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Research Article

PHYTOCHEMICAL AND ANTIBACTERIAL ANALYSIS OF AQUEOUS AND ALCOHOLIC EXTRACTS OF VERNONIA AMYGDALINA (DEL.) LEAF

*Tsaku Alumbugu Paul, Ibrahim Taibat, Ekeleme Ike Kenneth, Nkenne Istifanus Haruna, Oti Victor Baba and Abimiku Rejoice Helma

ABSTRACT

PDF

[DOI : [10.20959/wjpr20187-11567](#)]

DETECTIONS OF SOME RESPIRATORY VIRUSES BY MOLECULAR TECHNIQUES AMONG TWO SUDANESE TARGETS INDIVIDUALS

Ibrahim H. S.*, Kafi S. K., Musa H. A., Karsany M. S. and Abdel Hamid M. M.

ABSTRACT

PDF

[DOI : [10.20959/wjpr20187-11543](#)]

POLYUNSATURATED FATTY ACIDS INTAKE AND ITS ASSOCIATION WITH BREAST CANCER AMONG PATIENTS ATTENDING THE RADIATION AND ISOTOPE CENTER IN KHARTOUM STATE

Hafsa A. Elsafi* and Hassan A/Aziz Musa

ABSTRACT

PDF

[DOI : [10.20959/wjpr20187-11199](#)]

ASSESSMENT OF CHRONIC KIDNEY FAILURE PATIENTS' AWARENESS ABOUT DISEASE AND HEMODIALYSIS COMPLICATIONS IN ALHASA REGION OF SAUDI ARABIA

Dr. Amal Khaleel Abu Alhommos* and Fahad Sami Al-Fahaid

ABSTRACT

PDF

[DOI : [10.20959/wjpr20187-11644](#)]

EVALUATION OF RISK FACTORS IN BREAST CANCER AMONG IRAQI FEMALE PATIENTS

Bushra Hasan Hashim*, Khulood Kareem Abed, Lina Ziyad Tareq, Arwa Ra'id Mishaal and Haneen Sedqi Ismael

ABSTRACT

PDF

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SYNTHESIS OF N-4-METHOXYBENZOYL-N'-PHENYLUREA COMPOUND AND ACTIVITY TEST OF ANTICANCER AGAINST HeLa CELL LINE

Bambang Tri Purwanto*

ABSTRACT

PDF

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DOCKING, SYNTHESIS AND CYTOTOXIC TEST ON HUMAN BREAST CANCER CELL LINE T47D OF N- (PHENYL CARBAMOTHIOYL)-BENZAMIDE

Dini Kesuma*, Siswandono, Bambang Tri Purwanto and Marcellino Rudyanto

ABSTRACT

PDF

[DOI : [10.20959/wjpr20187-11474](#)]

ASSESSMENT OF CINNAMON EFFECT TO REDUCE PRIMARY DYSMENORRHEA AND IMPROVE QUALITY OF LIFE AMONG FEMALES IN EASTERN REGION OF SAUDI ARABIA: A CROSS SECTIONAL SURVEY

Dr. Amal Khaleel Abu Alhommos* and Muneerah Al-Hadi

ABSTRACT

PDF

[DOI : [10.20959/wjpr20187-11682](#)]

ASSESSMENT OF SAUDI WOMEN AWARENESS ABOUT PAIN RELIEF AGENTS IN LABOR IN AL-HASA CITY OF SAUDI ARABIA

Dr. Amal Khaleel Abu Alhommos* and Malak AlDossary

ABSTRACT

PDF

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ANALYSIS OF PRESCRIPTION PATTERN OF ANTIBIOTICS USED FOR THE MANAGEMENT OF URINARY TRACT INFECTION IN A KIDNEY CENTER

Dr. Maqsood Ahmed Khan*, Syed Imran Ali, Sadia S. Kashif, Dr. Rabia Bushra, Aqsa Fatima Wali, Aiman Ashraf, Summaiya Mukhtar, Maryam Nasar, Aruba Hassan and Saba Shahid

ABSTRACT

PDF

[DOI : [10.20959/wjpr20187-11418](https://doi.org/10.20959/wjpr20187-11418)]

PROTECTIVE ROLE OF MORINGA OLEIFERA LEAF EXTRACT AS A NATURAL ANTIOXIDANT AGAINST TOXIC EFFECT OF PARACETAMOL ON MALE MICE

Samia A. El-Fiky, Nagwa A. Hassan, Hassab El-Nabi S. E., Hasnaa A. Radwan* and Elsayad R. I.

ABSTRACT

PDF

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LOCAL SURVEY FOR AWARENESS OF HYPERACIDITY AND/OR OTHER ASSOCIATED PROBLEMS

Dr. Geeta D. Parulkar*, Dr. Manjushri Kharat, Dr. Abhishek Taksale, Dr. Ajitkumar Sahu, Dr. Vaishali Kokani, Dr. Ganesh Patare and Dr. Minal Bhusewar

ABSTRACT

PDF

[DOI : [10.20959/wjpr20187-11439](https://doi.org/10.20959/wjpr20187-11439)]

KNOWLEDGE OF ANTENATAL CARE IN WOMEN OF REPRODUCTIVE AGE RESIDING IN SUB-URBAN AREAS OF LAHORE

Musa Raza*, M Farooq Amjad, Samiyah Tasleem, Alweena Awan, Maarif Shehzad and Ayesha Humayun

ABSTRACT

PDF

[DOI : [10.20959/wjpr20187-11747](https://doi.org/10.20959/wjpr20187-11747)]

CHARACTERIZATION OF A NOVEL TAYLOR-COUETTE ULTRAVIOLET REACTOR FOR NON-THERMAL PASTEURIZATION OF MILK

Rawa Abdul Redha Aziz* and Keith Warriner

ABSTRACT

PDF

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TO EVALUATE GANDHRVAHASTYADI CHURNA IN MOOTRASHMARI

*Vd. Santosh Yashwant Solaskara and Dr. Umesh Avinash Vaidya

ABSTRACT

PDF

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TOTAL PHENOLIC CONTENTS OF SOME SEAWEED COLLECTED FROM RAIGAD COASTS OF KONKAN (MS)

J. S. Ambhore and *Whankatte V. R.

ABSTRACT

PDF

[DOI : [10.20959/wjpr20187-11107](https://doi.org/10.20959/wjpr20187-11107)]

CHOLINE CHLORIDE-UREA: AN EFFICIENT, GREEN MEDIA FOR PARTIAL HYDROLYSIS OF DI- AND TRI-PROTECTED GANCICLOVIR AND SELECTIVE O-ACYLATION IN THE SYNTHESIS OF ANTIVIRAL DRUG VALGANCICLOVIR

Anita Goswami-Giri* and Pranaya P. Dhawle

ABSTRACT

PDF

[DOI : [10.20959/wjpr20187-11305](https://doi.org/10.20959/wjpr20187-11305)]

ASSESSMENT OF QUALITY OF LIFE IN ADULT DIABETIC PATIENTS: PHARMACOLOGICAL THERAPY AND NON PHARMACOLOGICAL THERAPY

G. Prasanth*, R. Yogananda and N. J. Suba Sree

ABSTRACT

PDF

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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF THIADIAZOLE DERIVATIVES

*Dr. Rashmi Kumari, Dr. Vachaspati Dubey, Dr. S. K. Mishra and Anshu Raj

ABSTRACT

PDF

[DOI : [10.20959/wjpr20187-11417](https://doi.org/10.20959/wjpr20187-11417)]

FORMULATION AND EVALUATION OF FLOATING PULSATILE TABLET IN TABLET DRUG DELIVERY SYSTEM FOR HYPERTENSION

Mansi Shah* and Dipti Patel

ABSTRACT

PDF

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SYNTHESIS, CHARACTERISATION AND BIOLOGICAL EVALUATION OF A NEW SERIES OF BENZOFURANS

N. Vijaya Lakshmi*, V. Sandhya Reddy, S. K. Johny Basha, S. K. Shakeela Begum and D. Pavana Deepthi

[ABSTRACT](#)
[PDF](#)
[\[DOI : 10.20959/wjpr20187-11488 \]](#)


SCREENING OF PHYTOCHEMICALS, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF ELEPHANTOPUS SCABER LEAVES

Abarna N., Stephy Verghese and Jasmine R.*

[ABSTRACT](#)
[PDF](#)
[\[DOI : 10.20959/wjpr20187-11507 \]](#)


