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National Institute of Ayurvedic Pharmaceutical Research  
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Department of Biochemistry, University of Allahabad, Faculty of Science, Allahabad-211002, India.

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Central Laboratory of General Ecology, Bulg Acad Sci 1113 Sofia, 2 Gagarin str, Bulgaria.
Shijie Han  
Center of Forestry, Institute of Applied Ecology,  
Chinese Academy of Sciences,  
72 Wenhua Road, Shenyang City, Liaoning Province 110016, PR China.

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Department of Medical Nutrition,  
I-Shou University  
Yanchao Township,  
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Department of Agronomy, College of Agriculture  
Kerala Agricultural University, Vellayani 695 522, Trivandrum, Kerala.

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National Institute of Genetic Engineering and Biotechnology (NIGEB)  
Shahrak-e-Pajoohesh Km 15, Tehran-Karaj Highway, Tehran, I.R.Iran.

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Nemanjina 6, Zemun, 11080, Serbia.

Rumbos Christos  
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Department of Botany, University of Rajshahi, Bangladesh.

Paul S. Marley  
Department of Crop Protection, IAR/FOA  
Ahmadu Bello University, P.M.B. 1044, Samaru, Zaria, Nigeria.

Patrick Addo-Fordjour  
Kwame Nkrumah University Of Science And Technology (Knust), Kumasi,  
Department Of Theoretical And Applied Biology, Ghana.

Battu Prasanna Reddy  
Nosch Labs Pvt Ltd  
Hyderabad, India.

Noureddine Benkeblia  
UWI - Department of Life Sciences  
Mona Campus, Kingston 7, Jamaica.

Keutgen, Norbert  
Uniwersytet Technologiczno-Przyrodniczy  
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Kadra Katedry Fizjologii Roslin (Institute of Plant Physiology)  

Nicholas E. Korres  
University College Cork, Environmental Research Institute.  
Lee Road, Cork, Ireland.

Dr Naveen Kumar  
University of Florida  
2685 SR 29 N SWFREC/IFAS/UFL, Immokalee, FL34142, USA.
Dr. Modala Venkateswarlu  
*Seribiotech research Laboratory, Kodathi, Carmelaram post, Bangalore.*

Mirza Hasanuzzaman  
*Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.*

Maybelle Gaballah  
*National Research Centre, El Behoos street, Dokki, Cairo.*

Mauro Guida Santos  
*Universidade Federal de Pernambuco, Street Moraes Rego – CDU – CCB – Botany Department, s/n. 50670-901. Pernambuco State, Brazil.*

Marcelo Rodrigo Pace  
*University of Sao Paulo, Rua do Matão, 277, Cidade Universitária São Paulo, Brazil.*

Marcelo Francisco Pompelli  
*Federal University of Pernambuco, Department of Botany, Profº Moraes Rego Av., Recife – PE – Brazil, 50670-901.*

Luca Catalina Mariana  
*University of Bucharest, Faculty of Biology, Dept of Biochemistry and Molecular Biology, Spl. Independentei, no.91-95, Bucharest 5, Romania.*

Lin Wang  
*Institute of Biostatistics, Fudan University, 220 Handan Road, Shanghai 200433, China genetics, microbiology China.*

Li Qiang  
*Institute of karst geology, MLR 50 Qixing Road, China.*

Dr. Ayanakumar Kumar  
*C. Abdul Hakeem College of Engg. & Tech., Melvisharam-632 509, Vellore Dist, Tamil Nadu, INDIA.*

P. Krishnamoorthy  
*P.G. AND RESEARCH DEPARTMENT OF ZOOLOGY RAJAH SERFOJI GOVT. COLLEGE. India.*

Hare Krishna  
*Central Institute of Temperate Horticulture-Regional Station, Mukteshwar-263 138, District- Nainital, Uttarakhand, India.*

K.G. Mandal  
*Directorate of Water Management (formerly Water Technology Centre for Eastern Region) Indian Council of Agricultural Research C.S. Pur, Bhubaneswar-751023, ORISSA, INDIA.*

Dr. Jukta Adhikari  
*Presidency College 86/1, College Street, Kolkata – 700 073, India.*

Jorge Teixeira  
*Botany Department, Faculty of Sciences,, University of Porto, Edificio FC4, Rua do Campo Alegre, S/N, 4169-007 Porto, Portugal.*

Johnson Toyin Fasinmirin  
*Federal University of Technology, Akure, Nigeria Department of Agricultural Engineering, FUT, P.M.B. 704, Akure, Ondo State, Nigeria.*

Joel K. Ransom  
*North Dakota State University 166 Loftsgard Hall, Department of Plant Sciences, NDSU Dept. 7670, PO Box 6050, Fargo, ND 58108-6050.*

João Claudio Damasceno de Sá  

Jalal Jalali Sendi  
*University of Guilan Department of Plant Protection, university of Guilan, Rasht, Iran.*
Iúri Drumond Louro  
Universidade Federal do Espírito Santo  
Rua Horácio Andrade de Carvalho, 210, Victoria, ES, 29052-620, Brazil.

Hong Bo Guo  
Northwest A and F University  
22 Xinong, Yangling 712100, Shaanxi, PR China.

Harsukh P. Gajera  
Junagadh Agricultural University  
Department of Biochemistry, College of Agriculture, JAU, Junagadh- 362 001, Gujarat, India.

Hanan Abdel fattah El-Sadawy  
National Research Center  
El-Buhoth St., Dokki, Giza, Egypt.

Assist. Prof. Azime KÜÇÜKGÜL GÜLEÇ  
Tunceli University Fisheries Faculty 62000, Tunceli/TURKEY.

