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ORIGINAL RESEARCH ARTICLE

Phytochemical Screening, Antimicrobial Activity and Cytotoxicity of Nepalese Medicinal Plants *Swertia chirayita and Dendrobium amoenum*

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Abstract
Research on medicinal plants are important to Nepal because most of its rural population relies on it as mode of medicine. Medicinal plants namely *Swertia chirayita and Dendrobium amoenum* were collected from mid hills of Nepal. The present study was undertaken to find the antimicrobial activity, phytochemical presence and their cytotoxicity in different extraction medium. The percentage yield from the plants were highest in warm methanol extraction with 12.6%, followed by ethyl acetate and lowest was for cold methanol. Plant extract showed the presence of antioxidants like alkaloid, terpenoids, flavonoids, tannin, glycosides. The Brine Shrimp Bioassay of methanol and ethyl acetate extract showed cytotoxicity. Chiraito extract showed LC50 of 199 ppm for Dhunche sample, 128.82 ppm for Daman sample and 131.82 ppm of Illam sample. The antibacterial activity of methanol extract of Chiraito and *Dendrobium amoenum* showed significant bioactivity by inhibiting growth of microbial species selected for the test. The zone of inhibition shown by the extracts was comparable to the standard antibiotics. Similarly, methanol extract of Chiraito also showed significant antifungal activity with the zone of inhibition comparable to amphotericin.

Keywords: Antioxidant, LC50 (Lethal concentration-50), nauplii, ZOI (Zone of Inhibition)

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Introduction
For centuries, medicinal plants are being used as Ayurveda or traditional medicine in Nepal by local tribes and also in most of the rural areas of the Indian subcontinent. Medicinal plants and Ayurveda practice in the sub continent are intertwined. Different parts of plant are used in different herbal medicine as one of the constituents of final composition. Some phytochemicals present in these medicinal plants, have medicinal values and are expected to yield positive biological activities. Nepal, in the middle of the Himalayan belt which extends from Myanmar in the East to Karakorum in the West, possess vast diversity of plants because of its geographical distribution [1,2,3,4]. Some of these Medicinal plants are used in indigenous rural remedies, homoeopathic medicines, and allopathic pharmacopeia [5,6,7,8]. The exploitation of locally available medicinal plants in health care and economic advancement is a necessity of Nepal. Among all those plants *Swertia chirayita* and *Dendrobium amoenum* is the focus of this research.

Among 100 species of Swertia genus, 27 species are found in Nepal. Among them around nine species are reported to be traded for medicinal purpose. *Swertia chirayita* is biennial erect herb which is approximately 50 to 125 cm tall. The plant is a native of temperate Himalayas, found at an altitude of 1200–3000 m. Its stem is robust, branched and cylindrical below, four angled upward and containing large pith, broadly lanceolate leaves with 5-nerve and sub-sessile. It has lurid greenish yellow flowers tinged with purple in large panicles, with egg-shaped capsules and minute seed which are smooth and many angled [13]. Chiraito grows mainly in temperate Himalayas and is reported from 40 districts of Nepal. Chiraito grows between open forest and margin of cultivated land, it is predominant in Dolakha and spreads mainly in altitude of 1500 m to 3000 m of Eastern and Central region of Nepal [2,9].

Dendrobium contains about 1,200 species and is also known as orchid [10]. This genus also occurs in diverse habitats throughout much of South and East Asia, stretching to Oceania and some of Pacific islands. This plant is commonly distributed between 660 m to 2000 m temperate forest of Himalayan region and grow in little light exposure [11,12]. The orchids are mostly found in terrestrial, epiphytic and

saprophytic habitat. *Dendrobium amoenum* grows in clustered pendulous five to six slender stems. Morphologically, this species is highly evolved, tall, straight with elongated pseudobulbs covered by modest sized leaves. It has unique floral pattern which is fairly constant varying in size from very small to large, the shape and form of the stems and leaves are divergent. This traditional medicinal species is also ornamental plant and have been cultivated for decorative purpose. The flowers flourish on an older stem in cluster of two to three per node and the flower is amusingly perfumed [13,14,15].

Materials and Methods

Sample preparation

Swertia chirayita plants were collected from or near from Dhunche, Daman and Illam and Dendrobium amoenum from Pokhara, during their flowering season. The plants were air dried under room temperature. The dried plant samples were cut and grinded to make it in powder form and kept for storage at room temperature.