MICROPROPAGATION OF AN ENDANGERED TERRESTRIAL ORCHID GEODORUM DENSIFLORUM (LAM.) SCHLTR. OF KANYAKUMARI DISTRICT, INDIA

G.V. Gegl, Dr. B. Christudhas Williams & Dr. R. Mary Suja

[ABSTRACT](#)
[PDF](#)
[\[DOI : 10.20959/wjpr20187-11520 \]](#)


SCREENING OF RHIZOMICROFLORA ISOLATED FROM THE RHIZOSPHERE OF AEGLE MARMELOS FOR MULTIPLE PLANT GROWTH PROMOTING AND ANTIMICROBIAL ACTIVITIES

Damle N. R.* and S. W. Kulkarni

[ABSTRACT](#)
[PDF](#)
[\[DOI : 10.20959/wjpr20187-11528 \]](#)


SCREENING OF FUNGAL PATHOGENS FOR BIOLOGICAL MANAGEMENT OF NOXIOUS WEED EICHHORNIA CRASSIPES

R. P. Mishra and Sohni Jain*

[ABSTRACT](#)
[PDF](#)
[\[DOI : 10.20959/wjpr20187-11537 \]](#)


EVALUATION OF ANTIMICROBIAL ACTIVITY OF EXTRACTS OF PIPER BETEL LEAVES AGAINST SOME COMMON PATHOGENS

V. P. Sutar*

[ABSTRACT](#)
[PDF](#)
[\[DOI : 10.20959/wjpr20187-11540 \]](#)


SYNTHESIS AND CHARACTERIZATION OF NICKEL OXIDE BY USING UV/VIS SPECTROMETRY, SCANNING ELECTRON MICROSCOPY AND ITS ANTIMICROBIAL ACTIVITY AGAINST BACILLUS SPP., PSEUDOMONAS SPP. ESCHERICHIA COLI

J. C. Pradeep Kumar* and Dr. F. V. Dandawate

[ABSTRACT](#)
[PDF](#)
[\[DOI : 10.20959/wjpr20187-11541 \]](#)


NON INVASIVE ASSESSMENT OF NUTRITIONAL STATUS AMONG SCHOOL CHILDREN OF A SEMI-URBAN AREA IN TAMILNADU

Suja Pandian R.*

[ABSTRACT](#)
[PDF](#)
[\[DOI : 10.20959/wjpr20187-11549 \]](#)


CROSS MATCHING TRIBAL ETHNO MEDICINAL PRACTICES IN DIARRHEA & DYSENTERY BY BHIL- MINA TRIBE OF BANSWARA, RAJASTHAN WITH PUBLISHED PHARMACOLOGICAL RESEARCH

Dr. P. K. Dam* and Pankaj Kumar

[ABSTRACT](#)
[PDF](#)
[\[DOI : 10.20959/wjpr20187-11550 \]](#)


EFFECT OF BIOCHAR AMENDMENT AND AGEING ON SORPTION AND DISSIPATION OF TETRACYCLINE'S IN SOILS

*Dr. O. P. Bansal

[ABSTRACT](#)
[PDF](#)
[\[DOI : 10.20959/wjpr20187-11553 \]](#)


KNOWLEDGE AND AWARENESS ABOUT PHARMACOVIGILANCE AMONG 2ND YEAR MBBS STUDENTS AT GOVERNMENT MEDICAL COLLEGE AND HOSPITAL

Dr. Rajat Mishra*, Syed Meraj Fatmi and Dr. Sunil Kumar Singh

[ABSTRACT](#)
[PDF](#)
[\[DOI : 10.20959/wjpr20187-11557 \]](#)


EFFICACY OF JALANETI AND PRANAYAMA IN MANAGEMENT OF VATAJA PRATISHYAYA (ALLERGIC RHINITIS)

Dr. Manish Arora*, Dr. Patwardhan Ravindra, Dr. Shrikant G. Ingole and Dr. Rahul Gujarati

[ABSTRACT](#)
[PDF](#)
[\[DOI : 10.20959/wjpr20187-11559 \]](#)


CLINICAL EVALUATION OF BILVADI CHURAN IN THE MANAGEMENT OF GRAHANI ROG W.S.R. TO IBS (IRRITABLE BOWEL SYNDROME)

Dr. Avneet Kaur* and Dr. Manu Sharma

ABSTRACT

PDF

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IN VIVO STUDIES & SEM OF CONTROLLED POROSITY OSMOTIC PUMP (CPOP) TABLET OF ATENOLOL

Ayesha Sultana* and D. Varun

ABSTRACT

PDF

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DEVELOPMENT AND VALIDATION OF AN UVSPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF TENELIGLIPTIN AND METFORMIN

Leena Ajay Sawaikar*

ABSTRACT

PDF

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DEVELOPMENT OF POLYHERBAL PREPARATION FOR HAIR GROWTH PROMOTING ACTIVITY

Roshni Sahu*, Narendra Vyas, Shivendra S. Raghuwanshi, Sapna Malviya and Anil Khariya

ABSTRACT

PDF

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STUDY AND DESIGN OF BENZAMIDE AND PYRIDINECARBOXAMIDE DERIVATIVES AS A GLUCOKINASE ACTIVATOR

Dhanraj Patidar*, Sailesh Narayan, Rajeev Malviya and Phool Singh Yaduwanshi

ABSTRACT

PDF

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PURIFICATION OF BETA GALACTOSIDASE ENZYME FROM DAIRY EFFLUENT BACILLUS SPECIES

V. Pavithra and *Dr. J. Thirumagal

ABSTRACT

PDF

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NEW AND EFFICIENT GREEN CATALYST ONE-POT SYNTHESIS OF BENZIMIDAZOLE AND DERIVATIVES

Gurumeet C. Wadhawa*, Vitthal S. Shivankar, Yashwant A. Gaikwad, Nilam S. Dhumale, Charansingh H. Gill and Laxman V. Gavali

ABSTRACT

PDF

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IN VITRO ANTICANCER ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *Acampe praemorsa* (Roxb.)

G. Soumiya*, Dr. B. Christudhas Williams and Dr. R. Mary Suja

ABSTRACT

PDF

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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF EXTENDED SPECTRUM B-LACTAMASE PRODUCING ESCHERICHIA COLI

Parveen W.* and Subhashini A.

ABSTRACT

PDF

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PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITIES OF APPLE BER, A HYBRID VARIETY OF ZIZIPHUS MAURITIANA

Rajni Nigam*

ABSTRACT

PDF

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FORMULATION AND EVALUATION OF GELRITE BASED PHASE CHANGE OPHTHALMIC SOLUTION OF NEPAFENAC- As QBD APPROACH

Deepak G. Wagh* and Dr. Sadhana R. Shahi

ABSTRACT

PDF

[DOI : [10.20959/wjpr20187-11627](https://doi.org/10.20959/wjpr20187-11627)]

SCREENING FOR CONGENITAL HYPOTHYROIDISM IN GREATER NOIDA BY MEASUREMENT OF UMBILICAL CORD THYROXINE (FREE) AND THYROID STIMULATING HORMONE: A MULTICENTRIC HOSPITAL BASED STUDY

Chandra Prakash Sharma, Widhi Dubey and Dr. Suryakant Nagtilak*

ABSTRACT

PDF

[DOI : [10.20959/wjpr20187-11632](https://doi.org/10.20959/wjpr20187-11632)]

IN-VITRO ANTIHELMINTHIC ACTIVITY, PHOTO CHEMICAL SCREENING AND TLC STUDIES OF METHANOL EXTRACTION ON CELOSIA CRISTATA FLOWER USING IN - STATE FESTIVAL OF TELANGANA (BATHUKAMMA)

