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ORIGINAL RESEARCH ARTICLE

Comparative Study of Antibacterial and Cytotoxic Activity of Two Nepalese Medicinal Plants- *Allium wallichii* Kunth and *Allium sativum* L.

Kishor Acharya[#], Harihar Pokhrel[#], Laxman Maharjan[#], Ravi Bhattarai[#], Pravin Karki[#], Tirtha Maiya Shrestha and Rajendra Gyawali*

Kathmandu University, Department of Pharmacy, Dhulikhel, Kathmandu, Nepal

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ABSTRACT

Antimicrobial and cytotoxic activity of methanolic extract of two Nepalese species *Allium wallichii* Kunth and *Allium sativum* L has been evaluated. Phytochemical screening revealed the presence of terpenoids, flavanoids and reducing sugars as main phytochemical groups. The extracts screened for their antimicrobial activity on eight different strains of human pathogenic microorganisms; *Escherichia coli, Salmonella typhi, Salmonella paratyphi, Pseudomonas species, Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecalis* and *Klebshiella oxytoca* has shown selected antimicrobial effects. LC_{50} values were calculated by using brime shrimp lethality test. The LC_{50} value for *Allium wallichii* Kunth was 64.714 ppm while for *Allium sativum* L it was found to be 172.48 ppm.

Key words: *Allium wallichii, Allium sativum*, Nepal, antimicrobial and cytotoxic activity, phytochemicals

INTRODUCTION

Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants, particularly to control resistant bacterial diseases by this type of drug. Numerous investigations have been performed aimed at identifying the chemical composition of potential plants of various traditional systems [1]. Different kind of studies on the mechanisms of action, interaction with antibiotics or other medicinal plants or compounds and the pharmacokinetic profile of the extracts has been studied extensively and stimulated the use of natural products throughout the world. Because of the innumerable biologically active compounds that are found in plants possess antibacterial properties; now days numerous investigations are going on in isolation of potent compounds for antimicrobial therapy [2]. Ethnobotanical plants have a greater number of positive results than randomly selected plants.

High altitude medicinal plants provide quality products, and this is the reason why they are often the first choice of local users as immediate therapy and by pharmaceutical companies as precious ingredients. In the other hand, the Himalayan region shows the highest richness for endemic species and some of the plants found in the Himalayas can not be found elsewhere. Therefore the number of people and institutions seeking information on Himalayan medicinal plant is increasing very rapidly. But only limited studies has been carried out on antimicrobial properties of Nepalese medicinal plants [3-11]. In continuation of our efforts to verify the efficacy of traditional medicine we have collected several medicinal plants from various geographical locations of Nepal based on the ethnopharmacological information [12-14]. Thus all these conditions were taken in account in order to conduct this research aimed to asses the phytochemical and biological properties of Nepalese plants *Allium wallichii* Kunth and *Allium sativum* L.

MATERIALS AND METHODS

Plant materials

Entire plants of *Allium wallichii* Kunth were collected in June 2008, at the Phulchoki Mountain about 2200 m altitude, and *Allium sativum* L was collected from same site of cultivated land of Kathmandu, Nepal. Samples were identified by Mrs Tirtha Maiya Shrestha, a taxonomist of Nepal and voucher specimen was deposited in the department of pharmacy, Kathmandu University, Nepal.

***Corresponding Author:** Dr. Rajendra Gyawali, **Email:** ragyawali@gmail.com, gyawali@ku.edu.np

Preparation of extracts

The air dried and powdered plants (120g) were extracted in room temperature with methanol 80%. The solution was filtered using Whatman N° 1 filter paper under suction and concentrated to dryness at 50 °C under reduced pressure (yield was 8.79 % and 12.89 % for *Allium wallichii* Kunth and *Allium sativum* L respectively). The extracts from these processes were evaluated for phytochemical test, antimicrobial property cup plate method, lethality to brine shrimp larvae (*Artemia salina* Leach).