Extraction

Three types of extraction method were carried out, viz. warm methanol extraction, cold methanol extraction and ethyl acetate extraction.

For warm extraction, Soxhlet apparatus was used. 10 g of the crushed sample along with 200 ml of methanol was put into the soxhlet apparatus. The soxhlet was run for 28 hours at 65^{0}C. The methanol extract was taken out from the soxhlet apparatus. The pigment was removed using Hexane in the separating funnel. The methanol fraction was then dried using water bath.

For cold extraction methanol at room temperature was used. 100 g of powdered form of sample was taken and 450 ml methanol, that was just enough to cover the upper layer of sample, was poured on it and was shaken regularly. After 48 hrs in room temperature, filtration was done and the filtrate was stored at room temperature.

For ethyl acetate extraction, 10 g of powdered material was dissolved in 25 ml ammonium hydroxide. 300 ml of ethyl acetate was then added and left for 72 hrs at room temperature. Extract was then filtered. Ethyl acetate was then left to dry in water bath [16,17,18].

Phytochemical Screening was done to check the presence of alkaloid, sterols, triterpenes, tannins and polyphenols, reducing sugar, saponins, flavonoids, glycosides and coumarin according to protocol described in [19,20,21].

Brine Shrimp Bioassay

Preparation of Test sample

Stock solution was prepared by dissolving 100 mg of the extract in little amount of dimethyl sulfoxide (DMSO) for initial solublization and then addition of water to final volume of 25 ml to make a stock of concentration of 4000 ppm. The stock solution was further diluted to 1000, 100, 10 ppm concentration.

For Hatching of Brine shrimp, 50 mg of Brine shrimp eggs were sprinkled in a beaker with 300 ml of sea water. The transferred sample as then allowed incubating at 32 – 35°C for 24 hrs.

Bioassay

Cleaned test tubes were divided into four groups each group consisting of five test tubes. After 24 hrs of incubation, the nauplii were recovered with a pipette and 10 nauplii were transferred in each test tube. The groups were then treated with different dilutions of sample. The test tubes were then incubated at 32– 35°C overnight. The incubated tubes were observed for the number of survived nauplii and graph was plotted for death percentage versus Log of concentration of the extract. This gives linear equation in the form of $y = mx + c$.

Calculation of LC50

The death of nauplii was calculated as death percentage as,

% death = deaths/initial x 100. The % death was corrected for any control deaths by subtracting the -%death control from % death test. The Lethal concentration 50 (LD50) was derived from the graph from the equation of the straight line [19].

Antimicrobial Screening

Micro-organisms used Six clinical samples of bacteria were collected from Kathmandu University Teaching Hospital (KUTH), Dhulikhel namely *Staphylococcus aureus, Escherichia coli, Kleibsella pneumonia, Salmonella paratyphi, Salmonella typhi, Pseudomonas aerugenosa*.

The Yeast *Saccharomyces cerevisiae* (YCS2) were taken from microbiology lab of Department of Biotechnology, Kathmandu University.

Preparation of Sample

The different extraction of plant was dissolved in DMSO and water, which was made into different concentrations.