P. Sravan Kumar*



[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11633 \]](#)**ANTIBACTERIAL SCREENING OF AMORPHOPHALLUS CAMPANULATUS ROXB. AGAINST SELECTED HUMAN PATHOGENIC BACTERIA****Dr. T. Francis Xavier, G. Dhanasekaran* and D. Sathish Kumar**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11636 \]](#)**ISOLATION AND MOLECULAR IDENTIFICATION OF HALOPHILIC ARCHAEA (Part –I) FROM SALINE SOIL****Arpita R. Dave* and Dr. Ajit V. Pandya**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11637 \]](#)**GREEN SYNTHESIS OF GOLD NANOPARTICLES USING CASSIA ALATA LEAF EXTRACT AND EVALUATION OF ANTIMICROBIAL ACTIVITIES****Le Thi Thanh, Thejesh Kumar M. P. and Rajkumar H. Garampalli***[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11640 \]](#)**N-LINKED GLYCOSYLATION AND CHARGE VARIANT ANALYSIS OF BIOSIMILAR ANTI HER2 ANTIBODY IN TREATMENT OF BREAST CANCER****Pratik R. Gajjar*, Dasharath M. Patel, Amar K. Shrivastava, Umesh S. Shaligram, Noelle Sunstrom**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11645 \]](#)**GC-MS AND ANTIOXIDANT POTENTIAL OF METHANOLIC LEAF EXTRACT OF PUTRANJIVA ROXBURGHII WALL. (PUTRANJIVACEAE)****Emasushan M.* and John Britto S.**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11651 \]](#)**ISOLATION AND IDENTIFICATION OF XYLANASE PRODUCING MICROORGANISMS FROM SOIL****Rajakumar R.* and Rameshwara Reddy Naru**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11655 \]](#)**DESIGN AND PHARMACOLOGICAL STUDIES OF NEW COUMARIN DERIVED CHALCONE SYNTHESIZED COMPOUNDS****Sai Chandra J.***[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11657 \]](#)**A NOVEL VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF NALOXEGOL****Y. Sirisha* and Anilkumar Y.**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11661 \]](#)**STUDY OF THYROID FUNCTIONS IN TYPE 2 DIABETES MELLITUS AND RELATIONSHIP WITH OBESITY IN A TERTIARY CARE HOSPITAL, BANKURA, WEST BENGAL****Dr. Sumanta Banerjee*, Dr. Santa Saha (Roy), Dr. Pinaki Sarkar, Dr. Biswanath Sharma Sarkar and Dr. Dibakar Halder**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11666 \]](#)**A COMPARISON OF VESTIBULAR REHABILITATION APPROACHES ON BALANCE IN HEARING IMPAIRED CHILDREN WITH VESTIBULAR DYSFUNCTION****Shilpa Khandare, Nigel Gonsalves*, Dr. Tushar Palekar PhD., Vidhi Shah and Trupti Siddapur**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11669 \]](#)**VALIDATION OF STABILITY INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF ASSAY OF CARBAMAZEPINE DRUG IN THE PHARMACEUTICALS TABLET FORMULATIONS USING PHENYTOIN AS AN INTERNAL STANDARD****Shaikh Javed Shaikh Afzal*, Suresh C. Ameta and Pathan Mohd Arif Ali Khan**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11670 \]](#)**STUDY OF POLYPHARMACY IN GERIATRIC PATIENTS IN A TERTIARY CARE HOSPITAL: A PROSPECTIVE OBSERVATIONAL STUDY****I. Neelam*, A. Kavya, P. Priyanka, P. Vinod and P. Jeevan**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11674 \]](#)

CHARACTERIZATION OF SALMONELLA SPP- PRODUCING EXTENDED-SPECTRUM B-LACTAMASE (ESBL) ISOLATED FROM CHICKEN SLAUGHTERHOUSES PROCESSING LINE IN NAMAKKAL

*R. Sowmiyadevi, M. Anitha, D. Jegadeeshkumar and B. Madhumathi

ABSTRACT

PDF

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AN ASSESSMENT OF GROUND WATER QUALITY IN RURAL AREA OF ARAVALLI DISTRICT IN WINTER SEASON BY PHYSICOCHEMICAL PARAMETERS

Preksha Patel, Shiv Singh Dulawat* and Mangal Shree Dulawat

ABSTRACT

PDF

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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF SOME SUBSTITUTED-BENZOYL HYDRAZINO DERIVATIVES OF PYRIMIDINE

Darshitkumar M. Dave* and Dr. A. H. Bapodra

ABSTRACT

PDF

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EFFICACY OF KANTAKARI MARICHA LEHA ON KAPHAJA KASA IN CHILDREN OF AGE GROUP 1 TO 5 YEARS

Dr. Shital Sawant*, Dr. J. A. Nandgaonkar and Dr. Rahul H. Gujarathi

ABSTRACT

PDF

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CHEMOMETRICS ASSISTED SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF ALOGLIPTIN AND METFORMIN IN PHARMACEUTICAL DOSAGE FORM

Premakumari K. B.*, Sandhyavali M. S., Murugan V. and Shanaz Banu

ABSTRACT

PDF

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ASSESSMENT OF BIOMOLECULES FROM LEAVES EXTRACT OF ETHNIC PLANTS

B. D. Nagle, Anil Kumar*, K Khasdeo, S. R. Gayakwad, A. D. Lazrus

ABSTRACT

PDF

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EXPLORATION OF ACTINOBACTERIA FROM MANGROVE ECOSYSTEM OF KRISHNA DISTRICT, ANDHRA PRADESH

Krishna Naragani*

ABSTRACT

PDF

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METABOLIC SYNDROME AND RISK OF CARDIOVASCULAR DISEASE IN RURAL AND URBAN PATIENTS IN NORTH INDIA

Manoj Kumar Sharma, Sonali Pandey and Suryakant Nagtilak*

ABSTRACT

PDF

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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC FOR SIMULTANEOUS ESTIMATION OF ETODOLAC & THIOLCHOLCHOSIDE

N. Sai Prudhvi*, M. Prasadarao and G. Anil Kumar

ABSTRACT

PDF

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POTENTIAL USE OF WILD GANODERMA SP BASED PETROLATUM PASTE AGAINST SOME MIXED DERMATOLOGICAL CONDITIONS IN GOATS IN MAIDUGURI, NIGERIA

*Shamaki B. U., Sandabe U. K., Abdulrahman F.I., Sa'idu S. N. A., Tekdek L. B. and Ya'uba A. M.

ABSTRACT

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SYNTHESIS OF GOLD NANOPARTICLES FROM NATURAL HONEY AND ITS APPLICATION IN ANTIFUNGAL ACTIVITY

Dr. R. Manju* and D. Savi

ABSTRACT

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FORMULATION AND EVALUATION OF NANOPARTICLES OF EPROSARTAN MESYLATE

Nihar Ranjan Kar* and Manisa Patel

ABSTRACT

PDF

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PREVALENCE OF COLOR BLINDNESS AMONG THE STUDENTS OF RIYADH COLLEGES OF DENTISTRY AND PHARMACY

Ebtesam Al Fayeze, Ebtehal Al Shammari, Ghada Al Haiid and Dr. Shahzeb H. Ansari*

[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11738 \]](#)**ASSESSMENT OF THE KNOWLEDGE, ATTITUDE, AND PRACTICE OF SUDANESE MOTHERS TOWARD CHILDHOOD VACCINATION, AT KHARTOUM NORTH HEALTH CENTERS: SUDAN****Tahany Mohamed Osman Mohamed and *Kamal Addin Mohammad Ahmad Idris**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11739 \]](#)**ASSESSMENT OF THE KNOWLEDGE, ATTITUDE AND PRACTICE OF SUDANESE ADOLESCENT SECONDARY SCHOOL STUDENTS ABOUT THE APPROPRIATE AND SAFE HANDLING OF MEDICINES****Sahar Mohamed Osman Mohamed and Kamal Addin Mohammad Ahmad Idris***[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11740 \]](#)**ASSESSMENT OF IN-PATIENTS' SATISFACTION WITH THE QUALITY OF HEALTH CARE SERVICES PROVIDED IN SOBA UNIVERSITY TEACHING HOSPITAL, KHARTOUM, SUDAN****Fatima Ismail Hussein Badri and *Kamal Addin Mohammad Ahmad Idris**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11741 \]](#)**EVALUATION OF IN VITRO CYTOTOXICITY OF DIMETHOATE ON HUMAN PERIPHERAL BLOOD LYMPHOCYTES USING MTT ASSAY****Dr. Daya Shankar Gautam*, Meenal Raikwar, Roshni Soni and Sheeba Begum**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11773 \]](#)**SCREENING OF PRE-HYPERTENSION RISK FACTORS AMONG TEACHING PROFESSIONALS****Nigel Gonsalves*, Amita Aggarwal, Dr. Tushar J. Palekar PhD. and Chetna Jadhav**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11775 \]](#)**DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF ROSUVASTATIN AND EZETIMIBE IN CAPSULE DOSAGE FORM****Patel Aesha A.* and Dr. Paresh Patel**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11776 \]](#)**HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF GRACILARIA CORTICATA J.A.G. IN HARE ISLAND, THOOTHUKUDI, TAMIL NADU, INDIA*****John Peter Paul J. and Raja P.**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11780 \]](#)**MONONUCLEAR LINEAR-SHAPED ASSEMBLIES OF N,N'-DIETHYLBENZIMIDAZOLYDINE BASED IONIC LIQUID COORDINATED TO AG(I)- AND CU(I)-NHC AND THEIR ANTIBACTERIAL AND ANTIOXIDANT APPLICATIONS****A. G. Bharathi Dileepan, R. Mathumidha, D. Maruthamuthu, R. Ranjith, S. Priscilla Prabavathi and Shameela Rajam***[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11792 \]](#)**RESIDUE DEPLETION OF TILMICOSIN IN CHICKEN TISSUES****Abdelrahman Mostafa*, Nuha M. A. Agbna, Samia A. Wahab and Sania Shaddad**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11046 \]](#)**ETHANOBOTANICAL CHARACTERIZATION OF COCCINIA INDICA AGAINST URINARY TRACT INFECTION****Deepa M. and Sharly Elgal N*.**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11196 \]](#)**EVALUATION OF ANXIOLYTIC ACTIVITY OF SUBSTITUTED 1, 2, 4-TRIAZOLE BEARING IMINO, FIVE MEMBERED HETEROCYCLIC MOIETY****D. Kumudha*, T. Kalavathi and B. A. Viswanath**[ABSTRACT](#)[PDF](#)[\[DOI : 10.20959/wjpr20187-11440 \]](#)