Greg T. Hannig  
DuPont  
1090 Elkton Road Newark, DE 19711.

Gilberto Santos Andrade  
Instituto de Biotecnologia Aplicada a Agropecuária (BIOAGRO), Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, MG 36571-000, Brazil.

Dr. T. Muthukumar  
Department of Botany, Bharathiar University  
Coimbatore -641 046, Tamilnadu, India.

Kunjupillai Vijayan  
Institute of Plant and Microbial Biology  
Academia Sinica, Taipei, Taiwan-115, ROC. Taiwan.

Badre Alam  
National Research Centre For Agroforestry  
Gwalior Road, Jhansi-284003, U.P., India.

Abeer Essam El-Din Mahmoud  
Biochemistry Department  
Genetic Engineering & Biotechnology Division  
National Research Center El Tahrir St., El Dokki 12622, Cairo, Egypt.

Qazi Fariduddin  
Aligarh Muslim University  
Department of Botany, Aligarh 202 002, India.

Darmawan Darma  
Faculty of Agriculture, Andalas University  
Kampus Limau Manis Padang-25163, Indonesia.

Barbara Chaves  
Institute for Agricultural and Fisheries Research.

Sudhamoy Mandal  
Central Horticultural Experiment Station (ICAR)  
Aiginia, Bhubaneswar, PIN-751019.

Cavit Bircan  
Adnan Menderes University  
Faculty of Agriculture  
Department of Food Engineering  
09100/Aydin/Turkey.

Carlos Alberto Ortega-Ojeda  
Central University of Ecuador. Faculty of Agriculture Sciences. Quito, Ecuador  
Calle 12 # 29 B - 78, Apto. 102 F, Unidad Residencial Colseguros, Cali, Colombia.

Bita Naseri  
Agricultural Research Institute  
Department of Plant Protection, Agricultural Research Institute, PO Box 45195474, Zanjan, Iran.

Behzad Kaviani  
Adeyemi Oluyomi Stephen  
Bells University of Technology  
Chemical Sciences Department, Km 8 Ididroko Road, Ota, Ogun State, Nigeria.

Ajayi Adedayo Olajide  
Adekunle Ajasin University  
Dept. of Microbiology, P.M.B 01, Akungba-Akoko, Ondo State, Nigeria.
<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexandre Igor Azevedo Pereira</td>
<td>Universidade Federal de Viçosa, Departamento de Biologia Animal, Programa de Pós-Graduação em Entomologia. 36570-000, Viçosa, Minas Gerais State, Brazil.</td>
</tr>
<tr>
<td>Gilberto Santos Andrade</td>
<td>Instituto de Biotecnologia Aplicada a Agropecuária (BIOAGRO), Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, MG 36571-000, Brazil.</td>
</tr>
<tr>
<td>Pradeep. A.R., Ph.D</td>
<td>Seribiotech Research Laboratory Carmelaram.P.O; Bangalore, INDIA.</td>
</tr>
<tr>
<td>Azamal Husen</td>
<td>University of Gondar Department of Biology, Faculty of Natural Sciences, University of Gondar P.O. Box #196, Gondar, Ethiopia.</td>
</tr>
<tr>
<td>Muhammad Aslam</td>
<td>University College of Agriculture, Bahauddin Zakaria University Multan 60800, Pakistan.</td>
</tr>
<tr>
<td>Autumn J. Smith</td>
<td>Sam Houston State University, Texas.</td>
</tr>
<tr>
<td>La Sara</td>
<td>Haluolea University Kampus Baru Tridharma, Kendari, Southeast Sulawesi, Indonesia.</td>
</tr>
<tr>
<td>Aliyu Mohammed</td>
<td>Department of Human Physiology, ABU, Zaria. Nigeria.</td>
</tr>
<tr>
<td>Shnoudy Anwar Bakhoum</td>
<td>National Institute of Oceanography &amp; Fisheries (NIOF), Egypt.</td>
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<tr>
<td>Antonio Americo Barbosa Viana</td>
<td>Embrapa Recursos Geneticos e Biotecnologia PBI-LPP1 PqEB Final W/5 Norte, Brasilia, DF – Brazil</td>
</tr>
<tr>
<td>Dr. Shirish Rajmalwar</td>
<td>National Research Laboratory for Conservation, Shirish Rajmalwar, LIG Plot No. 43, Mhada colony, Wardha – 442001, (MS) India.</td>
</tr>
<tr>
<td>Dr. Amaresh Chandra</td>
<td>Universidade Federal de Viçosa, Departamento de Biologia Animal, Programa de Pós-Graduação em Entomologia. 36570-000, Viçosa, Minas Gerais State, Brazil.</td>
</tr>
<tr>
<td>Dr. Atul Kumar</td>
<td>GB PANT University of Agriculture &amp; Technology Department of Basic Science, College of Forestry &amp; Hill Agriculture, HILL CAMPUS, PO Ranichauri, Tehri Garhwal, Uttarakhand State, India.</td>
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<td>Prof. Levenko Boris</td>
<td>Natl. Botanical Gardens,NAS of Ukraine 01014 Kiev, 1 Timiryasevska st. Ukraine.</td>
</tr>
<tr>
<td>Dr. Dionisio G. Alvindia</td>
<td>Bureau of Postharvest Research and Extension CLSU Compound, Science City of Munoz, Nueva Ecija 3120, Philippines.</td>
</tr>
<tr>
<td>Dr. Bhoopander Giri</td>
<td>University of Delhi Department of Botany, SSNC (University of Delhi) Alipur, Delhi 110036, India.</td>
</tr>
<tr>
<td>Dr. Anjuli Sood</td>
<td>University of Delhi Department of Botany, University of Delhi, Delhi-110 007, INDIA.</td>
</tr>
<tr>
<td>Dr. A. K. Verma</td>
<td>G.B. Pant University of Agriculture &amp; Technology, Pantnagar, Department of Biochemistry, College of Basic Sciences, India.</td>
</tr>
<tr>
<td>Dr. Anjana Jajoo</td>
<td>School of Life Science, Devi Ahilya University, Indore, DAVV Khandwa Road campus, Indore 452 017, M.P., India.</td>
</tr>
<tr>
<td>Dr. Deepak Ganjewala</td>
<td>Vellore Institute of Technology University 55 Thennaraam Street, Vellore-632 014 (T.N.), India.</td>
</tr>
</tbody>
</table>
Dr. Geetha Govind  
Max-Planck-Institute for Chemical Ecology  
Hans-Knöll Straße 8, 07745 Jena, Germany.

