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ORIGINAL ARTICLE

Phytochemicals, Antioxidant, Anti-inflammatory Studies, and Identification of Bioactive Compounds Using GC–MS of Ethanolic Novel Polyherbal Extract

Amit Kumar Singh[1] · Pradeep Kumar[2] · Vishnu D. Rajput[3] · Sunil Kumar Mishra[1] · Kavindra Nath Tiwari[2] · Anand Kumar Singh[4] · Tatiana Minkina[3] · Ajay Kumar Pandey[5]

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Abstract
Hyperglycemia is the hallmark of diabetes, which is a collection of related metabolic disorders. Over time, diabetes can cause a variety of problems, including cardiovascular disease, nephropathy, neuropathy, and retinopathy. Ethanolic novel polyherbal extract (PHE) was prepared by mixing equal amounts of the following ingredients: *Terminalia chebula* Retz. (TC), *Terminalia bellerica* Roxb. (TB), *Berberis aristata* DC. (BA), *Nyctanthes arbostratis* L. (NA), *Premna integrifolia* L. (PI), and *Andrographis paniculata* Nees. (AP). Analysis of PHE results revealed phytochemicals like glycosides, flavonoids, alkaloids, tannins, phytosterols, and saponins. The aim of the study was to prepare an ethanolic extract of PHE using the cold maceration technique, and identify bioactive molecules from gas chromatography–mass spectrometry (GC–MS) analysis, and evaluate biological responses by using in vitro studies like antioxidant and anti-inflammatory activity. PHE was found to contain a total of 35 phytochemicals in GC–MS of which 22 bioactive compounds were obtained in good proportion. There are a few new ones, including 2-buten-1-ol, 2-ethyl-4-(2, 2, 3-trimethyl-3-cyclopenten-1-yl (17.22%), 1, 2, 5, 6-tetrahydrobenzonitrile (4.26%), 4-piperidinamine, 2, 2, 6, 6-tetramethyl-(0.07%), undecanoic acid, 5-chloro-, chloromethyl ester (0.41%), are identified. Antioxidant activity was estimated using EC_{50} values of 392.143 μg/ml, which were comparable to the standard value of EC_{50} 310.513 μg/ml obtained using DPPH. Antioxidant activity was estimated with EC_{50} 392.143 μg/ml, comparable to standard EC_{50} 310.513 μg/ml using DPPH. In vitro anti-inflammatory potential was found with IC_{50} of 91.449 μg/ml, comparable to standard IC_{50} 89.451 μg/ml for membrane stabilization and IC_{50} of 36.940 μg/ml, comparable to standard IC_{50} 35.723 μg/ml for protein denaturation assays. As a result, the findings of this study show an enrichment of bioactive phytochemicals that can be used to investigate biological activity. To better understand how diabetes receptors work, in silico studies like docking could be carried out.

Keywords Polyherbal extract · Antioxidant · Anti-inflammatory activity · GC–MS · Diabetes

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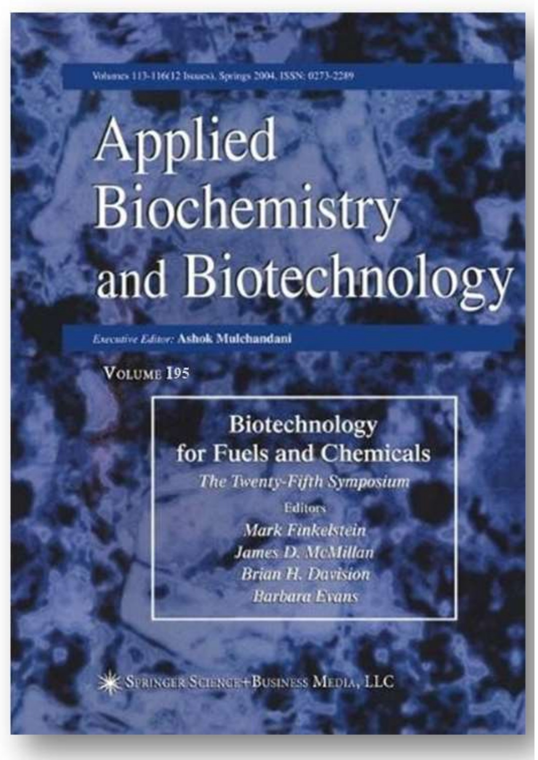