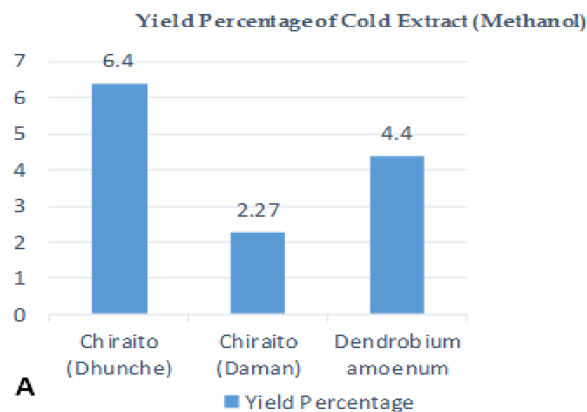
Inoculation

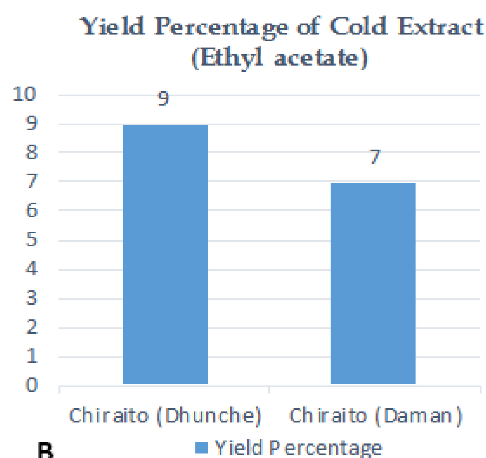
Sterilized petri dishes were filled to a uniform depth, with respective sterilized medium (Nutrient agar for bacteria and Muller Hinton agar for fungus respectively). The solidified media was then inoculated with respective organisms. Holes were cut into the medium using a sterile borer. Solution of various concentrations of the extracts under test was poured into the bored holes with the help of a micropipette and the standard antibiotic discs or solutions were gently placed at the different sites of the petri-dish containing the inoculated medium in the case of cup plate method. In case of filter disc method, filter paper discs prepared from Watman's filter paper were soaked with the test solution and the filter discs are then gently placed into the inoculated medium with the help of a sterile forceps. The plates were then maintained at room temperature for few minutes to allow the antibiotic and the extract solution to diffuse into the medium. In the uniform media, diffusion of the extract solution and the antibiotic discs will occur uniformly around the cup and the concentration gradient will be established around it and at a certain distance from the cup. The plates were then incubated at a suitable temperature (usually 37-39^{0}C) for 24-48 hours. After 48 hours, the zones of inhibition were measured with the help of a measuring ruler or antibiotic zone reader [22].

Result

The percentage yield for a given plant is calculated as:

$$\%\ yield = \frac{\text{weight of extract obtained}}{\text{Total weight of sample used for extraction}} \times 100\%$$





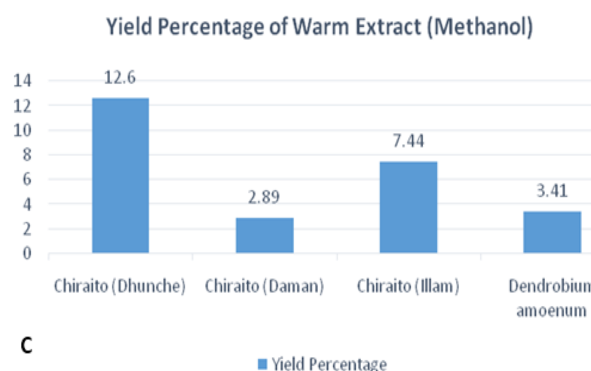


Figure 1: Yield percentage of different plant from various solvents. [A] representsyield percentage from cold extract (Methanol), [B] represents yield percentage from cold extract (Ethyl Acetate) and [C] represents yield percentage from warm extract (Methanol).

The various phytochemicals tested for Chiraito and *Dendrobium amoenum* extracts with their corresponding results can be tabulated in **Table 1**

Table 1: Screening of phytochemicals presence (represented by + for presence and by – for absence)

Phytochemicals	Methanol warm Chiraito (Dhunche)	Methanol cold Chiraito (Dhunche)	Methanol cold Chiraito (Daman)	Methanol cold Dendrobium amoenum
Alkaloids	+	+	+	–
Terpenoids	+	+	+	+
Coumarin	+	+	+	+
Tannin and polyphenols	+	+	+	+
Reducing sugar	+	+	+	+
Saponin	+	–	–	–
Glycosides	+	+	+	+
Flavonoids	+	+	+	–
Sterols	–	+	+	+

