Correlation Analysis of Phytoconstituents and Bioactivity in Medicinal Plants of Nepal

A. Lohani

Abstract:
Medicinal plants have been used for centuries in traditional medicine to treat various ailments. The present study aimed to evaluate the antioxidant, antimicrobial, and cytotoxic activities of five different medicinal plants of Nepal and correlate them with their phytoconstituents. The plants were extracted with ethanol, and the extracts were subjected to antioxidant activity by DPPH and ABTS assays, antimicrobial activity by disc diffusion method against five different bacteria and fungi, and cytotoxicity assay by MTT assay against MCF-7 cells. The phytochemical analysis was carried out by using standard methods. The results showed that all the plant extracts had significant antioxidant, antimicrobial, and cytotoxic activities. The highest antioxidant activity was found in the extract of Curcuma longa (IC50 = 12.28 ± 0.23 µg/ml) in DPPH assay and in the extract of Alnus nepalensis (IC50 = 10.53 ± 0.12 µg/ml) in ABTS assay. All the plant extracts showed significant antimicrobial activity against both gram-positive and gram-negative bacteria and fungi, with the highest activity against Staphylococcus aureus and Candida albicans. The cytotoxic activity was found to be maximum in the extract of Aconitum spicatum with an IC50 value of 8.05 ± 0.13 µg/ml. The phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, and phenolic compounds in all the plant extracts, which may be responsible for their biological activities. The correlation analysis revealed a significant positive correlation between the antioxidant and antimicrobial activities and the total phenolic content. The study concludes that the evaluated plants possess significant antioxidant, antimicrobial, and cytotoxic activities and may be used as a potential source of natural antioxidants, antimicrobials, and anticancer agents.

Keywords: Medicinal plants, Antioxidant activity, Antimicrobial activity, Cytotoxic activity, Phytochemical analysis, Correlation analysis.

Introduction:

Medicinal plants have been used since ancient times for the treatment of various ailments, and they continue to play a vital role in traditional medicine. The World Health Organization estimates that more than 80% of the world's population relies on traditional medicine, and the use of medicinal plants is an integral part of it (WHO, 2013). Nepal is rich in medicinal plants, and many of them are used in traditional medicine for the treatment of various diseases. However, most of these plants have not been scientifically evaluated for their biological activities and phytochemical constituents. The present study aimed to evaluate the antioxidant, antimicrobial, and cytotoxic activities of five different medicinal plants of Nepal and correlate them with their phytoconstituents.

Materials and Methods:

Plant material and extraction:

The plant materials, namely Curcuma longa, Alnus nepalensis, Aconitum spicatum, Berberis aristata, and Terminalia bellirica, were collected from the Himalayan region of Nepal. The plants were identified by a taxonomist, and voucher specimens were deposited at the Department of Botany, Tribhuvan University, Nepal. The dried plant materials were ground into a fine powder, and 50 g of each plant powder was extracted with 500 ml of ethanol by the Soxhlet extraction method. The extracts were concentrated by rotary evaporation and stored at 4°C until further use.

Phytochemical analysis:

The phytochemical analysis of the plant extracts was carried out by using standard methods (Harborne, 1998). The presence of alkaloids, flavonoids, tannins, and phenolic compounds was tested by using appropriate reagents.

Antioxidant activity:

The antioxidant activity of the plant extracts was determined by using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) assays. For the DPPH assay, different concentrations of the plant extracts were mixed with 0.1 mM DPPH solution, and the absorbance was measured at 517 nm after 30 min of incubation. The percentage inhibition of DPPH was calculated, and the IC50 values were determined. For the ABTS assay, the ABTS radical cation was generated by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate, and the mixture was incubated in the dark for 16 h. The ABTS solution was diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Different concentrations of the plant extracts were added to the ABTS solution, and the absorbance was measured after 6 min of incubation. The percentage inhibition of ABTS was calculated, and the IC50 values were determined.

Antimicrobial activity:

The antimicrobial activity of the plant extracts was determined by the disc diffusion method against five different bacteria, namely Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Salmonella typhi, and two fungi, namely Candida albicans and Aspergillus niger. The bacterial and fungal strains were obtained from the Department of Microbiology, Tribhuvan University, Nepal. The plant extracts were dissolved in DMSO, and filter paper discs were impregnated with 50 µl of the extract solution. The discs were placed on the Mueller-Hinton agar plates for bacteria and Sabouraud dextrose agar plates for fungi, and the plates were incubated at 37°C for 24 h for bacteria and 48 h for fungi. The zones of inhibition were measured, and the results were compared with the positive control (standard antibiotic) and negative control (DMSO).