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GREENER APPROACH OF SYNTHESIS OF AZO DERIVATIVES AND BIS- PYRAZOLE DERIVATIVES ALONG WITH ANTIMICROBIAL SCREENING

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COMPARATIVE PHYTOCHEMICAL AND SPECTROPHOTOMETRIC ANTIOXIDANT EVALUATION OF THE ROOT BARK, STEM BARK, LEAF AND SEED OF COLA NITIDA (STERCULACEAE)

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ANTIPYRETIC EFFECT OF ORAL VERSUS RECTAL ACETAMINOPHEN IN CHILDREN - A RANDOMIZED COMPARATIV STUDY

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Rahnuma Khan*, Dr. Manish Mishra and Dr. Mukta Shrivastav

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DOCKING, SYNTHESIS AND CYTOTOXIC TEST ON HUMAN BREAST CANCER CELL LINE T47D OF *N*-(PHENYLCARBAMOTHIOYL)-BENZAMIDE

Dini Kesuma^{1*}, Siswandono², Bambang Tri Purwanto² and Marcellino Rudyanto²

¹Faculty of Pharmacy, Surabaya University, Surabaya, Indonesia.

²Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia.

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*Corresponding Author

Dini Kesuma

Faculty of Pharmacy,
Surabaya University,
Surabaya, Indonesia.

ABSTRACT

Introduction: New breast anticancer compound *N*-(phenylcarbamothioyl)-benzamide have been synthesized, initiated with molecular modeling in silico which is docking between test compounds with SIRT-1 receptor PDB code: 4I5I to predict the bonding of test compounds with receptors. **Methods:** The compounds are synthesized from benzoyl chloride reaction with *N*-phenylthiourea. The molecular structure is confirmed using FTIR, ¹H-NMR, ¹³C-NMR and Mass Spectra. Anticancer activity is tested in vitro against human breast cancer cells (T47D) using an MTT assay. **Result:** RS (Rerank

Score) value and anticancer activity of test compound obtained are higher than Hydroxyurea as the reference compound. The Value of IC₅₀ *N*-(phenylcarbamothioyl)-benzamide is 0.53 mM (T47D cell) and 49.40 mM (Vero cel) and also IC₅₀ Hydroxyurea is 4.58 mM (T47D cells). It is predicted that the mechanism of action of these new compounds is targeted cell because it has toxic effect on cancer cells and is not toxic in normal Vero cells. **Conclusion:** This new compound are highly potential as an anticancer agent against the human breast carcinoma cell line (T47D).

KEYWORDS: *N*-(phenylcarbamothioyl)-benzamide, anticancer, T47D cell, SIRT 1.

INTRODUCTION

Cancer is one of the major causes of death in developing countries and also worldwide. In Indonesia, cancer becomes the third largest contributor to death after heart disease. Since 2007 breast cancer has reached first ranks for in-patients in hospitals, followed by cervical

cancer. Thiourea is a compound containing sulfur and nitrogen atoms whose chemical structures are similar to the urea compound already used today as an anticancer, including hydroxyurea, nitrosourea and 5-fluorouracil.^[1]

Resistance/intolerance has been reported against hydroxyurea in patients with essential thrombocythaemia.^[2] The clinical use of hydroxyurea begins to diminish, but in biochemical research and development of the anticancer drug hydroxyurea is used as a DNA replication inhibitor.^[3] The research data show that hydroxyurea activity is not maximum because it is hydrophilic with poor membrane penetration capability so it is necessary to develop new anticancer drugs of urea and thiourea derivatives which is more lipophilic with better membrane penetration so that its activity is more potent. Pirrie, 2012, has synthesized and evaluated the anticancer activity of thiourea derivatives, as 1-(4-acetamidophenyl)-3-(4-tert-butylbenzoyl) thiourea which is later named Tenovin-1. These compounds are known to increase p53 protein levels in vitro (inhibits SIRT1).^[4] Widiandani, 2016, has also proven breast anticancer activity (T47D cells) of the *N*-(allylcarbamothioyl)benzamide compound.^[5]

In this study, a new compounds *N*-(phenylcarbamothioyl)-benzamide is obtained with the presence of phenyl and benzoyl rings enhancing the lipophilic and electronic nature of the compound which in turn will improve the bonding of drugs and receptors. Breast cancer cell T47D is used to express the mutated p53 protein initiated with activity predictions by molecular modeling in silico, docking test compound with Histon deacetylase SIRT1 inhibitor code of PDB: 4I5I. This receptor has an important role in the growth of tumor cells.^[6] The test compounds are synthesized from *N*-phenylthiourea with benzoyl chloride using acyl nucleophilic substitution reactions.^[7] The compounds resulted from the synthesis are then identified in structures with IR spectrophotometers, ¹H-NMR spectrometers, ¹³C-NMR and mass spectrometers.^[8]

The anticancer activity of the test compound is determined by cytotoxic test using MTT assay (3-(4,5-dimethylthiazole-2-yl) -2,5-diphenyltetrazolium bromide) in vitro on T47D breast cancer cells and normal Vero cells. The results of anticancer activity test on T47D cells are IC₅₀ compared with Hydroxyurea as the reference compounds, also observed in normal cell Vero. The results of this study is expected to obtain a candidate for a new anticancer drug from thiourea derivatives that have potent anticancer activity on breast cancer cells T47D.^[9] This study will proceed with the synthesis of *N*-(phenylcarbamothioyl)-benzamide derivatives and molecular mechanisms, prior to preclinical and clinical trials.

MATERIALS AND METHODS

WORK PROCEDURES

Molecular Modeling

Predicting activity with molecular modeling of *N*-(phenylcarbamothioyl)-benzamide by docking with Histone deacetylase SIRT1 inhibitor PDB code: 4I5I, continued with using the MVD 5.5 software. Hydroxyurea is used as a reference compound.^[10]

Test Compounds Synthesis

The Synthesis of *N*-(phenylcarbamothioyl)-benzamide

In a round flask, *N*-phenylthiourea is mixed with tetrahydrofuran and TEA. a benzoyl chloride solution in tetrahydrofuran is added into the mixture, bit by bit through a dropping funnel over the ice bath, stirred with a magnetic tool. The mixture is refluxed and stirred in a water bath and in TLC every hour. The reaction is terminated whenever a single stain in TLC is formed. After the termination, THF is evaporated on the rotary evaporator proceeded with recrystallization.^[5]

The identification of the compound structure of the synthesis results is conducted based on the results of the examination: Infrared, ¹H-NMR, ¹³C-NMR and MS.

Cytotoxic Test On T47D Breast Cancer Cells and Normal Vero Cells

Anticancer activity is performed in vitro using T47D the breast cancer cells. 5 mg of test compound in 100 µL DMSO is dissolved to obtain 50.000 µg / mL concentration. The mother liquor is diluted gradually with RPMI culture medium for T47D cells to obtain a series of standard working solution. T47D cancer cell culture is prepared in microplate with 96 wells in a form of a cell suspension used in the experiment with a density of 10,000 cells / pitting. Empty wells are set on the plate for media control and afterwards CO₂ 5% incubator is added for 24 hours.

