

# Abstract

The North-Eastern hill region in India is a significant agro-biodiversity hotspot with underutilized ethnomedicinal fruits essential for rural life. However, these fruits are often unknown due to lack of documentation, shrinking forest areas, and disappearance of indigenous culture. Plant-derived products, particularly fruits, offer numerous benefits and are a promising alternative to current health-promoting medications due to their less expensive and less harmful side effects. Hence, the present study was conducted to collect wild edible fruits with ethnomedicinal values from India's North-Eastern hill region, considering their ethnomedicinal importance. Since these fruits are ethnomedicinal and nutritionally essential, this present investigation aims to explore bioactive phytochemicals from underutilized fruits and their health-beneficial role

against some diseases.

Fourteen fruit samples were collected from different locations in three states (West Bengal, Meghalaya, and Tripura) among them four fruits: *Elaeagnus pyriformis* (EP), *Baccurea ramiflora* (BR), *Phyllanthus acidus* (PA), and *Prunus nepalensis* (PN) were selected for further experiments while the remaining fruits were not considered based on DPPH assay. These four fruit extracts [EP (80.91±0.264%), BR (78.864±0.11%), PA (78.053±0.78%), PN (81.56±0.57%) at 200 µg/ml] exhibited higher free radical scavenging activity than the others when compared to the respective standard (ascorbic acid). With increasing concentrations of extracts (50-200 µg/ml), these fruit samples showed promising reactive oxygen species (ROS) scavenging power in the case of other *in*

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*vitro* assays [hydroxyl radical, hydrogen peroxide, nitric oxide, and hypochlorous and total antioxidant assay]. The study showed a strong positive correlation between antioxidant scavenging assay and total phenol and flavonoid content for all four fruit extracts, where EP showed the highest phenol ( $73.74631 \pm 1.171$  mg GAE/g DW) and flavonoid content ( $39.3726 \pm 0.6661$  mg QE/g DW). High phenol and flavonoid content have been proved by FTIR analysis where EP, BR, PA, and PN extracts indicated the presence of significant peaks for polyphenolic OH, alkane, carboxylic acid, and amide-I at around ( $3419-3439$   $\text{cm}^{-1}$ ), ( $2920-2927$   $\text{cm}^{-1}$ ), ( $1732-1740$   $\text{cm}^{-1}$ ) and ( $1625-1648$   $\text{cm}^{-1}$ ) respectively. The present study was extended to analyze the identification of active phytochemicals in EP, BR, PA, and PN extracts using the GC-MS method. A total number of 38 phytochemicals have been identified in EP (11 compounds), BR (8 compounds),

PA (8 compounds) and PN (11 compounds). It has been reported that fatty acids like octadecanoic acid (stearic acid), dodecanoic acid (lauric acid), and hexadecanoic acid (palmitic acid) were present in the fruit extracts that humans need in their diets. Apart from these, ellagic acid,  $\gamma$ -sitosterol, 1,2-Longidione, n-hexadecanoic acid, and octadecanoic acid, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl each have anticancer, antidiabetic, nephroprotective, hypocholesterolemic, antibacterial, and anti-inflammatory properties. Therefore, based on the study above, it is possible to indicate that EP, BR, PA, and PN extracts can potentially become powerful antioxidative agents in the future. Further LC-MS analysis allowed the identification of more than 75 polyphenols, including flavanols, flavonoids, anthocyanidins, alkaloids, steroids, vitamins, triterpenoids, phenylpropanoids, phenolic acids, and derivatives from four fruit samples

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(EP, BR, PA, PN). It has been reported that vitamin B<sub>9</sub> has antioxidant and neuroprotective properties among these bio compounds and reduces the chances of lipid peroxidation. Reports suggested that flavonoids (trifolin, quercetin-3 $\beta$ -D-glucoside, kaempferol), flavonols (rutin), anthocyanidins (malvidin), phenolic acids (quinic acid, gallic acid, phenolic acid), phenylpropanoids (rosmerinic acid) are potent antioxidant and ROS scavengers. These have high antioxidant, anticancer, anti-inflammatory, hypolipidemic, and immunomodulatory properties. Fruit extracts were investigated against two Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and two Gram-negative (*Escherichia coli*, *Salmonella typhimurium*) pathogenic bacterial strains to see antimicrobial activity. Among all fruit extracts, PA showed perfect growth inhibitory activity against gram-positive and gram-negative bacteria at low doses (5mg/ml). At high