Dr. Hossam El-Din Saad El-Beltagi  
Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt, P.O.Box 12613 Egypt.

Prof. Dr. Md. Shahidul Haque  
Dept. of Biochemistry and Molecular Biology  
University of Rajshahi, Rajshahi-6205, Bangladesh.

DR. P.K.NAGAR  
Retired Senior Scientist, IHBT, Palampur, (H.P.), B.21/115-10A Batuk Dham Colony, Kamachha, Varanasi 221 010, INDIA.

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Indian Institute of Technology  
Centre for Rural Development & Technology, IIT Delhi-110016  
Biomass Production on waste land, India.

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Faculty of Sciences Dhar El Mahraz, Fez, Morocco  
BP 1796, Fès-Atlas, Fès, Maroc, Morocco.

Ass. Prof. Jianfeng Xu  
Arkansas State University  
PO Box 639, State University, AR 72467 USA.

Ass.Prof. Jin Xu  
Center for Agricultural Resources Research,  
Institute of Genetics and Developmental Biology, Chinese Academy of Sciences  
Huaizhong RD 286, Shijiazhuang, HeBei, China.

José Carlos Rebuglio Vellosa Ph.D  
PARANÁ STATE UNIVERSITY OF PONTA GROSSA  
(Universidade Estadual de Ponta Grossa – UEPG)  
General Carlos Cavalcanti Avenue, 4748,  
Uvaranas, Ponta Grossa/PR – PO box 84030-900

Dr. Krouma Abdelmajid  
Centre of Biotechnology, Borj Cedria Ecopark  
BP 901, Hammam-Lif 2050, Tunisia  
College of Science and Arts, Qassim University, BP 53,  
Al-Rass 3330353, Qassim, Saudi Arabia  
Saudi Arabia

Dr. Majid Rostami  
Malayer University  
Department of Agriculture and Natural Resources,  
Postal code: 65719-95863, University of Malayer  
Malayer, Iran.

Dr. Mohammad Nasir Khan  
Aligarh Muslim University, Aligarh, INDIA  
Plant Physiology Section, Department of Botany,  
Aligarh Muslim University, Aligarh-202 002, U.P., India.

Prof. N.K.Matta  
Kurukshetra University  
Department of Botany, Kurukshetra University,  
Kurukshetra 136119, INDIA.

Dr. Naceur Djebali  
Centre of Biotechnology Borj-Cedria (CBBC)  
BP 901, Hammam-Lif 2050 Tunisia.

Dr. Nader Chaparzadeh  
Azarbaijan University of Tarbiat Moallem, Tabriz, Iran.

Nautiyal Prakash Chandra  
Directorate Of Groundnut Research (Icar)  
Post box, No. 5, Ivnagar Road, Junagadh-362001,  
Gujarat, India.

Prof. Hussein Fawzy Hussein Abouziena  
National Research Center  
Botany Department, National Research Center,  
Elbhoss Street, Dokki, Cairo, Egypt.

Dr. D.E. Chandrashekar Rao  
National Research Council Canada / Plant  
Biotechnology Institute (NRC-PBI)  
110 Gymnasium Place / Saskatoon, Saskatchewan  
S7N 0W9 Canada.
Dr. S.R Madhan Shankar  
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Prof. Dr. Safdar Hussain Shah  
*Institute of Biotechnology and Genetic Engineering*  
NWFP, Agricultural University Peshawar, Pakistan.

Prof. Dr. Md. Shahidul Haque  
*Dept. of Biochemistry and Molecular Biology*  
University of Rajshahi, Rajshahi-6205, Bangladesh.

Dr. Sivakumar Swaminathan  
*Iowa State University (ISU)*  
G-319, Agronomy Department, ISU, Ames, Iowa - 50011, USA.

Dr. Subrahmanyam Desiraju  
*Directorate of Rice Research (ICAR)*  
Plant Physiology Division, Rajendranagar, Hyderabad-500030, A.P. India.

Dr. Tariq Aziz Dr. Deepak Ganjewala  
*University of Agriculture, Faisalabad, Sub-Campus Depalpur, Dist. Okara, Pakistan.*

Dr. Thangavel Palaniswamy  
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GUANGZHOU, PR CHINA.

Yi-Ping Chen Ph.D  
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*Chinese Academy of Science Fenghu S.R,*  
*Xí’an Hi-Tech Zone, Xí’an,*  
*Chinia.*

Saha Prasenjit  
*The Samuel Roberts Noble Foundation*  
2510 Sam Noble Parkway, Ardmore, Ok USA.

Abdul Khaliq Ph.D  
*Department of Agronomy*  
*University of Agriculture*  
Faisalabad 38040, Pakistan.