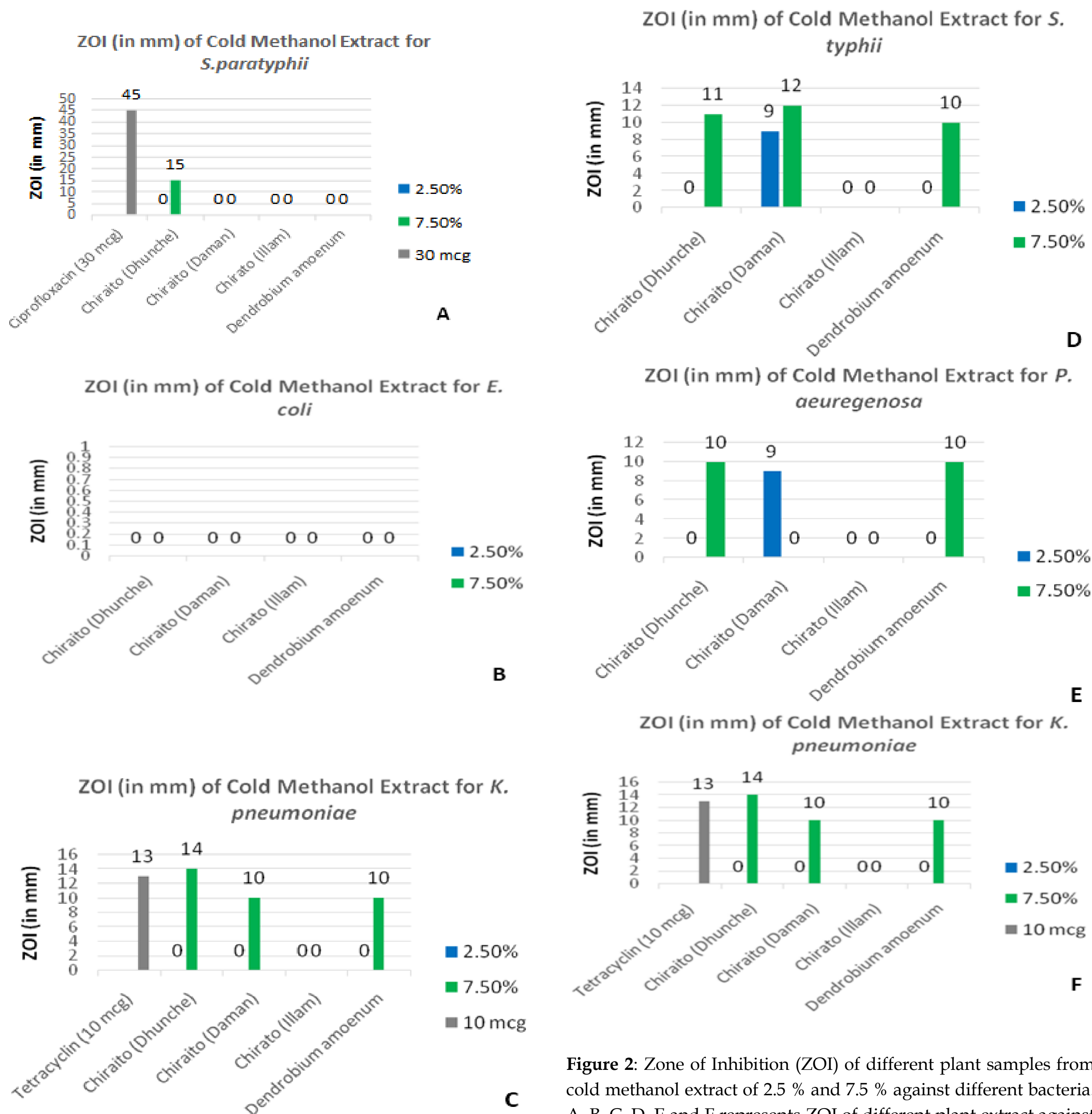


Figure 2: Zone of Inhibition (ZOI) of different plant samples from cold methanol extract of 2.5 % and 7.5 % against different bacteria. A, B, C, D, E and F represents ZOI of different plant extract against *S. paratyphi, E. coli, K. pneumoniae, S. typhi, P. aerugenosa* and *S. aureus* respectively.