doses (10mg/ml), EP, BR, PA, and PN (all four fruit extracts) showed a significant zone of inhibition against *S. typhi* (11.576 mm), *E. coli* (14.256 mm), *B. subtilis* (13.964 mm), *S. aureus* (15.382 mm) respectively. The study demonstrated that active phyto-compounds (beta-sitosterol, octadecanoic acid,  $\gamma$ -sitosterol, and squalene from fruits like EP, BR, PA, and PN, respectively) have potential growth inhibitory activities against pathogenic strains, which suggests these fruits could be used as herbal remedies for microorganism-related disorders. When the ACHN human renal adenocarcinoma cell line was exposed to varying doses of four fruit extracts (EP, BR, PA, and PN), after 48 hours of treatment, the MTT test results offered an interesting concentration-dependent decrease in cell viability, which ranges between 45% to 49% at the highest dose (300  $\mu$ g/ml) of four (EP, BR, PA, and PN) fruit extracts. This MTT assay data was also sup-

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ported by *in silico* molecular docking analysis where selected phytochemicals (Malvidin, Rosmerinic acid, Ellagic acid, Quercetin for EP, BR, PA, and PN respectively) showed potential inhibitory activities against the MDM2 protein, which is a positive regulator for adenocarcinoma. Probably, this was the first report on the cytotoxic effects of these underutilized fruit extracts on the ACHN cell line.

An increasing dose ((5, 10, 20, 40 mg/L) of resveratrol (a potent polyphenol from EP) demonstrated protection against H<sub>2</sub>O<sub>2</sub>-induced oxidative injury and increased sperm viability by up-regulating several frontline antioxidant and non-antioxidant enzymes such as SOD (165-172%), GSH (18.5-91.43%) respectively, reducing ROS level [NOX (39-55%)] improving altered lipid metabolism and histological architectures (Sertoli cells and seminiferous tubules regenerates). *In silico* molecular docking study also supports this data where resveratrol

showed good inhibitory potential (-7.8kcal/mol) against stress protein NOX2. The different resveratrol treatments had better antioxidant activities than the standard antioxidant, ascorbic acid (AA). Therefore, this phytochemical might be a promising medicine against stress-mediated male infertility problems.

Fruit wines (fermented alcoholic beverages) are traditionally popular in the cool climatic zones of Northeast India. A study on fruit wines in Northeast India found high antioxidant activity, medicinal potential, and significant anti-cancer activity in breast cancer cell-line studies. Metagenomic analysis revealed diverse probiotic bacterial species in the wine sample's microbial population, playing an essential role in anti-cancer properties. Overall, the study suggests wine has therapeutic and health benefits.

The study also describes the green synthesis of silver nanoparticles (AgNPs) from silver nitrate utilizing

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EP fruit juice in an optimized process, which caused the solution's color to shift from yellow to brownish. UV-Vis spectroscopy, SEM, FESEM, EDX, XRD, and FTIR were used to characterize the AgNPs thoroughly to determine the existence and degree of bioactive compounds as a capping and reducing agent. Additionally, the antibacterial activity of AgNPs against human pathogenic microorganisms was shown to be substantial, and their antioxidant effectiveness was assessed. The anti-apoptotic protein Bcl-X<sub>L</sub> was employed in an *in-silico* molecular docking investigation to determine the efficacy of AgNPs as a powerful anti-cancerous agent. According to the results, nanoparticles are deemed harmless as they can alter how cancer cells typically initiate cell death by blocking their ABC transporters. A similar study was done using BR fruit juice, and surface morphology, size, and stability of biogenically synthesized AgNPs were deter-

mined by SEM, FESEM, HRTEM, EDX, XRD, and DLS. In this study, DPPH and other *in vitro* antioxidant assays explored the effect of these nanoparticles in treating oxidative stress-related disorders. The antimicrobial assay revealed that AgNPs have potent antibacterial activity against both above mentioned gram-positive and gram-negative bacteria. Two human breast cancer cell lines were used in an MTT test to determine the anticancer effects. Results indicated that AgNPs were cytotoxic to the cell lines in a dose-dependent manner, with IC<sub>50</sub> values of 110 mg/ml and 140 mg/ml, respectively, after 48 hours of treatment. We may conclude from this work that AgNPs are highly effective in preventing cancer. The study synthesized nanoparticles from *Phyllanthus acidus* fruit juice to investigate its clinical effects. The study compared the efficacy of raw fruit juice and nanoparticles on gentamicin-induced nephrotoxicity in a mouse model. The