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Full Length Article

A network pharmacology approach with experimental validation to discover protective mechanism of poly herbal extract on diabetes mellitus

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Poly herbal extract (PHE)
Alpha Tocospiro A (ATA)
Diabetes mellitus (DM)
KEGG
Molecular simulation
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ABSTRACT

Objective: Polyherbal extracts (PHE) contain six traditional medicinal plants, and the efficacy of the medicinal plants used in the preparation of this PHE has been confirmed for the treatment of diseases like diabetes mellitus (DM). The aim of this study was to evaluate the efficacy and therapeutic mechanism of PHE through a network pharmacology approach to reveal the protective mechanism of Alpha-Tocospiro A (ATA) present in PHE on DM with experimental validation.
Methods: In this study, Lipinski's rule (Swiss ADME) and drug-likeness score (MolSoft's) web pages were used to confirm the drug-likeness of identified constituents in PHE. Swiss Target Prediction (STP) genes were found for ATA-related genes. The DisGeNet database was used to screen genes associated with DM. String created a network diagram of the interactions between the ATA and DM genes. Top-scoring genes from the string network through CytoNCA plugged into Cytoscape 3.8.2 were selected as hub genes. In addition, the ShinyGO database is used to predict GO and KEGG pathway enrichment analyses.
Results: A total of 675 and 105 therapeutic genes (STP) were associated with all bioactive compounds and ATA in the PHE screen, respectively. Additionally, a maximum of 2,803 DM-related genes (DisGeNet) were observed. Further, in the analysis, 331, 57 potential (intersecting) genes were identified in the correlation between the target genes of all compounds and ATA, respectively, of PHE and the target genes of DM. The identified hub gene "TNF" for both ATA and PHE was found to be precisely strengthened in 49 pathways, along with 14 signaling pathways out of more than 100 enriched KEGG pathways. This study predicted that ATA activates PI3K/Akt and MAPK pathways enriched with TNF by phosphorylating the insulin receptor (IR) β-subunit. The anti-diabetic activity of PHE was found to be good and primarily confirmed by in vitro α-glucosidase enzyme inhibition activity.
Conclusion: The anti-diabetic activity of PHE was found to be effective and was confirmed by the enzyme inhibition activity in the primary study. This study predicted that ATA is a novel drug molecule in PHE that has a targeted mechanism of action and therapeutic effect on DM.

1. Introduction

Although immense improvement has been achieved in drug development and pharmaceutical technology over the last century, some polygenic chronic diseases, such as cardiovascular events, brain hemorrhage, cancer, and diabetes mellitus (DM), remain difficult to treat due to their multi-factorial nature (Sagner et al., 2017). DM is rapidly increasing globally, exacerbating its enormous social and economic impact (Seehusen et al., 2019). According to the most recent information available from the International Diabetes Federation (IDF-

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ORIGINAL ARTICLE

A Dual Therapeutic Approach to Diabetes Mellitus via Bioactive Phytochemicals Found in a Poly Herbal Extract by Restoration of Favorable Gut Flora and Related Short-Chain Fatty Acids

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Abstract
Diabetes mellitus (DM), a metabolic and endocrine condition, poses a serious threat to human health and longevity. The emerging role of gut microbiome associated with bioactive compounds has recently created a new hope for DM treatment. UHPLC-HRMS methods were used to identify these compounds in a poly herbal ethanolic extract (PHE). The effects of PHE on body weight (BW), fasting blood glucose (FBG) level, gut microbiota, fecal short-chain fatty acids (SCFAs) production, and the correlation between DM-related indices and gut microbes, in rats were investigated. Chebulic acid (0.368%), gallic acid (0.469%), andrographolide (1.304%), berberine (6.442%), and numerous polysaccharides were the most representative constituents in PHE. A more significant BW gain and a reduction in FBG level towards normal of PHE 600 mg/kg treated rats group were resulted at the end of 28th days of the study. Moreover, the composition of the gut microbiota corroborated the study's hypothesis, as evidenced by an increased ratio of *Bacteroidetes* to *Firmicutes* and some beneficial microbial species, including *Prevotella copri* and *Lactobacillus hamster*. The relative abundance of *Bifidobacterium pseudolongum*, *Ruminococcus bromii*, and *Blautia producta* was found to decline in PHE treatment groups as compared to diabetic group. The abundance of beneficial bacteria in PHE 600 mg/kg treatment group