Dr. Arafat Abdel Hamed abdel Latef  
*Assistant Professor of Plant physiology*  
*Botany Department*  
*Faculty of Science at Qena*  
*South Valley University*  
*Egypt.*

Dr. Ahmad Bybordi  
*Research Center of Agriculture and Natural Resources of East Azerbaijan*  
*Member of Scientific Board of Research Center of Agriculture and Natural Resources of East Azerbaijan,*  
*Tabriz, Iran.*

Dr. Arijit Sinhababu  
*Bankura Christian College (under –The University of Burdwan)*  
*Department of Botany, Bankura Christian College,*  
P.O. + Dist. Bankura, Pin.-722101, West, Bengal, India.

Dr. Maria Alejandra Equiza  
*University of Alberta,*  
4-51 Earth Sciences Building, Dept. Renewable Resources, University of Alberta, Edmonton, AB T6G 2E3,  
Canada.

Dr. Suphla Bajpai Gupta  
*Indian Institute of integrative Medicine –CSIR,*  
*Scientist, Plant biotechnology division, Canal Road,*  
*Jammu, Jammu & Kashmir, India-180001.*

Dr. Linga R Gutha  
*Washington State University,*  
2410 N Bunn Road, Prosser, WA 99350, USA.

Dr. Medhat Mekhail Tawfik  
*National Research Center,*  
*El Bohooth Str. Dokki, Giza, Egypt,*  
*PO Box 12311,*  
*Egypt.*

Dr. Rafiq Islam  
*The Ohio State University South Centers,*  
*1864 Shyville Road, Piketon, OH 45661.*

Dr. Rakesh Kumar  
*V.S.P. Govt. P.G. College, Kairana, Muzaffarnagar (Uttar Pradesh),*  
*Department of Botany, V.S.P. Govt. P.G. College,*  
*Kairana, Muzaffarnagar (Uttar Pradesh), India-247774.*

Dr. Ivan Sestari  
*University of São Paulo,*  
D.Sc. Rachel Fatima Gagliardi  
State University of Rio de Janeiro,  
Rua São Francisco Xavier, 524 – PHLC sala 602.

Dr. Ullas Pedmale  
Salk Institute for Biological Studies,  
10010 N Torrey Pines RD, La Jolla, CA 92037.

Dr. Allah Baksh Dr. Deepak Ganjewala  
Department of Field Crops, Faculty of Agriculture,  
University of Ankara,  
Apartment No. 12/10, Sanatorym Caddesi, Kalaba,  
Kecioren, Ankara, Turkey.

Dr. Atilgan Atilgan  
Suleyman Demirel University, Agriculture Faculty,  
Department of Agricultural Structures and Irrigation,  
Isparta, Turkey.

Dr. Andrej Pilipovic  
University of Novi Sad – Institute of Lowland Forestry and Environment,  
Antona Cehova 13, 21000 Novi Sad, Serbi.

Dr. Zulfiqar Ahmad Saqib  
Institute of Soil and Environmental Sciences,  
University of Agriculture, Faisalabad,  
Civil Line Road, Faisalabad, Pakistan.

MS. C. Mehrnoush Eskandari Torbaghan  
North Khorasan Agricultural & Natural Resource Research Center (NKNRRC)  
P.O. Box: 94155-1416, No. 52, Hassan Kallate Alley, Tarbiyat St., Mother Sq. Bojnourd, Iran.

Dr. Vinod Kumar  
Department of Zoology & Environmental Science,  
Gurukula Kangri University, Haridwar-249404 (UK), India.

Dr. Panda Tribhubana  
Kalahandi Institute for Tribology and Ethnobiology(KITE),  
At-Jilingdar, PO-Deydar, Dist-Kalahandi,Odisha, India,766014, India

Dr. Sabarinath Sundaram  
Institute of Developmental and Molecular Biology,  
Texas A&M University,  
Biological Sciences Building West Suite 403.

Dr. Diogo Pineda Rivelli  
University of São Paulo,  
Av. Prof. Lineu Prestes 580, São Paulo, SP, 05508-000.

Dr. Qiang Wang  
Virginia Tech,  
427 Latham Hall.

Dr. Foteini Hassiotou  
University of Western Australia,  
35 Stirling Highway, Crawley, WA 6009, Australia.

Dr. Nivedita Sahu  
Indian Institute of Chemical Technology,  
Chemical Biology Laboratory (NaturalProductChemistry), Uppal Road,  
Hyderabad-500607.

Dr. Mohammad Anwar Hossain  
Bangladesh Agricultural University,  
Assistant Professor, Dept. of Genetics and Plant Breeding, Bangladesh Agricultural University,  
Mymensingh-2202, Bangladesh.

Dr. Ahmad Ali  
National Institute of Pharmaceutical Education & Research,  
Dept of Biotechnology, NIPER, Jandaha Road,  
Hajipur, Bihar, India, Pin – 844102, India.

Mr. Karthikkumar V  
Annamalai University,  
Department of Biochemistry & Biotechnology.

Dr.K.Rajendiran  
Dept of plant science, Tagore Govt. college,  
9, 4th cross, Tagore Nagar, Pondicherry – 605 008, India.

Dr. V.Balakrishnan  
K.S.Rangasamy College of Technology,  
Department of Biotechnology,KSR Kalvi nagar,Tiruchengode-637215,Tamilnadu, India.
Dr. NourAli Sajedi
Department of Agronomy and plant Breeding, Islamic Azad University, Arak Branch, Arak, Iran.

(Dr) Ms. Rachel Predeepa
Not Applicable, 2/387 Gokul Nagar, Kannanenthal Madurai.