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present study administered gentamicin (negative control) intraperitoneally to induce nephrotoxicity in the mice model. Several *in vivo* antioxidant and biochemical tests were performed to measure the efficacy of low and high doses of fruit juice and juice-synthesized nanoparticles in preventing renal injury. Results revealed that creatinine and urea levels significantly deteriorated by up to 1.13% and 1.49% in the high-dose *P. acidus* nanoparticles (PANH) group compared to the negative control group. After the application of PANH, major antioxidant (SOD, CAT) and non-antioxidant (GSH) enzymes were significantly enhanced compared to the gentamicin-treated group. Histopathological examination revealed that the glomerulus structure reappeared significantly in the PANH group. *In silico* molecular docking, analysis showed an inhibitory effect of fruit juice and fruit juice synthesized nanoparticles against inflammatory protein

NF $\kappa$ b. Findings indicated that PANH was more ameliorative over a high dose of *P. acidus* fruit juice (PAJH) towards kidney injury. Our findings generate scopes to explore the therapeutic potentials of PANH in treating kidney disorders.

Now a days arsenic, a toxic metalloid, poses a significant threat to human health and agriculture. In India, the highly nutritious *Vigna mungo* (L.) Hepper (Blackgram) pulse crop, Urad, is highly contaminated, leading to failure in germination and reduced crop yield. This contamination is particularly prevalent in West Bengal, Murshidabad, and Malda districts, where blackgram is cultivated alongside other cash crops. The build-up of arsenic in grains endangers agricultural productivity and food security because arsenic stress inhibits seed germination and plant growth. To reduce As-phytotoxicity in plants and seedlings before they reach the reproductive stage, this study investigated the via-

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bility of seed-priming black gram plants with biogenic zinc oxide nanoparticles (ZnONPs). ZnONPs under stress considerably increased the germination rate of blackgram seeds (20–23.33%) and other morpho-physiological characteristics. Adding ZnONPs reduced the accumulation of malondialdehyde (21–69%) and reactive oxygen species (ROS) (hydrogen peroxide) by 23–67%. ZnONPs also led to an increase in the activity of antioxidant enzymes (superoxide dismutase by 28–68%, catalase by 20–42%, guaiacol peroxidase by 19–49%, and ascorbate peroxidase by 26–39%) and the accumulation of osmoregulators. The findings suggest that ZnONPs significantly reduce As-toxicity by preventing its transfer from the root to the shoot. This work provides the first proof that ZnONP-based nano-priming effectively reduces As-stress in blackgram, indicating the possible application of this technique as a stress-relieving agent in As-

contaminated regions to boost yield and growth.

Another important legume in the food chain such as peas (*Pisum sativum* L.) are rich in nutrients. However, arsenic (As) in the agroecosystem provides a considerable bottleneck to its productivity because of its severe effects on ontogeny, particularly in Southeast Asia. This work proposes biogenic ZnONPs as a rhizospheric nano-remediation technique to avoid As-genotoxicity and increase crop production. In the negative control, germination rate, shoot length, and root length dropped by 62%, 16%, and 14.9%, respectively, above the normal control. As-toxicity caused fast oxidative bursts with worsening morpho-physiological properties. Negative control experienced membrane damage (82.75% increase) and electrolyte leakage (2.6-fold increase) as a result of ROS buildup (12.8 and 9-fold increase in leaves and roots), overworked antioxidant defense, and loss

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of cellular homeostasis. According to the study, root tip cells under stress significantly increase nuclear area, nuclear fragmentation, and micronuclei formation. These findings suggest severe genomic instability and increased programmed cell death (with a 3.3-fold increase in early apoptotic cells) due to leaky plasma membranes and unrepaired DNA damage. ZnONPs adsorb into the rhizosphere and reduce As-toxicity, they improve the antioxidant response and reduce As-uptake in peas. As-interference is reduced by increased vacuolar sequestration of As due to improved phytochelatin fabrication. Better photosynthetic activity (1.3–1.9-fold increased chlorophyll autofluorescence), increased pollen viability, and less genotoxicity (reduced tail DNA in comet assay) were observed. The flowering time was also better (7.74–19.36% reduction in flowering delay) with greater transcript abundance of GIGANTIA (GI), CONSTANS (CO),

and FLOWERING LOCUS T (FT) genes. Pod number and seed zinc concentration increased up to a maximum of 37.5% (1.67-fold), whereas seed arsenic content was reduced when treated with ZnONPs. In our experiment, the maximum ZnONPs dose (400 mg/L) caused NP-toxicity in pea plants; however, up to 300 mg/L, the best stress alleviation was seen. Although blackgram and pea are highly significant pulse crops, the use of nanoparticles in reducing heavy metals or metalloid stress in these crops has never been reported. Through this investigation, we hope to fully assess the viability of using ZnONPs to reduce stress caused by As.

Therefore the detailed study on underutilized fruits have shown their potentiality against several diseases like cancer, infertility, nephrotoxicity and improves human health in a drastic way.