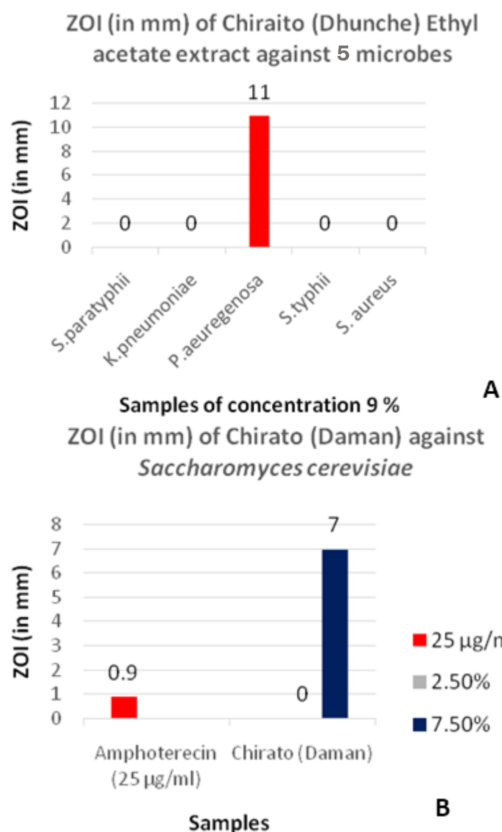


Figure 3: Zone of Inhibition (ZOI) of different Chiraito samples. A represents ZOI of Chiraito (Dhunche) prepared from ethyl acetate against 5 microbes and B represents ZOI of Chiraito (Daman) against *Saccharomyces cerevisiae*.

ZOI (in mm) of plant extract against following fungus and comparison with fungicide solution **Figure 3**

Brine Shrimp Bioassay

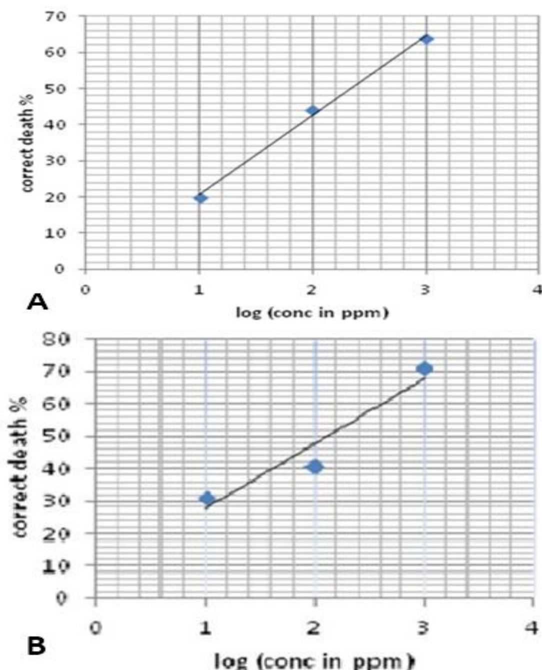


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Figure 4: Brine Shrimp assay graph for Chirato sample of different places Graph A= Dunche, Graph B= Illam and Graph C=Daman

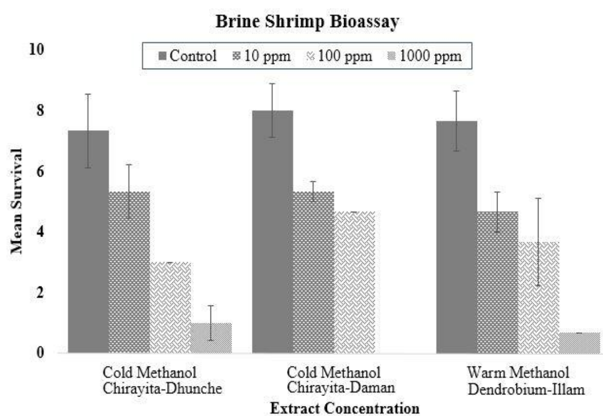

Figure 5: Brine Shrimp Assay for different Chirato samples in different concentration

Calculation:

Graph was plotted as death percentage versus Log of extract concentration in ppm. This shows the linear equation in the form of y=mx+c. By substituting the value of y=50, the corresponding value of x gives the Log value of LC50 value and antilog of that value gives the LC50 value in ppm.

From Fig 4, Graph 1, equation: y=22x -1.33

For LC50, Y=50, then X=2.3, Antilog of X is Antilog(2.3) = 199ppm

Therefore, LC50 of Chirato (Dhunche) methanol extract for brine shrimp is 199ppm.

From Fig 4, Graph 2, From equation: Y= 26.5x-6

For LC50, Y=50, then X=2.11, Antilog of X is Antilog(2.11) = 128.82ppm

Therefore, $LC50_0$ of Chirato (Daman) methanol extract for brine shrimp is 128.82 ppm.

From Fig 4, Graph 3, From equation: Y= 20x + 7.6

For LC50, Y=50, then X=2.12, Antilog of X is Antilog (2.12) = 131.82ppm

Therefore, LC50 of Chiraito (Illam) methanol extract for brine shrimp is 131.82ppm.