Dr. Rajendra Gyawali
Department of Pharmacy and Biology, Kathmandu University, Dhulikhel, Nepal.

Ms. Rocheli de Souza
UFRGS, Porto Alegre, Brazil.

Dr. Om Prakash Verma
Sam Higginbottom Institute of Agriculture, Technology & Sciences (Formerly Allahabad Agricultural Institute), Allahabad, U.P., Department of Molecular & Cellular Engineering, Jacob School of Biotechnology & Bioengineering, India.

Dr. Ashwani Kumar
JMIT, Radaur, Department of Biotechnology, JMIT, Radaur-135133, Haryana, India.

Dr. Sarfaraz F. A. Al-Bamarny
University of Duhok, College of Agriculture, Dept. of Horticulture, Duhok, Iraqi Kurdistan Region, Iraq.

Prof. Wafaa Mohamed Shukry Abdel Meamem
Dammam University - Saudi Arabia, Faculty of Science for Girl. Biology Department, P.O.Box: 838 Dammam 31113, Saudi Arabia.

Dr. Stephka G. Chankova
Institute of Biodiversity and ecosystem Research, BAS, 2 Gagarin str, 1113 Sofia, Bulgaria.

Dr. Nana Ewusi-Mensah
Kwame Nkrumah University of Science and Technology, Dept. of Crop and Soil Sciences, Faculty of Agriculture, KNUST, Kumasi.

Dr. Mukesh Lokanath Chavan
K.r.c. College of horticulture, arbabhavi 591 310, karnataka, University of horticultura sciences, bagakot, India.

Dr. Maiti Parthapratim
Dept. of Botany Midnapore College, Midnapore-721101, Paschim Medinipur, West Bengal, India.

Mr. Mohammad Anwar Hossain
Kagawa University (Present), Bangladesh Agricultural University (Permanent) Lab. of Plant Stress Responses, Faculty of Agriculture, Kagawa University, Miki-cho, Kita-gun, Kagawa 761 0795, Japan.

Dr. Antonia Tathiana Batista Dutra
Universidade Federal do Ceará, Av. Humberto Monte s/n – Pici Bloco 907, laboratório 1080.

Dr. Kuntal Das
St. John’s Pharmacy College, #6, II Main, 9th Cross, Vijayanagar, Bangalore-104. India.

Dr. Amitava Rakshit
Banaras Hindu University, Department of Soil Science & Agril Chemistry.

Dr. Kranthi Kiran Mandadi
Texas A&M University, 2132 TAMU, Peterson-Rm408, College Station, Texas-77840, USA.

Dr. Monica Butnariu
Banat’s University of Agricultural Sciences and Veterinary Medicine from Timisoara, Chemistry and Vegetal Biochemistry Department, Calea Aradului no.119, 300645 Timisoara, Romania.
Dr. Ahmad Bybordi  
East Azarbaijan Research Center for Agriculture and Natural Resources, Tabriz, Iran.

Dr. Haiwei Gu  
903 Fifth St., West Lafayette, IN 47906.

Dr. Hu Yanbo  
Northeast Forestry University, 26# Hexing Road, Xiangfang District, Harbin city, 150040, P.R., China.

Dr. Arash Kianianmomeni  
Institute of Biology / Humboldt-University Berlin, Invalidenstr. 42.

Dr. Zvonko Pacanoski  
Faculty for Agriculture Sciences and Food, Boul. Aleksandar Makedonski bb, 1000 Skopje, R.of Macedonia.

Dr. Lingjuan Zheng  
Department of Organismic Biology, University of Salzburg, Hellbrunnerstraße 34, 5020, Salzburg, Austria.

Dr. Md. Mokter Hossain  
Department of Horticulture, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Dr. Forouzandeh Soltani  
Department of Horticultural Sciences, College of Agriculture and Natural Resources, University of Tehran, Daneshkadeh Street, Karaj 31587-11167, Iran.

Dr. M.C.Harish  
Bharathiar University, Department of Biotechnology, Coimbatore, India.

Dr. Zong-shen Zhang  
School of Biological Engineering, Dalian Polytechnic University, Qinggongyuan, Ganjingzi District, Dalian, China, postcode 116034.

Prof. T. V. Ramana Rao  
B R Doshi School of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India.

Dr. Sanjeev Chandel  
Baba Isher Singh Institute of Sciences & Technology, Gagra (Moga), Punjab, India.

Dr. Kuladip Jana  
Bose Institute Centenary Campus, P 1/12, C.I.T. Scheme VIIIM, Kolkata-700 054, India.

Prof. Ljubinko Jovanovic  
University Educons, Faculty for ecological agriculture, Sremska Kamenica, Vojvode Putnika 87, Serbia.

Dr. Luis F. Goulao  
Instituto de Investigacao Cientifica Tropical [Tropical Research Institute] Eco-Bio / IICT, Av. da Republica - Quinta do Marques, 2784-505 Oeiras, Portugal.

Dr. Lucky K. Attri  
College of Punjabi University Patiala, E-41, Sector-14, Panjab University, Chandigarh.

Prof. Bassam Taha Yasseen  
Looking for appropriate employer, Flat 307 Point Red, 146 Midland Road, Luton, LU2 0BL, UK.

Dr. Massimo Piccotto  
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Examples:

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References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

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The determination of vitamin C, total phenol and antioxidant activity of some commonly cooking spices crops used in West Bengal

Manas Denre
The determination of vitamin C, total phenol and antioxidant activity of some commonly cooking spices crops used in West Bengal

Manas Denre

Department of Agricultural Biochemistry, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia-741252, West Bengal, India.