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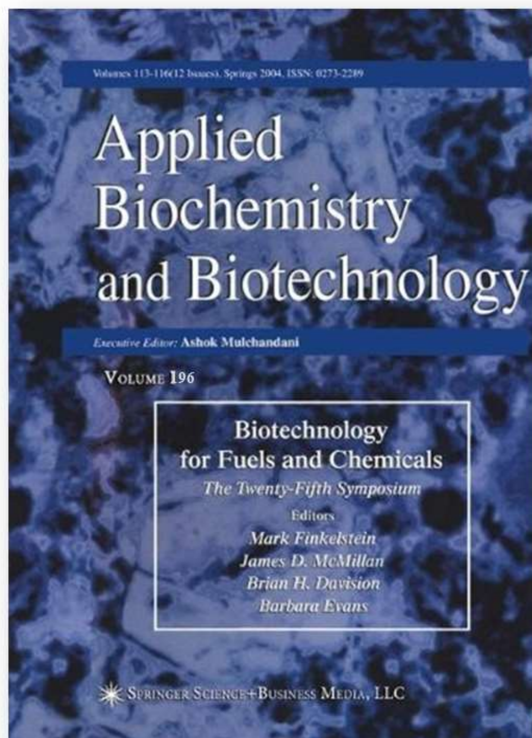

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22. Dual modes of poly herbal extract in the mitigation of STZ-induced diabetes through enrichment of polyphenols and restoration of the gut microbiota-metabolites-targets network
23. Phytochemical profiling, safety evaluation and oxidative stress study of novel polyherbal extract in Wistar rats
24. Berberine a biomarker of poly herbal extract attenuates ferroptosis in diabetes mellitus via ESR1/CDC42/AR/PTGS2 signaling
25. In silico and network pharmacology analysis of Fucosterol: A potent anticancer bioactive compound against HCC
26. Luteolin: A Promising Drug against Liver Cancer Analyzed by Network Pharmacology Approach
27. Acceptable, Affordable, and Applicable Nano-Assisted Delivery Tools for Studying Plant Genetic Engineering

Appendix 1

काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

AN INSTITUTION OF NATIONAL IMPORTANCE ESTABLISHED BY AN ACT OF PARLIAMENT

Prof. Nawal Kishore Dubey
FNASc, FNAAS

Date: February 15, 2020

To Whom It May Concern

This is to certify that the following plant specimens collected by **Amit Kumar Singh,** Research scholar, Department of Pharmaceutical Engineering and Technology, IIT, Banaras Hindu University, Varanasi have been authenticated by me and the voucher specimens has been kept in our Herbarium.

1. *Terminalia chebula* Retz. **(Voucher specimen no. Combreta. 2020/02)**
   Combretaceae
2. *Terminalia bellirica* (Gaertn.) Roxb. **(Voucher specimen no. Combreta. 2020/02)**
   Combretaceae
3. *Andrographis paniculata* (Burm.f.) Nees **(Voucher specimen no. Acantha. 2020/01)**
   Acanthaceae
4. *Berberis aristata* DC. **(Voucher specimen no. Berberida. 2020/02)**
   Berberidaceae
5. *Nyctanthes arbor-tristis* L. **(Voucher specimen no. Olea. 2020/01)**
   Oleaceae
6. *Premna integrifolia* L. **(Voucher specimen no. Lamia. 2020/01)**
   Lamiaceae

(Prof. N. K. Dubey)

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Seeking an environment where my knowledge and hard working attitude can be put for mutual benefit

Professional Profile

CORE COMPETENCIES

- Handling of Pharmaceutical Equipments

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- Excellence in Analysis and Problem Solving
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- Strong Influencing Skills
- Achievement oriented Team Builder
- Persuasive Communicator