After incubated, the plate is removed and the media is discarded by 180 degrees reversion . After washed with PBS 100 µL for each well, then PBS was discarded by reversing the plate 180 degrees. A total of 100 µL of standard working solution, positive control and solvent control are included in microplate pours. Each concentration is replicated three times. Sums that do not contain T47D cancer cells are filled with media as media controls. Then incubated at incubator 5% CO₂ for 24 hours, at 37°C. After 24 hours the plate is removed from the incubator and the media is discarded. Within each well, 0.5 mg / ml of MTT is added to 100

μl / well, then incubated for 3 hours. After 3 hours of incubation, microplate is removed and the reaction is terminated by adding 100 μl SDS 10% in 0.1 N HCl to the hole. Microplate is wrapped with aluminum paper and incubated for 24 hours at 5% CO_2 incubator, then Elisa Reader is inserted at $\lambda = 595 \text{ nm}$. With probit analysis we get IC_{50} values from test compound.^[11]

RESULTS AND DISCUSSION

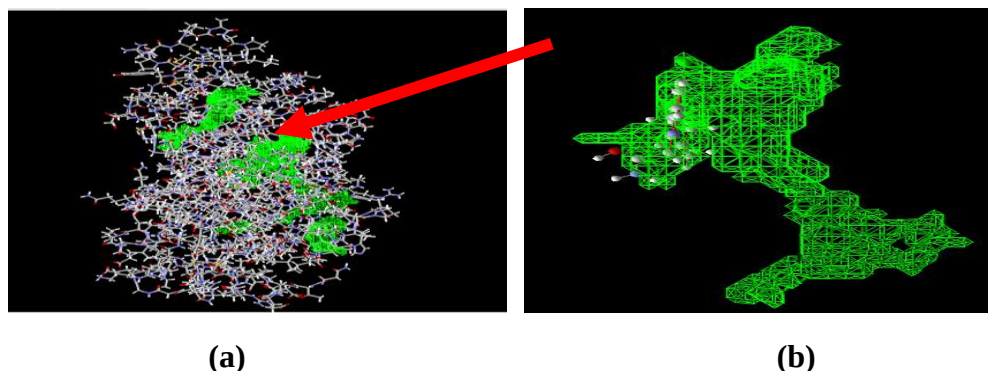


Fig 1. Preparation of protein (a) Histone deacetylase SIRT1 inhibitor code pdb: 4I5I (b) selected hole (corresponding arrow) on the 4I5I receptor where interacting ligand and receptor (cavity 1). Images are obtained from Molegro version 5.5.

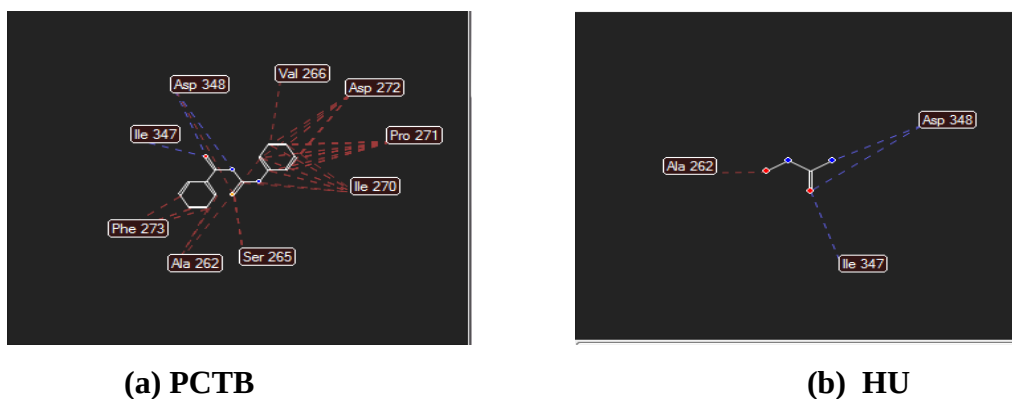


Fig. 2: 2D pictures show hydrogen bonds (blue dashed-line) and steric interaction (red dashed-line) of *N*-(phenylcarbamothioyl)-benzamide compound (PCTB) (a), reference drug HU (b) with amino acid in SIRT1 binding site. Images are obtained from Molegro version 5.5.

Table 1. The results of the docking value as a Rerank Score (RS).

No	Compounds	RS (PDB 4I5I)
1	PCTB	-119.4130
2	HU	-40.0237

Table 2: Hydrogen and steric bonds performed by interaction between tested compounds and amino acids in binding site of SIRT1.

N O	COMPOUNDS	AMINO ACIDS											
		ALA 262	ASN 346	ASP 272	ASP 348	ILE 270	ILE 347	PHE 273	PRO 271	SER 265	GLY 263	VAL 266	GLY 261
1.	PCTB	2S		4S	1H/1S	5S	1H	3S	5S	1S		1s	
2.	Hydroxyurea	1S			2H		1H						

Description: H: Hydrogen bond and S: Steric bond (Van der Waals and Hydrophobic)

The RS values describe drug activity in silico. Based on the results of in silico test (table 1) obtained the results of RS PCTB: --119.4130 and RS HU: -40.0237. The smaller the RS value, the more stable the resulting drug-receptor bonds are, the more predictable the activity will be (Hinchliffe, 2008).^[12] The number and types of amino acids in Table 2 and Figure 2. Based on the bonding of drugs and amino acids, the greater the number of hydrogen bonds and steric bonds (Van der Waals and Hydrophobic), it can be predicted that the bonds between the drug and the receptor will be more stable.

The new compound *N*-(phenylcarbamoithiyl)-benzamide is synthesized from benzoyl chloride with *N*-phenylthiourea in one stage. The synthesized result compound is yellow solid and in water insoluble substance. The structure of the synthesis compound is identified by IR, ¹H-NMR, ¹³CNMR, and HRMS spectroscopy as follows.

***N*-(phenylcarbamoithiyl)-benzamide.** Yield 71% as yellow crystal, m.p. 108⁰C. ¹H-NMR (DMSO-D6, 400 MHz) : δ 7.30 (t, J=7.2 Hz, 1H, Ar-H); δ 7.43 (dd, J=7.2;8.0 Hz, 2H,Ar-H); δ 7.54 (t, J=7.2 Hz, 2H, Ar-H);); δ 7.65 (dd, J=7.2;1.2 Hz, 1H, Ar-H);); δ 7.69 (d, J=8.0 Hz, 2H, Ar-H); δ 7.98 (dd, J=7.2;1.2 Hz, 2H, Ar-H); δ 11.56 (s, 1H,O=C-NH-C=S); δ 12.61 (s, 1H,S=C-NH-CH₂); NMR ¹³C (DMSO-D6, 400 MHz) ; δ 124.87 (2C, Ar); δ 126.87 (2C, Ar); δ 128.99 (2C, Ar); δ 128.99 (1C, Ar); δ 129.21 (2C, Ar); δ 132.69 (1C, Ar); δ 133.67 (1C, Ar); δ 138.53 (1C, Ar); δ 168.83 (1C, C=O); δ 179.65 (1C, C=S). IR (KBr),v maks (cm⁻¹) : 1672 (C=O amide) ; 1672&1451 (C=C Ar); 3219&1592 (NH strech sec.amides); 1085&811 (C=S). HRMS (m/z) C₁₄H₁₁N₂OS: (M-H)⁻: 255.0590, Calc. Mass : 255.0592 δ m/z = 0.0002 < 0.005.

Cytotoxic Activity**Table 3: Cytotoxic Effect of PCTB compound on T47D Cell Line.**

S.NO	CONCENTRATION ($\mu\text{g/mL}$)	ABSORBANCE	CELL VIABILITY (%)
1	12.5	0.619	87
2	25	0.609	86
3	50	0.565	78
4	100	0.437	57
5	200	0.336	40
6	400	0.247	25
7	CELL CONTROL	0.693	100

IC₅₀ : 0.53 mM**Table 4: Cytotoxic Effect of Hydroxyurea compound on T47D Cell Line.**

S.NO	CONCENTRATION ($\mu\text{g/mL}$)	ABSORBANCE	CELL VIABILITY (%)
1	31.25	0.621	90
2	62.5	0.589	84
3	125	0.554	78
4	250	0.424	56
5	500	0.330	40
6	1000	0.245	25
7	CELL CONTROL	0.680	100

IC₅₀ : 4.58 mM**Table 5: Cytotoxic Effect of PCTB compound on Vero Cell Line.**

S.NO	CONCENTRATION ($\mu\text{g/mL}$)	ABSORBANCE	CELL VIABILITY (%)
1	31.25	0.200	100
2	62.5	0.197	98
3	125	0.195	96
4	250	0.191	92
5	500	0.187	89
6	1000	0.183	86
7	CELL CONTROL	0.200	100

IC₅₀ : 49.40 mM**Table 6: RS, IC₅₀ T47D cell and IC₅₀ Vero cell values of test and reference compounds.**

Compound	RS	IC ₅₀ T47D Cell Line	IC ₅₀ Vero Cell Line
PCTB	-119.4130	0.53 mM	49.40 mM
HU	-40.0237	4.58 mM	-

Table 3 to table 5 are the data of the cytotoxic test result on T47D cancer cells and the normal cell Vero. The IC_{50} values of the test compound obtained from probit analysis are better than the Hydroxyurea reference compound. There is a corresponding result between the insilico and invitro tests of the test compound and the reference compound (Table 6). The better the value of the RS (stable bonding of the drug and the receptor) the better the anticancer activity is.