Discussion

The % yield from the Chiraito extract was highest in warm methanol extraction with 12.6% as compared to cold methanol extraction and that for ethyl acetate was 9% as shown in Figure 1. However in the case of *Dendrobium amoneum* the % yield was higher for cold extraction at 4.4 % as compared to warm extraction at 3.4 %.This indicates that there are many compounds present in the plants soluble in methanol and ethyl acetate and that there is higher amount of bioactive component in Chiraito compared to *Dendrobium amoneum*. Generally all alkaloids are highly soluble in methanol. High extract yield for methanol also suggests that there are high amount of alkaloids in the plant.

The Phytochemical screening of the plant extract showed the presence of alkaloid, terpenoids, tannins, coumarins, flavanoids and sterols, as shown in **Table 1**, indicating that there are high value natural compounds in the plants. Since there are presence of alkaloids, terpenoids, coumarins, flavanoids and sterols these plants could have anti cancer activity.

The antibacterial activity of methanol and ethyl acetate extract of Chiraito and *Dendrobium amoenum* showed bioactivity by inhibiting growth of microbial species selected for the test as shown in Figure 2 and 3. The zone of inhibition shown by the extracts was comparable to the standard antibiotics. Cold methanol extract of Chiraito didn't show any activity against *E Coli*. Cold methanol extract of Chiraito from Dhunche had activity higher than that of the control antibiotic tetracycline. Bioactivity of ethyl acetate extract was not significant as that of methanol extract and showed activity against only *Pseudomonas auregenosa*. This may be due to absence of alkaloids.

Cold methanol extract of Chiraito (Daman) showed significant activity against *Saccharomyces cerevisiae*. The zone of inhibition was significantly higher than that of control, amphotericin at 25µg/ml.

The Brine Shrimp Bioassay of methanol extract showed cytotoxic nature of plant extracts. If LC50 value of test sample is less than 1000 ppm the extract is considered to be biologically active. Chirato extract showed LC50 of 199 ppm for Dhunche sample, 128.82 ppm for Daman sample and 131.82 ppm for Illam sample. Since cytotoxicity value given by Brine Shrimp assay directly correlates with its cytotoxicity ability, toxicity at low concentration of Chiraito extracts can have toxicity against cancer cell lines and has the potential to be developed as drugs for cancer.

Conclusion:

Researchers have identified compounds used in mainstream medicine derived from plant resources. Similarly, this research has assessed the phytochemical, biological screening of Chiraito and *Dendrobium amoenum.* This natural product can bring new and effective antimicrobial agents and serve as alternate source of combating infections in human beings. Hence this research can have a promising potential in various traditional, complementary and alternate systems of treatment of human diseases.

Further work on isolation and characterization of these plants and their pharmacodynamics study can be a great contribution. The research also proved to be beneficial in exploiting medicinal plants having biological activities namely cancer.

References

1. Radcliffe-Smith A, Hara H, Steam WT, Williams LHJ: **An Enumeration Of The Flowering Plants Of Nepal**. *Kew Bulletin* 1979, **34(1)**:198.
2. Joshi K: **Swertia L. (Gentianaceae) In Nepal: Ethnobotany and Agenda For Sustainable Management**. *Ethnobotanical Leaflets* 2008, **12**:1-6.
3. Cseke JL, Kirakosyan A, Kaufman PB, Warber SL, Duke JA, Brielmann HL: ***Natural Products From Plants***. In 2nd edition. CRC Press Taylor and Francis Group, 2006.
4. Siddiqui S, Verma A, Rather AA, Jabeen F, Meghvansi MK: **Preliminary Phytochemicals Analysis of Some Important Medicinal And Aromatic Plants**. *Advances in Biological Research* 2009, **3**(5-6):188-195.
5. Joshi B, Lekhak S, Sharma A: **Antibacterial Property of Different Medicinal Plants: Ocimum Sanctum, Cinnamomum Zelanicum, Xanthoxylum Armatum and Origanum Majorana**. *Kathmandu University Journal of Science, Engineering and Technology* 2009, **5**:1-6.
6. Bhattarai KR, Ghimire M: **Commercially Important Medicinal and Aromatic Plants of Nepal and Their Distribution Pattern and Conservation Measure Along the Elevation Gradient of the Himalayas**. *Banko Jankari* 2007, **16**(1)**:**1-6.
7. Okamura T, Ayajiki K, Fujioka H, Toda M, Fujimiya M,

Toda N: **Effects of Endothelial Impairment by Saponin On the Responses to Vasodilators and Nitrergic Nerve Stimulation in Isolated Canine Corpus Cavernosum**. *British Journal of Pharmacology* 1999, **127**(3):802-808.