Phytochemical Test

The methanolic extract of whole plant of *Allium wallichii* Kunth and *Allium sativum* L was screened for the presence of alkaloids, glycosides, tannins, saponins and flavonoids according to standard procedure [15].

Antimicrobial assays

In this study, strains of human pathogenic microorganisms, *Salmonella typhi, Salmonella paratyphi, Pseudomonas sps, Streptococcus aureus, Klebshiella oxytoca , Enterococcus faecalis, Klebshiella pneumonia* and *Escherichia coli*, collected from Dhulikhel hospital, Kathmandu University teaching hospital, were used to investigate the antimicrobial potential of the extracts by cup plate method. Stock solutions of the samples were prepared in 1 % aqueous DMSO and then in RO water. The solution was then diluted to give final concentrations ranging from 2, 4 and 6 % w/v. Ciprofloxacin 2 % (w/v), 4 % (w/v) and amoxicillin 2 % (w/v), 4 % (w/v) were used as positive controls.

The microorganism cultures were grown in the appropriate media after susceptibility test in both Muller Hinton agar and MaConcy Agar. The microorganisms were kept under refrigeration (4 °C) until use. Each microorganism, at a concentration of 1.5×10^6 cells/mL (adjusted to the 0.5 McFarland turbidity standards) was inoculated on the surface of respective media. Diluted extracts were impregnated in 6 mm diameter cup plates, in triplicate of each. After holding the plates at room temperature for 1 h to allow diffusion of test samples into the agar, they were incubated at 37 ^oC for 24 hr. After that, the results were recorded by measuring the zones of growth inhibition around the cup-plates, and presented as the arithmetic average. Overall, cultured microorganisms with halos equal to or greater than 7 mm were considered susceptible to samples tested.

Cytotoxicity assay

Methanolic extracts of *Allium wallichii* Kunth and *Allium sativum* L samples were evaluated for lethality to brine shrimp larvae (*A. salina* Leach) according to the procedures described by Meyer et al. 1982 [16]. Brine shrimp eggs were hatched for 48 h in a conical flask containing 300 mL of artificial seawater. The flasks were well aerated with the aid of an air pump and kept in a water bath at 29–30 °C. The extracts were dissolved in 1 % aqueous DMSO and then in sea water to obtain a concentration of 1,000 ppm, 750 ppm, 500 ppm, 250 ppm, 100 ppm and 10 ppm. An aliquot of each concentration (1 mL) was transferred, in triplicate, into clean sterile vials with pipette, and aerated seawater (9 mL) was added. Ten shrimp nauplii were transferred to each vial. Thymol 1% aqueous solution and 1% DMSO in seawater were used as positive and negative controls, respectively. After 24 h the numbers of survivors were counted and percentage of death calculated. The concentration that killed 50 % of the nauplii (LC_{50} in µg/mL) was determined. Criterion of toxicity for fractions was established according to Déciga-campos et al. 2007: LC_{50} values > 1000 µg/mL (non-toxic), ≥ 500 ≤ 1000 µg/mL (weak toxicity) and < 500 µg/mL (toxic) [18].

RESULTS AND DISCUSSION

The phytochemical screening of the methanolic extract of *Allium wallichii* Kunth and *Allium sativum* L revealed the presence of terpenoids, flavanoids and reducing sugars as main chemical groups.

The experiment to determine the antimicrobial property was carried out by using 2 %, 4 %, 6 % of the plant by using cup diffusion method. The zone of inhibition (ZOI) was observed in the culture of *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Salmonella paratyphi, Pseudomonas species, Enterococcus faecalis, Klebshiella pneumonia* and *Klebshiella oxytoca*. In this study, the extracts showed significant inhibition against microorganisms. In general, medium inhibition zones ranged from 7.0 to 13.25 mm for different organisms. The ZOI produced by extract are compared with the ZOI produced by antibiotic solution and the results can be easily evidenced from **Table 1**.