✓ Bioinformatics study through molecular docking simulation related software and databases (Autodock, Cytoscape, Discovery Studio, etc.)
✓ Network Pharmacology study for identification of key target genes
✓ A dynamic professional with better handling of Pharmaceutical equipments
✓ Successfully completed studies in the modules: Computer Concept, Operating System
✓ Usage of internet
✓ Ms office
✓ Self confidence
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✓ Hard working
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SeminarAttended

✓ Short Term course on Transforming Healthcare with AI-driven Drug Discovery, December 2023, at Department of Pharmaceutical Engineering & Technology, IIT (BHU), Varanasi
✓ Hands-on Workshop on Applications of Artificial Intelligence In Drug Discovery, March 2023, at IIT (BHU), Varanasi
✓ SAMSKARA, PG Orientation Program (First Year Scholar 2022-23), July 2023, at Faculty of Ayurveda, DravyaGuna, IMS, BHU
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✓ "Future Aspects and Challenges in Pharmaceutical Technologies" February 2015, at Shambhunath Institute of Pharmacy (SIP), Jhalwa, Allahabad
✓ "Lead Development and Drug Designing: Emerging Opportunities" September 2014, at United Institute of Pharmacy, Naini, Allahabad
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✓ National Seminar "Recent Innovation in Pharmaceutical Sciences & Technology" November 2011, at BITS-Pharmacy, Bhopal, MP
✓ "Pharma Vision 2020" National seminar April 2010, at Bhopal, MP
✓ "Health Awareness Camp" 1st to 3rd February 2010, at BITS-Pharmacy, Bhopal, MP
✓ Attended 59th Indian Pharmaceutical Congress (IPC) 2007 held at BHU, Varanasi, UP

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- Examination In-charge, KHBS College of Pharmacy, Jaunpur, UP, from August 2015 to December 2018
- Examiner as practical examinations, Center controller for UPSEE entrance examinations, Centre superintendent for UG and PG examinations at KHBS College of Pharmacy, Jaunpur, UP
- As a GPAT qualified candidate in year 2014
- Project in M. Pharm.IInd year Pharmacognostic Study with of medicinal plant-drug
- Internship in ZydusCadila Ahmadabad, Gujarat
- Industrial visit in IPCA and Divya Pharmacy in Uttaranchal

Publications (as**A**uthor) from**Ph.D. W**ork

Research articles

1. **Singh AK**, Kumar P, Rajput VD, Mishra SK, Tiwari KN, Singh AK, Minkina T, Pandey AK. Phytochemicals, Antioxidant, Anti-inflammatory Studies, and Identification of Bioactive Compounds Using GC–MS of Ethanolic Novel Polyherbal Extract. **Applied Biochemistry and Biotechnology. 2023**, 195:4447-4468.(IF 3.0)

2. **Singh AK**, Kumar P, Mishra SK, Tiwari KN, Singh AK, Pandey AK, Shatie AA, Alfaifie MY, Elbehairie SEI, Sayyed RZ.A network pharmacology approach with experimental validation to discover protective mechanism of Poly herbal extract on diabetes mellitus. **Journal of King Saud University-Science. 2024**, 36:04:103138.(IF 3.8)

3. **Singh AK**, Kumar P, Mishra SK, Rajput VD, Tiwari KN, Singh AK, Minkina T, Pandey AK, Upadhyay P.A dual therapeutic approach to diabetes mellitus via bioactive phytochemicals found in a poly herbal extract by restoration of favorable gut flora and related short-chain fatty acids. **Applied Biochemistry and Biotechnology. 2024**, 196:03:1-26.(IF 3.0)

Publications (as**C**0-**A**uthor) from**Ph.D. W**ork

Research articles

4. SinghK, KumarP, **Singh AK**, SinghN, SinghS, TiwariKN, AgrawalS, DasR, SinghA, RamB, TripathiAK, MishraSK. In silico and network pharmacology analysis of Fucosterol: A potent anticancer bioactive compound against HCC.**Medical Oncology. 2024**, xxx:xxx.(IF 3.4) Accepted

5. Kumar P, **Singh AK**, Tiwari KN, Mishra SK, Rajput VD, Minkina T, Cavalu S, Pop O. Identification and validation of core genes as promising diagnostic signature in hepatocellular carcinoma based on integrated bioinformatics approach. **Scientific Reports. 2022**, 12(1):19072.(IF 4.6)

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