concentration at 4, 5, 6, 7 and 8 hour did not differ significantly so that it can be concluded time its saturation solubility at 4<sup>th</sup> hour.

While the results of the determination of the PMCA solubility in acetate buffer pH  $4.2 \pm 0.2$  during 4<sup>th</sup> hour was  $70.04 \pm 0.66$  mg/L can be seen in Table 1.

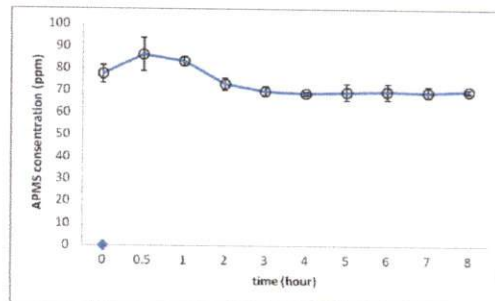


Fig. 3: Correlation curve between PMCA concentrations (ppm) in acetate buffer pH  $4.2 \pm 0.2$  vs. time (hour)

The apparent partition coefficient of PMCA (concentration of 50 and 60 ppm) in acetate buffer pH  $4.2 \pm 0.2$  and each oil (soybean oil/corn oil/VCO) with ratio of 10:0.5; 10:1.0; 10:2.0 are presented in Table 2. From Table 2 known apparent partition coefficients of PMCA in acetate buffer pH  $4.2 \pm 0.2$  and soybean oil, corn oil, VCO were  $2.39 \pm 0.11$ ;  $2.38 \pm 0.12$  and  $2.41 \pm 0.13$  respectively.

Table 2: PMCA apparent partition coefficient in acetate buffer pH  $4.2 \pm 0.2$  and each oil (soybean oil, corn oil, and VCO)

Oil	Buffer : Oil Ratio (mL)	PMCA Apparent partition coefficient						Mean $\pm$ SD	
		Concentration PMCA in buffer							
		50 ppm			60 ppm				
		1	2	3	1	2	3		
Soybean oil	10 : 0.5	2.53	2.52	2.51	2.57	2.55	2.56	2.54	2.39 $\pm$ 0.11
	10 : 1.0	2.36	2.33	2.34	2.38	2.39	2.37	2.36	
	10 : 2.0	2.24	2.29	2.26	2.21	2.33	2.31	2.27	
Corn oil	10 : 0.5	2.52	2.54	2.56	2.55	2.55	2.54	2.54	2.38 $\pm$ 0.12
	10 : 1.0	2.38	2.36	2.37	2.38	2.39	2.36	2.37	
	10 : 2.0	2.29	2.28	2.29	2.16	2.16	2.16	2.22	
VCO	10 : 0.5	2.51	2.48	2.46	2.69	2.64	2.62	2.57	2.41 $\pm$ 0.13
	10 : 1.0	2.39	2.39	2.42	2.48	2.49	2.51	2.45	
	10 : 2.0	2.25	2.26	2.29	2.33	2.28	2.29	2.28	

Table 3: The main contents of soybean oil, corn oil, and VCO fatty acids

Fatty acids main contents of vegetable oil					
Soybean oil	Corn oil	VCO		Fatty acid	Conc. (%)
Fatty acid	Conc. (%)	Fatty acid	Conc. (%)	Fatty acid	Conc. (%)
palmitic acid (C16)	19.57	palmitic acid (C16)	26.86	lauric acid (C12)	32.41
linoleic acid (C18:2)	45.20	linoleic acid (C18:2)	31.52	myristic acid (C14)	24.15
elaidic acid (C18:1) <i>trans</i>	25.36	elaidic acid (C18:1) <i>trans</i>	31.30	palmitic acid (C16)	15.68
stearic acid (C18)	7.07	stearic acid (C18)	4.68	linoleic acid (C18:2)	2.29
				elaidic acid (C18:1) <i>trans</i>	11.06
				stearic acid (C18)	5.22

Results of the determination of fatty acid content of soybean oil, corn oil and VCO using GC-MS can be seen in Table 3. From Table 3 it can be seen that the highest levels of soybean oil fatty acids was 45.20%

linoleic acid (C18:2), the highest levels of corn oil fatty acids was 31.52% oleic acid (C18:1-*cis*) and 31.30% elaidic acid (18:1-*trans*), while the highest fatty acid levels VCO is 32.41% lauric acid



(C12). Screening of oils HLB needs and determination of surfactants (combination surfactants Tween 80 - Span 80); cosurfactant

(ethanol) ratios were done by ratio 1:1 until 9:1 and combination surfactants Tween 80-Span 80 at HLB 10, 11, 12, 13 and 14.

That result known at surfactant combination with HLB 10, 11 and 12 the emulsion using all oils turbid, at surfactants combination with HLB 13 emulsion using soybean oil and corn oil were turbid, while emulsion using VCO start from surfactant and co-surfactant ratio 3:1 produced clear emulsion. At surfactants combination with HLB 14 emulsion using soybean oil and corn oil appear translucent start from surfactant and cosurfactant ratio 5:1, while emulsion that used VCO start from surfactant and cosurfactant ratio 3:1. So that for application suggest use surfactant and cosurfactant ratio 6:1 at combination surfactants with HLB 14 to produce more stable clear emulsion. The example of emulsion can see in Figure 4, 5, 6 and 7. From Figure 7 was shown that emulsion with VCO more clearly than emulsion with soybean oil and corn oil. It was causes atom C chain of soybean oil and corn oil fatty acid mean content longer than VCO, so that emulsion droplet will be bigger and transparency was decrease [4].



Fig. 4: Emulsion used VCO with surfactants-cosurfactants ratio of 4:1; 6:1; 7:1 and combination surfactants to produced HLB 12



Fig. 5: Emulsions used VCO with surfactants-cosurfactants ratio of 3:1; 4:1; 5:1; 6:1; 7:1; 8:1 and surfactants combination to produced HLB 13



Fig. 6: Emulsions used VCO with surfactants-cosurfactants ratio of 1:1; 2:1; 3:1; 4:1; 5:1; 6:1; 7:1; 8:1; 9:1 and surfactants combination to produced HLB 14



Fig.7: Emulsions used soybean oil (a), corn oil (b) and VCO (c) with surfactants-cosurfactants ratio of 5:1; 6:1; 7:1 and surfactants combination to produced HLB 14.

## CONCLUSION

From the results of this study concluded:

1. The solubility of PMCA in acetate buffer pH  $4.2 \pm 0.2$  was  $70.04 \pm 0.66$  mg/L.
2. PMCA apparent partition coefficient in acetate buffer pH  $4.2 \pm 0.2$  and soybean oil, corn oil, and VCO, were  $2.39 \pm 0.11$ ;  $2.38 \pm 0.12$  and  $2.41 \pm 0.13$  respectively.
3. The main contents of soybean oil fatty acids were 19.57% palmitic acid, 45.2% linoleic acid, 25.36% elaidic acid, and 7.07% stearic acid. The main contents of corn oil fatty acids were 26.86% palmitic acid, 31.52% linoleic acid, 31.30% elaidic acid and 4.68% stearic acid. The main contents of VCO fatty acids were 32.41% lauric acid, 24.15% myristic acid, 15.68% palmitic acid, 2.29% linoleic acid, 11.06% elaidic acid and 5.22% stearic acid.
4. Soybean oil, corn oil, and VCO HLB needs was 14 and the ratio of surfactant and cosurfactant which can formed a clear emulsion (nanoemulsion) was 6:1.
5. Nanomulsions with soybean oil and corn oil more turbid than nanoemulsion with VCO.

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