The antimicrobial screening of methanolic extract of the both the plant showed encouraging results by inhibiting almost all microbial species selected for the test but inhibitory effect was remarkable with *E. coli*. The zone of inhibition shown by the plant extracts were so prominent that they were

comparable to the standard antibiotics ciprofloxacin and amoxicillin. In the case of *E. coli*, it has been observed that both the plants possess bactericidal activity much higher than that of synthetic antibiotics when tested in vitro. Both of these plants may be effective on other Gram-ve and Gram+ve bacteria. More importantly, these can be included in the list of herbal medicines due to their high antimicrobial potential and lesser side effects. Based upon above findings, this can be recommended for therapeutic purposes and be used as an alternative medicine. However, the components of *Allium wallichii* Kunth should be immediately investigated for the isolation of active constituent as a potential antimicrobial drug. Terpenoids are generally recognized as safe and have been found to inhibit the growth of microorganisms [19]. As the extracts were rich in terpenoids, the additive and synergistic effects of phytochemicals in extract are responsible for their potent antimicrobial action against pathogenic microorganisms.

Graph was plotted as death percentage versus log of extract concentration in ppm. LC_{50} of *Allium wallichii* methanol extract and *Allium sativum* L methanol extract for *Aetemia salina* is 64.7 ppm and 172.48 ppm respectively and results can be easily evidenced from **Table 2**. The result shows, extract of *Allium wallichii* is more cytoxic, thus it could be taken for further study of antiproliferative activity on cancer cell lines. Several studies have shown that brine shrimp assay has been an excellent method for preliminary investigations of toxicity, to screen medicinal plants popularly used for several purposes and for monitoring the isolation a great variety of biologically active compounds [20]. From the present finding it has also been shown that the plants rich in flavanoids and terpenoids may possesses the cytotoxic property during *in vivo* test.

Table 1: Zone of inhibition (mm) exhibited by plant extracts and antibiotics against human pathogenic bacteria

Treatment	Concentration	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella typhi*	*Salmonella paratyphi*	*Pseudomonas species*	*Enterococcus faecalis*	*Klebshiella pneumonia*	*Klebshiella oxytoca*
Allium wallichii Kunth	2%	8.25	10	10	10.5	9.5	12.25	8.75	7.5
	4%	9	9.5	10	11.75	8.5	9.5	8.25	8.5
	6%	10	10.5	9.5	9.75	12.5	10.5	10	9.25
Allium sativum L	2%	9.25	8	10.5	9.5	7	8	9.25	8.5
	4%	9	11.75	10.5	10.75	8.5	11.75	8.5	10
	6%	9.25	13.25	11.25	11.5	9	13.25	9.75	9.25
Cifrofloxacin	2%	7	10	7	6.5	11	9	7	7
	4%	7.5	20	8	7	14	9	7.5	7.5
Amoxicillin	2%	9	8	7	6	11	8	8	8
	4%	7	10	7.5	7	12	8.5	8.5	9

Table 2: Brine shrimp bioassay for extract of *Allium wallichii* Kunth and *Allium sativum* L

Plant extract	Concn(ppm) (A)	Log (A)	Number of napulii	NaIpIulii survival (n=1)	Napulii survival (n=2)	Napulii survival (n=3)	Mean survival	Death %	LC 50 (ppm)
Allium wallichii Kunth	10	1.00	10	7.50	8.50	9.00	8.33	16.70	64.7
	100	2.00	10	4.00	5.50	6.00	5.16	48.33	
	250	2.39	10	0	0.50	5.50	2.00	8.00	
	500	2.69	10	1.00	0	0	0.33	9.66	
	750	2.87	10	0	0.50	0.50	0.33	9.66	
	1000	3.00	10	0	0	0	0	100	
	Control		10	10	10	10	10	0	
Allium sativum L	10	1.00	10	8.50	9.00	10	9.16	8.33	172.48
	100	2.00	10	6.50	9.50	10	8.67	13.33	
	250	2.39	10	6.00	6.00	8.00	6.67	33.30	
	500	2.69	10	0.50	2.00	2.50	1.67	83.30	
	750	2.87	10	2.00	0.50	0.50	1.00	9.00	
	1000	3.00	10	0.50	0	000	0.16	98.33	
	Control		10	10	10	10.0	10	0	