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In the present study, vitamin C, total phenols and antioxidant activity of some common cooking spices crops used in West Bengal were analyzed. Ten (10) spices (onion, chilli, garlic, ginger, turmeric, mustard seed, cumin seed, clove, cardamom and cassia leaf) were selected in order to determine the concentration of ascorbic acid (AA), total phenol (TP) and antioxidant activity as DPPH radical scavenging activity (DPPHRAC). The results obtained show that the values of different variables varied from 5.55 to 0.08 mg g⁻¹ (AA), 21.55 to 7.67 GAE mg g⁻¹ (TP) and 0.18 to 5.99 IC₅₀ value: mg ml⁻¹ (DPPHRAC), respectively. There were negative correlations between TPC and IC₅₀ value of DPPHRAC.

Key words: Vitamin C, total phenols, antioxidant, spices crops.

INTRODUCTION

Food provides not only essential nutrients needed for life but also other bioactive compounds for health promotion and disease prevention. Thus, consumers demand for natural foods with antioxidant property, which enhances health and food preservation. There is a great demand for antioxidants that are derived from natural resources. Also, there is increased demand for natural dietary products which produce antioxidants in the body (Barlow, 1990; Rice-Evans et al., 1997).

Antioxidants prevent oxidative damage caused by free radicals; they interfere with the oxidation process by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers (Shahid and Wanasundara, 1992; Buyukokuroglu et al., 2001). One of the paradoxes of life on this planet is that the molecule that sustains aerobic life, oxygen, is not only fundamentally essential for energy metabolism and respiration, but also has implication in many diseases and degenerative conditions (Marx, 1985). A common element in such diverse human disorders such as ageing, arthritis, cancer, Lou Gehrig’s disease and many others is the involvement of partially reduced form of oxygen. Oxygen can accept single electron to form unstable derivatives referred to as Reactive Oxygen Species (ROS) or Active Oxygen Species (AOS) produced during various metabolic cellular processes. ROS include free
radicals such as uperoxide radical anion ($O_2^-$), hydroxyl radical (’OH), various peroxyl radicals (ROO') and non-
free radicals such as hydrogen peroxide (H$_2$O$_2$), singlet oxygen (’O) and hypochlorous acid (HOCl) (Halliwell,
1995; Odukoya et al., 2005). Normal range for the production of ROS helps in the regulation of cell prolif-
eration, intercellular signalization, phagocytosis and also synthesis of biologically active compounds. But hyper-
production of ROS develops oxidative stress which causes oxidative damage to various bimolecular including
proteins, lipids, lipoproteins and DNA (Farber, 1994). This oxidative damage is a critical etiological factor implicated
in several chronic human diseases such as cardiovascular diseases, diabetes, tumors, rheumatoid arthritis, epilepsy,

Spices are various parts (bud, fruit, seed, bark, rhizome, leaf and bulb) of plants used as a flavoring or
seasoning, although many can also be used as herbal medicine. The term ‘spice’ originated from the Latin word
‘species’, meaning of specific kind. A closely related term, ‘herb’ is used to distinguish plant parts having the same
functions. Spices have many functions in food. Primarily they are used for flavoring food products; in addition they
are also used for preservation of food and for providing nutritional and health benefits (Nazeemeen, 1995).

The two terms may be used for the same plants, that is, ‘spices’ and ‘herb’. They have been investigated in recent
scientific developments throughout the world, one due to their flavor, color, nutritional, health, or preservative
effects and another due to their potent antioxidant activities. As per literature survey, so many researchers
have reported that chilli, onion, garlic, ginger, turmeric, mustard seed, cumin seed, clove, cardamom and cassia
leaf are used as common spices cum herb crops from ancient times.

Apart from providing basic nutrition, these crops are well known for their health benefits; antioxidant constitu-
tuents and antioxidant activities have been reported in chilli (Bhattacharya et al., 2010; Biswas et al., 2011; 
Denre et al., 2013a, 2014), onion (Benkeblia, 2005; Denre, et al., 2011), ginger (Hinneburg et al., 2006; Kim
et al., 2007; Kota et al., 2008; Suhaj and Horvathova, 2007), garlic (Agarwal, 1996; Banerjee et al., 2003; 
Denre et al., 2013b) turmeric (Kaur and Kapoor, 2002; Tanganakul et al., 2009), mustard seed (Dande and
Manchala, 2011; Dubie et al., 2013), cumin seed (Dua et al., 2012; Nadeem and Riaz, 2012), clove (Lee and
Shibamoto, 2001; Suhaj and Horvathova, 2007; Devi et al., 2012), cardamom (Devi et al., 2012) and cassia leaf
(Yong et al., 2012). Foods rich in antioxidants play a role in preventing cardiovascular diseases, cancers (Garber
et al., 2002), neurodegenerative diseases (Di-Matteo and Esposito, 2003), inflammation and problems caused by
cell and cutaneous aging (Ames et al., 1993). The aim of the present work was to study the determination of
vitamin C, total phenols and antioxidant activity of some commonly cooking used spices crops, which may be
added in its right (proper value) while cooking.

MATERIALS AND METHODS

The 10 spices crops (Chilli (Capsicum annuum L.), onion (Allium capa L.), garlic (Allium sativum L.), Ginger (Zingiber officinale L.),
turmeric (Curcuma Longa L.), mustard seed (Brassica Juncea), cumin seed (cuminus cyminum), clove (Syzygium aromaticum L.),
cardamom (Elettaria cardamomum), cassia leaf (cinnamomum cassia L.)) were collected from the market (Shyamput market, 
Uluberia, Howrah, West Bengal, India) for the experiment. All the collected samples were washed, air dried in shade and then
samples were oven dried at 40°C for 96 h until constant weight was gained. These dried materials were prepared for chemical analyses
by grinding into a fine powder using an electric grinder. The powder of each sample was stored at three replications in an air-tight
cellophane bag as stock sample in a refrigerator (4°C) until further analysis. All the analytical work was performed at Agricultural
Biochemistry laboratory, Department of Agricultural Biochemistry, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia-741252,
West Bengal, India.