8. Sharma M, Chapagain N, Sah S: **Phytochemicals as Emerging Antimicrobials**. *Plant Resources (A Scientific Publication)* 2010, **32**:96-101.
9. Joshi P, Dhawan V: **Swertia chirayita – an overview**, *Current Science*, 2005, **89**(04)**:**635-640
10. Venkateswarlu S, Mudunuri SSR, Gottumukkala VS: **Synthesis and Biological Activity of Isoamoenylin, A Metabolite of Dendrobium Amoenum**. *Bioscience, Biotechnology and Biochemistry* 2002, **66**(10):2236-2238.
11. Rajkarnikar K; **In Vitro Propagation of Dendrobium Amoenum Wall. Ex Lindi. From Seed Culture**. *Plant Resources (A Scientific Publication)* 2010, **32**:90-92.
12. Shrestha P: **Antibacterial Activity of Medicinal Plant Extracts**. *Plant Resources (A Scientific Publication)* 2010, **32**:51-54.
13. Martha R, Gutierrez P: **Orchids: A Review of Uses in Traditional Medicine, Its Phytochemistry and Pharmacology**. *Journal of Medicinal Plants Research* 2015, **4**(8)**:**592-638.
14. Zhang CF, Wang M, Wang L, Iinuma M, Zhang M, Xu LS, Wang ZT: **Chemical Constituents of Dendrobium Gratiosissimum and Their Cytotoxic Activities**. *Indian Journal of Chemistry* 2008, **47**B:952-956.
15. Buckingham J: ***Dictionary of Natural Products.*** *CD-ROM 13:1*. Boca Raton, Florida: Chapman & Hall/CRC Press, 2005.
16. Bhandari J, Naqvi A, Niraula P, Thapa P, Thapa N, Shrestha N and Shrestha BG: **Phytochemical Screening, Antioxidant Assay of *Junipers recurva* and study of its Invitro cytotoxicity against Breast Cancer Cell Lines**. *International Journal of Pharma and Bio Sciences.* 2015, **6**(3B)**:**1134-1145.
17. Lamichhane B, Adhikari S, Shrestha P, Shrestha BG: **Study of Phytochemical, Antioxidant, Antimicrobial and Anticancer Activity of *Berberis Aristata***. *Journal of Tropical Life Science*. 2014, **4**(1): 1-7.
18. Shrestha P, Adhikari S, Lamichhane B, Shrestha BG: **Phytochemical Screening of the Medicinal Plants of Nepal**. *IOSR Journal of Environmental Science, Toxicology and Food Technology*. Special Issue 2015, **1**(6):11-17.
19. Chhetri HP, Yogol NS, Sherchan J, Anupa KC, Mansoor S, Thapa P**: Phytochemical and Antimicrobial Evaluations of Some Medicinal Plants of Nepal.** *Kathmandu University Journal of Science engineering and technology*. 2008, **1**:49-54.
20. Setzer WN, Flair MN, Byler KG, Huang J, Thompson MA, Setzer AF, Moriarity DM, Lawton RO and Windham-Carswell.: **Antimicrobial and cytotoxic activity of crude extracts of Araliaceae from Monteverde. Costa Rica.** *Brenesia* 1992, **38**:123-130.
21. Zabri H, Kodjo C, Anoubilé B, Békro M, Bekro Y A: **Phytochemical Screening and Determination of Flavonoids in Secamone Afzelii (Asclepiadaceae) Extracts**. *African Journal of Pure and Applied Chemistry* 2008, **2**:80-82.
22. Reuben KD, Abdulrahman FI, Akan JC and Egwu GO: **Phytochemical Screening and in Vitro Antimicrobial Investigation of the Methanolic Extract of Croton Zambesicus Muell ARG. Stem Bark**. *European Journal of Scientific Research* 2008, **23**:134-140.