The cytotoxic effect of the T47D cells from the test compound could be seen on figure 3.

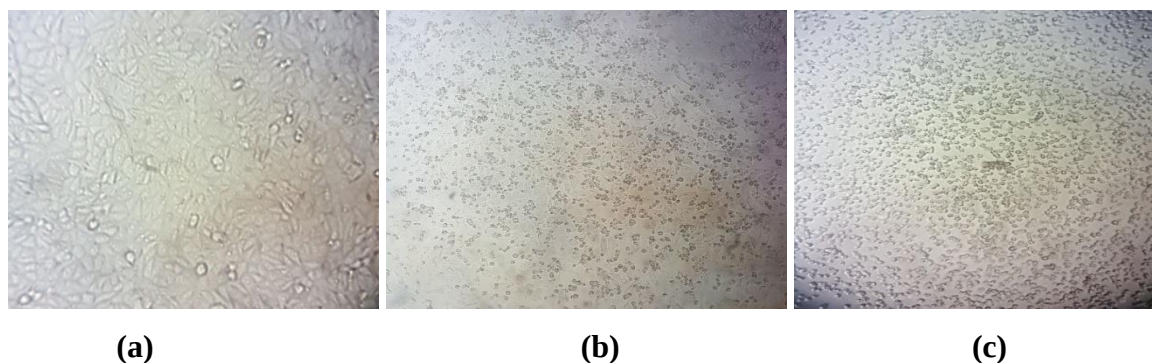


Figure 3:

- a. T47D cells before given a test compound
- b. T47D cells after given the test compound (dose 500 μg / mL)
- c. T47D cells after given a test compound (dose 1000 μg / mL)

CONCLUSION

This study concludes that a new compound of *N*-(phenylcarbamothioyl)-benzamide show in vitro anticancer activity against human breast cancer cells (T47D) higher than the anticancer drug hydroxyurea. In the in silico study it can be explained that better inhibitory activity is associated with higher affinity with SIRT1 binding sites. The value of IC_{50} *N*-(phenylcarbamothioyl)-benzamide is 0.53 mM, is more active than Hydroxyurea which is 4.58 mM. This new compound is more suitable for binding enzymes compared to Hydroxyurea, as it can illustrate better inhibitory activity. It is predicted that the activity of this new compound is targeted cell because it has toxic effects on cancer cells and are not toxic to normal Vero cells (Vero cell death <15%). Further researches are required to examine the molecular mechanism of this new compound.

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39	Dr. Mohammad Javed Ansari	javedpharma@gmail.com	Department of Pharmaceutics, College of Pharmacy Prince Sattam Bin Abdulaziz University Al-kharj, Saudi Arabia.	Saudi Arabia

40	Dr. Bhanwar Lal Jat	drblj1682@gmail.com	Assitant Professor, Department of Botany, Dayanand College, MDS University Ajmer, Rajasthan, India.	India
41	Dr. Rokeya Sultana	rokeya@ubhs.in	Head of the Department-Pharmacy at University of Bio-Chemic Health Sciences, Bangalore, India.	India
42	Dr. Abbas Yazdanpanah	abbas_yaz@yahoo.com	Assistant Professor, Department of Healthcare Management, Marvdasht Branch, Islamic Azad University, Marvdasht Iran.	Iran
43	Dr. Vijay Kumar	drvkdwivedi670@gmail.com	Associate Professor & Head, Department of Swasthavritta & North, Eastern Institute of Ayurvda & Homeopathy(NEIAH), Shillong, Meghalaya, India.	India
44	Asst. Prof. K. Murugan	micromurugan2013@gmail.com	Microbiologist, Quality Control Department, K. V. Marine Exports Private Limited, Padur Village, Kelambakkam, Chennai, INDIA.	India
45	Dr. Pradip Kumar Goswami	pkgoswamibhu@gmail.com	Director, North Eastern Institute of Ayurveda & Homoeopathy (NEIAH), Shillong, Meghalaya, India.	India
46	Dr. M. Prabhakaran	prabhasnc@gmail.com	Assistant Professor in P.G. & Research Department of Botany at Pachaiyappa's College, Chennai – 600 030, India.	India
47	Dr. Vijay N. Bhosale	vijaynbhosale77@gmail.com	Asst. Professor & Research Guide, P.G.Research Center, Dept. of Chemistry, Yeshwant Mahavidyalaya, Nanded(MS), India.	India
48	Dr. Ajay Kumar Pandey	pandeysavarna@gmail.com	Associate Professor PG Dept. of Shalakyatantra Gurukul Campus, UAU, Haridwar, U.K. (India).	India
49	Prof. Dr. N. Dora Babu	ndorababu81@gmail.com	Professor Mother Teresa Pharmacy College Sanketika Nagar, Sathupally, Khammam District, Telangana, India.	India
50	Dr. T. Satyanarayana	drtsatya@gmail.com	Prof. & Principal, Mother Teresa Pharmacy College, Kothuru, Sathupally, Khammam (Dist.), Telangana, India.	India
51	Dr. Sushant Sud	drsushantsud@gmail.com	Assistant Professor & Consultant Physician Shri Gulavbkunverba Ayurved Mahavidyalaya, Gujarat Ayurved University, Jamnagar (Gujarat), India.	India
52	Dr. Y. Thangam	thangambaskars@gmail.com	Assistant Professor in PG and Research Department of Zoology, J.K.K. Nataraja College of Arts and Science, Komarapalayam, Namakkal Dt, Tamilnadu, India.	India
53	Dr. A. Nusrath Unissa	nusrathunissa@gmail.com	National Institute for Research in Tuberculosis (NIRT), Chennai, India.	India
54	Dr. Kaveh Ostad-Ali-Askari	kaveh.oaa2000@gmail.com	Assistant Professor, Department of Civil Engineering, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.	Iran
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			Technology Vikrama Simhapuri University SPSR. Nellore-524003, Andhra Pradesh State, India.	
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61	Dr. Pushkar Mukund Kulkarni	PM.Kulkarni@northeastern.edu	Department of Pharmaceutical Sciences, Bouve College of Health Sciences, Northeastern University, Boston, MA – 02115, USA.	USA
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76	Dr. Satej T. Banne	satejbanne16@gmail.com	M.D.(Ayu.), E.M.S., Ph.D. Scholar, Department of Dravyaguna, Parul University, Parul Institute of Ayurveda, Gujarat, India.	India
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82	Dr. Moyad Jamal Said Shahwan	moyad76@hotmail.com	Associate Professor in Biochemistry College of Pharmacy and Health Sciences, Ajman University of Science and Technology, P.O.Box:346, Ajman - U.A.E.	U.A.E.
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84	Dr. K. Srinivasan	srinigene@gmail.com	Postdoctoral Fellow, Department of Consumer and Food Science, University of Pretoria, South Africa	South Africa
85	Dr. Benaka Prasad S. B.	benaka_prasad@yahoo.co.in	Head & Dean, Department of Basic Sciences School of Engineering & Technology Jain University, Jain Global Campus, Bangalore, India	India
86	Dr. Guruprasad K.	punithakb@gmail.com	Associate Professor, Department of Swasthavritta, Sri Jayendra Saraswathi Ayurveda College, Nazzarathpet Chennai – 600123, India.	India
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93	Dr. Mahesh Kumar Kataria	kataria.pharmacy@gmail.com	Professor and Head, Department of Pharmaceutics, Seth G.L. Bihani S.D. College of Technical Education, Sri Ganganagar, Rajasthan, India	India
94	Dr. Biswaranjan Paital	biswaranjanpaital@gmail.com	Assistant Professor, Department of Zoology, Orissa University of Agriculture and Technology, College of Basic Science and Humanities, Bhubaneswar-751003, Odisha, India	India
95	Dr. Pasupuleti Sreenivasa Rao	sraopasupuleti@yahoo.com	Research Scientist in Department of Advanced Research Centre Narayana Medical College and Hospitals Nellore, Andhra Pradesh, India	India
96	Prof. Dr. Osmar A. Centurión	osmarcenturion@hotmail.com	Professor of Medicine. Asuncion National University-UNA, Paraguay	Paraguay
97	Dr. A.V.L.N.S.H. Hariharan	ahharan@gmail.com	Professor & BOS – Chairman, Dept. of Chemistry, GITAM Institute of Technology, GITAM University, Visakhapatnam – 530 045, India	India
98	Prof. Dr. Bharat Mishra	bharatekansch@gmail.com	Professor & Head, Department of Pharmacology, Research & Development Cell, Nirmala College of Pharmacy, Muvattupuzha, Ernakulam Dist., Kerala, India	India
99	Dr. Reddy Sunil	drsunilsvsintuh@gmail.com	Professor (HOD), Department of Pharmaceutics. SVS Group of Institutions, School of Pharmacy (TK), Bheemaram, Hanamkonda, Warangal, Telangana, India	India
100	Dr. N. G. Raghavendra Rao	nraghu@rediffmail.com	Director and Professor, Dept. of Pharmacy, GRD [PG] Institute of Management and Technology, Rajpur Road, Dehradun - 248009, Uttarakhand, India	India
101	Dr. Saurabh Khasnavis	skhasnavis@bwh.harvard.edu	Department of Neurology, Brigham and Women's Hospital & Harvard Medical School 60 Fenwood Rd, Boston, MA-02115, USA	USA
102	Dr. Sonal Jain	sonal.jn1@gmail.com	Manager- Medical Services, Young Concepts, Andheri East-Mumbai- 400093, India	India