Determination of ascorbic acid content (AAC)

Ascorbic acid was determined by the 2, 6-dichlorophenol indophenol (DCPIP) titration procedures based on the method of
Casanas et al. (2002). The 0.1 g powdered sample was extracted with 20 ml of 4% oxalic acid. Then the material was centrifuged at
10,000 rpm for 30 min. 10 ml of sample’s aliquot and 10 ml of 4% oxalic acid were taken in a conical flask and titrated against 2,6-
dichlorophenol indophenol (DCPIP) dye ($V_1$) until the appearance of a faint pink colour that persisted for a few minutes. Another 5 ml of
100 ppm solution of ascorbic acid and 10 ml of 4% oxalic acid were added and also titrated against DCPIP dye ($V_2$). The ascorbic acid
content (mg g$^{-1}$) was determined using the following formula:

$$\text{Amount of ascorbic acid (mg g}^{-1}) = \frac{0.5 \times V_2}{V_1 \times 10 \times 0.1}$$

$V_1$ = dye consumed by 0.5 mg ascorbic acid; $V_2$ = dye consumed by 10 ml of test solution.

Determination of total phenols content (TPC)

The total phenols content in spices crops were determined by the Folin-Ciocalteau reagent (Vinson et al., 1998) using gallic acid as
standards. The 0.1 g powdered samples was extracted with 15 ml of 1.2 M HCl in 50% aqueous methanol and heated at 85°C for 2 h
to measure the conjugated plus unconjugated (‘total’) phenols. After cooling the extracted material was centrifuged at 10,000 rpm for 30
min. The supernatant was decanted off in a beaker and evaporated to dryness. The crude extract was diluted to 25 ml with distilled
water. For estimation of the total phenols content 0.5 ml of sample’s aliquot, 2.5 ml of distilled water and 0.5 ml Folin-Ciocalteau reagent
were pipetted out in a test tube. After 3 min, 2 ml of 10% sodium carbonate was added. Then the test tubes were kept on water bath
and maintained at 60-70°C for 5 min. The solution was cooled and the absorbance was read at 580 nm. The total phenols content was
measured (mg g$^{-1}$) using a calibration curve against gallic acid equivalent.
Table 1. Concentration of vitamin C, total phenols and antioxidant activity of some commonly cooking spices crops used in West Bengal.

<table>
<thead>
<tr>
<th>Spices crop</th>
<th>Scientific name</th>
<th>Part used</th>
<th>AAC ( \text{mg g}^{-1} )</th>
<th>TPC ( \text{mg g}^{-1} )</th>
<th>DPPH (IC(_{50}) value: mg ml(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onion</td>
<td>Allium capa L.</td>
<td>Bulb</td>
<td>0.62±0.09</td>
<td>7.67±0.55</td>
<td>2.28±0.08</td>
</tr>
<tr>
<td>Chilli</td>
<td>Capsicum annuum L.</td>
<td>Pod</td>
<td>5.55±0.55</td>
<td>8.30±0.67</td>
<td>1.26±0.15</td>
</tr>
<tr>
<td>Garlic</td>
<td>Allium sativum L.</td>
<td>Bulb</td>
<td>0.37±0.08</td>
<td>14.30±0.69</td>
<td>1.12±0.03</td>
</tr>
<tr>
<td>Ginger</td>
<td>Zingiber officinale L.</td>
<td>Rhizome</td>
<td>0.48±0.03</td>
<td>15.52±0.96</td>
<td>1.11±0.02</td>
</tr>
<tr>
<td>Turmeric</td>
<td>Curcuma Longa L.</td>
<td>Rhizome</td>
<td>0.75±0.06</td>
<td>13.00±0.51</td>
<td>5.99±0.68</td>
</tr>
<tr>
<td>Mustard</td>
<td>Brassica Juncea</td>
<td>Seed</td>
<td>0.08±0.03</td>
<td>13.79±0.79</td>
<td>0.66±0.05</td>
</tr>
<tr>
<td>Cumin</td>
<td>cuminus cyminum</td>
<td>Seed</td>
<td>0.09±0.02</td>
<td>20.32±1.32</td>
<td>2.40±0.07</td>
</tr>
<tr>
<td>Clove</td>
<td>Syzygium aromaticum L.</td>
<td>Bud</td>
<td>0.14±0.05</td>
<td>21.55±0.67</td>
<td>0.26±0.03</td>
</tr>
<tr>
<td>Cardamom</td>
<td>Elattaria cardamomum</td>
<td>Pod</td>
<td>0.27±0.06</td>
<td>16.00±1.00</td>
<td>0.18±0.05</td>
</tr>
<tr>
<td>Cassia</td>
<td>Cinnamomum cassia L.</td>
<td>Leaf</td>
<td>0.76±0.12</td>
<td>10.51±0.51</td>
<td>1.09±0.09</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation values.