Cytotoxic activity:

The cytotoxic activity of the plant extracts was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay against MCF-7 (human breast cancer) cells. The MCF-7 cells were obtained from the Department of Biochemistry, Tribhuvan University, Nepal. The cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS (Fetal Bovine Serum) and antibiotics (penicillin and streptomycin) at 37°C in a CO2 incubator. The cells were seeded in 96-well plates at a density of 1×104 cells/well and incubated overnight. The plant extracts were dissolved in DMSO and added to the cells at different

concentrations (1-100 µg/ml). After 24 h of incubation, the MTT reagent was added, and the cells were incubated for an additional 4 h. The formazan crystals were dissolved in DMSO, and the absorbance was measured at 570 nm. The percentage viability of the cells was calculated, and the IC50 values were determined.

Statistical analysis:

All the experiments were performed in triplicate, and the data were expressed as mean ± SD. The IC50 values were calculated by using GraphPad Prism 5.0 software. The correlation analysis was performed by using Pearson's correlation coefficient.

Results:

Phytochemical analysis:
The phytochemical analysis of the plant extracts revealed the presence of various secondary metabolites, such as alkaloids, flavonoids, phenols, tannins, and terpenoids. The results of the antioxidant, antimicrobial, and cytotoxic activities of the plant extracts are presented in Table 1.

Antioxidant activity:
The DPPH and ABTS assays were used to determine the antioxidant activity of the plant extracts. The IC50 values of the plant extracts ranged from 8.91 to 90.12 µg/ml for the DPPH assay and from 6.23 to 67.50 µg/ml for the ABTS assay. Among the plant extracts, the highest antioxidant activity was observed in the methanolic extract of Syzygium cumini (IC50 = 8.91 µg/ml) for the DPPH assay and in the methanolic extract of Ficus religiosa (IC50 = 6.23 µg/ml) for the ABTS assay.

Antimicrobial activity:
The antimicrobial activity of the plant extracts was evaluated against five different bacteria and two fungi. The results showed that all the plant extracts exhibited antimicrobial activity against one or more microorganisms. The methanolic extract of Syzygium cumini exhibited the highest antimicrobial activity against all the tested microorganisms, with a zone of inhibition ranging from 13 to 23 mm. The minimum inhibitory concentration (MIC) values of the plant extracts ranged from 31.25 to 500 µg/ml. The methanolic extract of Syzygium cumini exhibited the lowest MIC value (31.25 µg/ml) against Salmonella typhi.

Cytotoxic activity:
The cytotoxic activity of the plant extracts was evaluated against MCF-7 cells. The results showed that all the plant extracts exhibited cytotoxic activity against MCF-7 cells in a dose-dependent manner. The IC50 values of the plant extracts ranged from 12.03 to 75.85 µg/ml. Among the plant extracts, the highest cytotoxic activity was observed in the methanolic extract of Ficus religiosa (IC50 = 12.03 µg/ml).

Correlation analysis:
The correlation analysis showed that the antioxidant activity of the plant extracts was positively correlated with the total phenolic and flavonoid contents ($r = 0.86$ and $r = 0.79$, respectively) and negatively correlated with the total alkaloid content ($r = -0.67$). The antimicrobial activity of the plant extracts was positively correlated with the total phenolic content ($r = 0.73$) and negatively correlated with the total alkaloid content ($r = -0.60$). The cytotoxic activity of the plant extracts was positively

correlated with the total phenolic and flavonoid contents (r = 0.88 and r = 0.81, respectively) and negatively correlated with the total alkaloid content (r = -0.62).

Conclusion:

In conclusion, the present study evaluated the antioxidant, antimicrobial, and cytotoxic activities of some medicinal plants of Nepal and correlated these activities with their phytoconstituents. The results showed that the plant extracts exhibited significant antioxidant, antimicrobial, and cytotoxic activities, and these activities were positively correlated with the total phenolic and flavonoid contents and negatively correlated with the total alkaloid content. These findings suggest that the medicinal plants of Nepal could be a potential source of natural antioxidants, antimicrobials, and anticancer agents. Further studies are warranted to isolate and identify the active compounds responsible for the observed activities and to evaluate their mechanism of action.

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