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S.No.	Name	Email	Address	Country
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1	Dr Kumaraswamy Gandla	kumaraswamy.gandla@gmail.com	Associate Professor & H.O.D in Dept. of Pharmaceutical Analysis in Care College of Pharmacy- Warangal, Telangana, India.	India
2	Associate Professor, Ravindra Reddy Yaramala	ravindrareddyaramala@gmail.com	Teegala Ram Reddy College of Pharmacy, Meerpet, Saroomagar, Hyderabad, India.	India
3	Dr. Ajit Pandya	ajit1sujit@gmail.com	Department of Biochemistry, C U SHAH SCIENCE COLLEGE, Ashram road, Ahmedabad - 380014	India
4	Dr. Atindra Shukla	atindra.sscsn@ddu.ac.in	Shah-Schulman Center for Surface Science & Nanotechnology Dharmsinh Desai University, Nadiad. India.	India
5	Dr. Tauqeer Hussain	tauqeer.hussain.mallhi@hotmail.com	School of Pharmaceutical Sciences, USM, Penang, Malaysia.	Malaysia
6	Dr. Suryakanta Swain	swain_suryakant@yahoo.co.in	Assistant Professor Department of Pharmaceutics, Roland Institute of Pharmaceutical Sciences, P.O: Khodasingi, Berhampur (Ganjam), Pin-760 010, Odisha (INDIA),.	India
7	Dr. Jiyauddin Khan	jiyauddink@gmail.com	Department of Pharmaceutics/ Department of Research, School of Pharmacy, Management & Science University, Shah Alam, Selangor Darul Ehsan, Malaysia.	Malaysia
8	Dr. Chirag M. Modi	chiragvets@yahoo.co.in	Asistant Professor, Department Of Pharmacology, Veterinary College, Sdau, Sardarkrushinagar. India.	India
9	Dr. Sankalp Yadav	drsankalpyadav@gmail.com	General Duty Medical Officer-II at the Chest Clinic Moti Nagar, North Delhi Municipal Corporation, New Delhi, India.	India
10	Dr. Ravi Shankar Khatri	drskhatri@gmail.com	Service senior resident, Faculty of Ayurveda, IMS, BHU India.	India
11	Dr Bijay Kumar Gupta	aapkavj@gmail.com	Reader & Head, Dept. of Agadatantra, SBLD Ayurved Vishwabharati, Gandhi Vidya Mandir, Sardarshahar, Churu, Rajasthan.	India
12	Dr. Manoj Kumar	manojcipl@gmail.com	Scientific Officer Indian Pharmacopoeia Commission Ministry of Health & Family Welfare Government of India CIPL Campus Sector-23, Rajnagar Ghaziabad-201002 (UP), India.	India
13	Dr. Naresh Kumar Kumawat	dmareshbps3584@gmail.com	S.B.L.D. Ayurved Vishwabharti, Gandhi Vidya Mandir, Sardarshahr, Churu, Rajasthan. India.	India
14	Dr. Alaa H. Abuznait	abuznait@yahoo.com	Department of Pharmaceutical Sciences University of Oklahoma Health Sciences Center 1110 N. Stonewall Ave., Oklahoma City, OK.	Jordan
15	Dr. Muneesh Kumar	manishloves200787@gmail.com	Department of Zoology, University of Jammu, Jammu (J&K) INDIA 180006, India.	India

16	Dr. Ajit Singh	ajitsmarty01@gmail.com	Pathologist in charge of routine Hematobiochemical Profiling, Postmortems (domestic, laboratory, wild animals and poultry birds) and Histopathological laboratory at Department of Pathology, College of Veterinary Science and Animal Husbandry, SHIATS, Allahabad	India
17	Dr. Neeraj Kumar Patel	neerajkumar.p@himalayawellness.com	Laxmi Niwas, Behind Punjab Bank, Patel Nagar, Suhagi, Jabalpur, Madhya Pradesh 482002, India.	India
18	Dr. Ananda Kumar Konreddy	mrjayapal007@gmail.com	Post Doctoral Research Associate, College of Pharmacy, University of Georgia, Athens, 30602 Georgia, USA.	USA
19	Dr. Grandhe Usha Rani	ushaindian@yahoo.com	2091, South Milledge Ave, Apt C60, Athens, Georgia-30605, USA.	USA
20	Dr. Muneesh Kumar	manishloves200787@gmail.com	Department of Zoology, University of Jammu, Jammu (J&K) INDIA 180006, India.	India
21	Dr. Fasalul Rahiman OM	fasaluom@gmail.com	Tutor, Department of Pharmacology, MES Medical College, Perinthalmanna, Malappuram Dt, Kerala- 679338, India.	India
22	Dr. Kiran Mishra	dmishrakiran@gmail.com	Applied Sciences Department, Chandigarh Engineering College, Landran, Mohali (Pb.) India	India
23	Ramesh Chand	rchand@lrri.org	Senior Research Associate, Scientific Core Laboratories, Lovelace Respiratory Research Institute (LRRI), Albuquerque, NM USA	USA
24	Dr. T. Sivapriya	sivaamanick@gmail.com	Assistant Professor and Head, Department of Nutrition, Food Service Management and Dietetics, Prof. Dhanapalan College of Arts and Science, Chennai, India	India
25	Dr. Jessen George	georgejessen@gmail.com	Associate Prof. & Principal, Eben-Ezer Degree College, Bangalore-560077, Karnataka, India	India
26	Dr. Vikas Kumar	vkskumar49@gmail.com	Dept. of Silviculture & Agroforestry, Periyar Hostel, College of Forestry, Vellanikkara, Kerala Agricultural University, Thrissur, Kerala- 680656, India.	India
27	Dr. Pralhad Vinay Rege	pralhad1806@gmail.com	Assistant Professor Dept of Chemistry St. Xaviers College, Mumbai, India.	India
28	Dr. Ali Olfati	a.olfati66@gmail.com	Department of Animal Science, University of Tabriz, Iran.	Iran
29	Dr. Diptendu Sarkar	diptendu81@gmail.com	Assistant Professor, Dept. of Biotechnology Engineering, NHCE, Bangalore, India.	India
30	Dr. G. Swathi	swathi.gun@gmail.com	Govt. Degree and PG College for Women, Srikalahasthi, India.	India
31	Dr. Tajamul Islam	taju.zoology@gmail.com	Contractual Lecturer in Deptt. of Zoology at Govt Degree College	India