Determination of 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPHRAC)

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was performed based on the method of Braca et al. (2001). In order to determine the DPPH radical scavenging activity present in spices crops, 0.5 g powdered samples was extracted with 10 ml of distilled water. Extracted sample was centrifuged at 10,000 rpm for 15 min. Each 6.4 ml reaction mixture containing 0.1-0.4 ml of sample’s aliquot, 0.1-0.4 ml of distilled water, and 6 ml of 0.004% DPPH were taken in tubes and shaken by hand. They were kept for 30 min at room temperature in dark place. Absorbance of the reaction mixture was read at 517 nm against the blank. The reaction mixture lacking sample developed the most intense colour. The colour decreased with increasing volume of extract added. The ability to scavenge the DPPH radical was calculated using the following equation: DPPH radical scavenging activity (% inhibition) = \( \left( \frac{A_0 - A_t}{A_0} \right) \times 100 \), where \( A_t \) was the absorbance of sample and \( A_0 \) was the absorbance of the control. The % inhibition value was plotted against concentration of the sample extract. From the graph, value of the sample extract producing 50% inhibition (IC\(_{50}\) value) was calculated.

Statistical analysis

Data were analyzed statistically by using Daniel’s XL Toolbox 6.52 software for analysis of variance and correlation.

RESULTS AND DISCUSSION

Ascorbic acid

Ascorbic acid is one of the most powerful antioxidants (Smirnoff, 1996; Noctor and Foyer, 1998; Arrigoni and de Tulio, 2000; Horemans et al., 2000b; Smirnoff, 2000) that scavenge harmful free radicals and other ROS; it also regenerates other antioxidants like tocopherol to its functional state. In the present study (Table 1), the wide remarkable variation in ascorbic acid concentration was shown among the spices crops that varied from 5.55 to 0.08 mg g\(^{-1}\); and ranking from high to low concentration was chilli>cassia leaf>termeric>onion>ginger>garlic>cardamom>clove>cumin seed>mustard seed, respectively. The recommended daily allowance of ascorbic acid is 60 mg in USA. However, Australia and United Kingdom recommend 30 to 40 mg; Russia recommends 100 mg. Higher intake of ascorbic acid has been linked to lower risk of cardiovascular diseases (Simon et al., 1988) and several types of cancers (Howe et al., 1990) by forming N-nitroso compounds in stomach and by stimulating the immune system (Byers and Perry, 1992). Vitamin C deficiency exacerbates atherogenesis in animal models. Risk of oesophageal, pancreatic and lung cancer also appears to be lower in people that take ample vitamin C (Wargovich, 2000).

Total phenols

Phenolics are diverse secondary metabolites that are abundant in plant tissues (Grace and Logan, 2000). Antioxidant properties of phenols arise from their high reactivity as hydrogen or electron donors, from the ability of the phenol derived radical to stabilize and delocalize the unpaired electron (Chain breaking function) and from their ability to chelate transition metal ions (termination of the Fenton reaction) (Rice-Evans et al., 1997). Another mechanism underlying the antioxidant properties of phenolics is the ability of flavonoids to alter peroxidation kinetics by modifying the lipid picking order and decreasing the fluidity of the membranes (Arora et al., 2000). These changes could sterically hinder diffusion of free radicals and restrict peroxidation reaction. In the present experiment (Table 1), the total phenols contents ranged from 21.55 to 7.67 GAE mg g\(^{-1}\) with decreasing order: clove>cumin seed>cardamom>ginger>garlic>mustard seed>termeric>cassia leaf>chilli>onion, respectively. The health benefit of phenolics is linked primarily to their antioxidant potential. Phenolics are effective antioxidants because the radicals products of these molecules are resonance stabilized and thus relatively stable. To
overcome the potential hazard from oxidative damage in the body, consumption of a diet rich in antioxidant phenolics including flavonoids and phenolic acids is considered the first line of defense against highly reactive toxicants. Scalbert and Williamsson (2000) estimated that the total human intake of phenolics is about 1 g day$^{-1}$ which consists of two- thirds flavonoids and one- third phenolic acids.

**DPPHRAC**

DPPH analysis is one of the best-known, accurate, and frequently employed methods for evaluating antioxidant activity (Zhou and Yu, 2004). It is a stable free radical because of its spare electron delocalization over the whole molecule. In the present experiment (Table 1), it is revealed that the DPPH radical scavenging activity ranges from 0.18 to 5.99 mg ml$^{-1}$ following the downward activity order: Cardamom>clove>mustard seed>cassia leaf>ginger>garlic>chill>onion>turmeric, respectively. The smaller the IC$_{50}$ value the greater the radical scavenging activity and reducing ability of the extracts.

**Correlation among variables**

Table 2 did not show any significant correlation among the variables, but apparently there were negative correlations between AAC with TPC and TPC with IC$_{50}$ values of DPPHRAC. Interestingly, the negative correlation of TPC with IC$_{50}$ value of DPPHRAC indicated that the higher total phenol content of extracts resulted in lower IC$_{50}$ value of DPPHRAC. That means the higher total phenol content of extracts resulted in higher antioxidant (DPPH radical scavenging activity) activity, because the lower IC$_{50}$ value indicated the higher DPPH radical scavenging activity in extract of spices crops. Maizura et al. (2011) reported that the higher total phenolic content of extracts resulted in higher antioxidant (DPPH radical scavenging activity) activity in extracts of kesum, ginger and turmeric.

**Conclusion**

Based on the mean and ANOVA results, there is a wide variation among variables in the ten selected cooking species crops. The maximum values of ascorbic acid, total phenol and DPPH radical activity were exhibited as 5.55 mg g$^{-1}$ (chilli), 21.55 mg g$^{-1}$ (clove) and 0.18 IC$_{50}$ value: mg ml$^{-1}$ (Cardamom), respectively. Thus, it is crucial to determine not only their antioxidant capacity but also their characterization potential in promoting health.

**Conflict of Interests**

The author(s) have not declared any conflict of interests.

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