				for Women Baramulla, India.	
32	Dr. Mohanraj Rathinavelu	moley4u@rediffmail.com	Assistant Professor, Dept. of Pharmacy Practice, Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Andhra Pradesh, India.	India	
33	Dr. Anuj Singh Parihar	dr.anujparihar@gmail.com	Assistant Professor, Vyas Dental Collage and Hospital, Jodhpur, Rajsthan, India.	India	
34	Dr. Islam Mostafa Abdo Bayomi Elbaz	islam_mostafa_elbaz@yahoo.com	Lecturer of Pharmacognosy Faculty of Pharmacy, Zagazig University, 44519- Zagazig, Egypt.	Egypt	
35	Dr. Gopinath Shankar Khansole	gopinathskhansole@gmail.com	Assistant Professor, Department of Chemistry, D.A.B.N. Arts and Science College, Chikhali, Sangli, Maharashtra-415408, India.	India	
36	Dr. Namrata Singh	namrata.singhms@gmail.com	Asst. Professor in P. Cognosy Dept. Shreeyash Institute of Pharmaceutical Education and Research, Aurangabad (MS) India.	India	
37	Dr. Anoop Narayanan V.	anoopvn84@gmail.com	Assistant Professor, NGSM Institute of Pharmaceutical Sciences, NITTE University, Mangalore- 575018, India.	India	
38	Dr. Maheshkumar Prakash Patil	maheshprakashpatil1984@gmail.com	Lab of Cell Signaling, Department of Microbiology, Pukyong National University, Busan, Korea.	Korea	
39	Dr. Abhinandan Ghosh	abhinandan.vu@gmail.com	Dept. of BMLSM Vidyasagar University, Midnapore- 721102 West Bengal, India.	India	
40	Dr. Jagdish Labhubhai Kakadiya	jagdishkakadiya@gmail.com	Assistant Professor, Department of Pharmacology, Indubhai Patel College of Pharmacy and Research Centre, Petlad-Khambhat Road, Dharmaj- 388430, Anand, Gujarat, India.	India	
41	Dr. Kunal Kishor	kunal_biotech@yahoo.co.in	Head Department of Microbiology SGRITS Patel Nagar Dehradun, India.	India	
42	Dr. Kishor R. Chudasama	krchudasama84@gmail.com	Assistant Professor Department of Shalaky IPGT and RA Jamnagar- 361008, Gujarat, India.	India	
43	Dr. Myle Akshay Kiran	myleakshaykiran@gmail.com	Doctor of Pharmacy (pharm. D), JNTUH UNIVERSITY. Pratista Institute of Pharmaceutical Science, Durjipally, Chevimala Mandalam, Suryapeta, Telangana State, India.	India	
44	Dr. Prakash S. Goudanavar	pgoudanavar01@gmail.com	Professor and Head Department of Pharmaceutics Sri Adichunchanagiri College of Pharmacy B.G.Nagar-571448 Nagamangala Taluk Mandya Dist. Karnataka, India.	India	
45	Dr. Somil Dubey	somildubey11@gmail.com	Associate Professor Rasa Shastra and Bhaishajya Kalpana to Govt. College Burhanpur, M.P., India.	India	
46	Dr. Naga	rathna.supriya@utu.ac.in	Assistant Professor C.G. Bhakta	India	

	Rathna Supriya G.		Institute of Biotechnology Uka Tarsadia University Maliba Campus, Gopal Vidyanagar, Bardoli-Mahuva Road, Ta. Mahuva, Dist. Surat-394350, Gujarat, India.	
47	Dr. Dhivya Ravichandran	dhivya6@hotmail.com	Ph.D Biotechnology, Vels University India.	India
48	Dr. Sachin Kumar	sachin.amroha@gmail.com	DST-SERB National Post Doctoral Fellow in the Dept. Animal Science, M.J.P.Rohilkhand University, Bareilly, U.P., India.	India
49	Dr. Pravin Kendrekar	kkpravin@gmail.com	Department of Health Sciences, Faculty of Health and Environmental Sciences Central University of Technology (CUT) Bloemfontein 9300, Free State, South Africa.	South Africa
50	Dr. S. Sathish	sathishamg88@gmail.com	Assistant Professor and Head in Department of Automobile Engineering, Vels University, Chennai, India.	India
51	Dr. Sanjay Kumar Bharti	drskbharti24@gmail.com	Assistant Professor-cum- Junior Scientist, Department of Veterinary Anatomy. Bihar Veterinary College, Patna-800014, India.	India
52	Dr. Anurag Kulshreshtha	anuragfpt@iitr.ac.in	Faculty-Packaging Technology Ph.D., D.I.H., FICC Indian Institute of Technology, Roorkee Saharanpur Campus Saharanpur, India.	India
53	Dr. Nilesh Wagh	waghnk@gmail.com	Principal Scientist R&D at Radiomedix Inc, USA.	USA
54	Dr. Pradeep Kumar Soni	pradeepsonij@gmail.com	Faculty of Chemistry, Institute of Engineering, Jiwaji University, Gwalior (M.P.), India.	India
55	Dr. Siddharth	dr.siddharthtevatia@gmail.com	Master of Dental Surgery (M.D.S) Periodontology & Oral Implantology ITS-CDSR, Muradnagar, Ghaziabad, Uttar Pradesh, Ch. Charan Singh University, Meerut, India.	India
56	Dr. Tina Singhal	drtinasinghal@gmail.com	Assistant Professor, Deptt. of Rachana Sharir Govt. Ayurvedic College & Hospital, Varanasi, India.	India
57	Dr. Mridul Malakar	mridulmalakar1@gmail.com	District Microbiologist, IDSP I/C District Public Health Laboratory District Hospital, Sonapur, Kamrup (M), Assam, India	India
58	Dr. Dheeraj Nagore	nagoredheeraj@gmail.com	Senior Manager- Research and Development Center, Ari Healthcare Pvt. Ltd., Pune, Maharashtra, India	India
59	Dr. Tushita Thakur	dr_tushita@yahoo.co.in	Consultant (Homoeopathy), AYUSH Wellness Clinic, Rashtrapati Bhavan, New Delhi-110004, India.	India
60	Vd. Rohit Mehta	rohitayu04@yahoo.com	Senior Medical Officer - Panchakarma; Vivekanand Medical Research Trust; Kayakalp (Himalayan Research Institute for Naturopathy &	India

			Yoga), Palampur, Kangra, Himachal Pradesh, India.	
61	Dr. Sunita Verma	vermas.bot@gmail.com	Assistant Professor, Department of Botany, Rakesh P.G. College, Pilani (Raj.) India	India
62	Dr. Sujith Abraham	sujithabraham1@gmail.com	Professor & Principal, St John's College of Pharmaceutical Sciences & Research, Kattappana South (PO), Idukki Dist., Kerala- 685515, India	India
63	Dr. R. Mary Suja	rmsuja.83@gmail.com	Director William Research Centre at Nagercoil, India	India
64	Dr. Silpa Somavarapu	silpasomavarapu.1981@gmail.com	Faculty in Vikrama Simhapuri University, Andhra Pradesh, India	India
65	Dr. Teresa May B. Bandiola	bandiolateresamayb@gmail.com	Instructor A, School of Pharmacy, Far Eastern University- Dr. Nicanor Reyes Memorial Foundation, Quezon City, Philippines	Philippines
66	Dr. Janki B. Patel	janki0410pharma@gmail.com	Associate Professor, B.Pharmacy College Rampura, Kakanpur Godhra, Gujarat, India	India
67	Dr. Prasanna B. Ranade	prasannaranade@yahoo.com	Assistant Professor, Department of Chemistry, Vivekanand Education Society's Arts, Science and Commerce College, Sindhi Society, Chembur Mumbai 400071, India.	India
68	Dr. Md. Elias- Al-Mamun	elias_0276@yahoo.com	Associate Professor, Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.	Bangladesh
69	Dr. Harika Vemula	vharikashetty@gmail.com	Senior Scientist, Upstream R&D Millipore Sigma, Kansas, USA	USA
70	Dr. J. Sai Chandra	jsaichandra@gmail.com	Academic Assistant in Chemistry, Jawaharlal Nehru Technological University, Hyderabad- Sultanpur, Sangareddy Dist. – 502273, India.	India
71	Dr. Farah Naaz	farah.ali07@gmail.com	Research Associate Under Swasthya Rakshan/ Parikshan Programme of Ministry of AYUSH, Government of India	India
72	Dr. Rajendra H. M.	ayurrajhm@gmail.com	Assistant Professor (Reader) in Dravyaguna Vigyan in Sri Jayendrasaraswathi Ayurveda College & Hospital, Chennai, India	India
73	Dr. Javeed Ahmad Tantray	javeedh3@gmail.com	Assistant Professor in Department of Zoology, Centre for Biodiversity and Biotechnology, Baba Ghulam Shah Badshah University, Rajouri J. & K., India	India
74	Dr. M. Selvendran	selvendran008@gmail.com	Assistant Professor, Dept. of Microbiology, Sardar Raja College of Arts and Science, Vadakkankulam, Thirunelveli Dist. Tanil Nadu, India	India
75	Dr. S. Krishnamoorthy	drkrishna83@gmail.com	Assistant Professor, Department of Biotechnology, The Madura College, Madurai-625011, India	India

76	Dr. Kinage Pradip Ravindra	drkinagepradeep@gmail.com	Asst. Professor, Parul institute of Ayurveda and Research, Parul University Gujarat, India	India
77	Dr. Chandra Mohan	gurgaonmohan@yahoo.co.in	Assistant Professor in Chemistry, SBAS, K. R. Mangalam University, Sohna Road, Gurgaon-122103, Haryana, India	India

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