Fig 1: Mortality response of brine shrimp to 0.5 – 3.5 ppm after 48 h of exposure of *Allium wallichi* Kunth methanolic extract

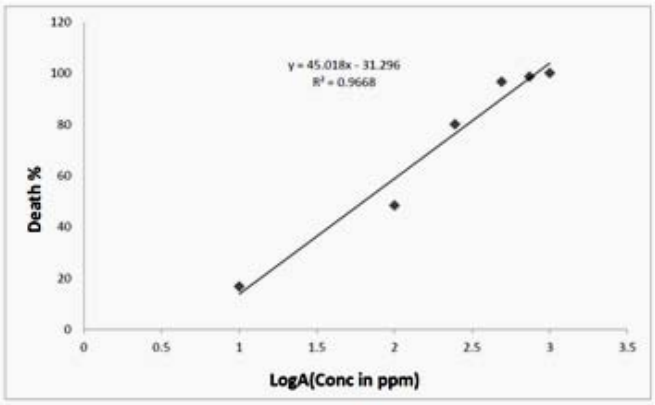
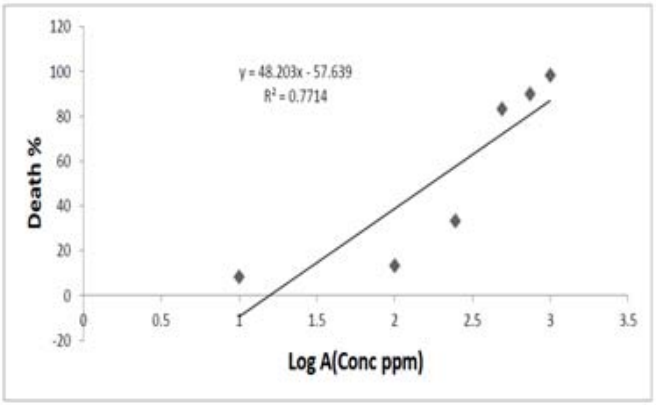


Fig 2: Mortality response of brine shrimp to 0.5 – 3.5 ppm after 48 h of exposure of Allium sativum L methanolic extract.



CONCLUSION

Nepalese species *Allium wallichii* Kunth and *Allium sativum* L are rich source of terpenoids and flavanoids. Methanolic extract from both plants showed broad spectrum of activity against human pathogenic microorganisms and cytotoxic property. The LC_{50} value for *Allium wallichii* is 64.714 ppm while for *Allium sativum* L it was found to be 172.48 ppm. However, in order to evaluate possible clinical application in therapy of infectious diseases, further studies about the safety and toxicity of the isolated compounds are needed.

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REFRENCES

1. Heinrich M. Ethnobotany and its role in drug development. Phytother. Res. 2000; 14: 478–488.
2. Clark AM and Hufford CD. Discco and development of novel prototype antibiotics for opportunistic infections related to the acquired immunodeficiency syndrome. In: Human Medical Agents from Plants. 1993; 534: 228-241.
4. Taylor RSL, Edel F, Manandhar NP and Towers GHN. Antimicrobial activities of southern Nepalese medicinal plants. Journal of Ethnopharmacology. 1996; 50: 97-102.
5. Taylor RSL, Manandhar NP, Hudson JB, Towers GHN. Antiviral activities of Nepalese medicinal plants. Journal of Ethnopharmacology 1996; 52: 157–163.
6. Taylor RSL, Hudson JB, Mananghar NP, Towers GHN. Antiviral activities of medicinal plants of southern Nepal. Journal of Ethnopharmacology 1996; 53: 97–104.
7. Taylor RSL, Manandhar NP and Towers GHN. Screening of selected medicinal plants of Nepal for antimicrobial activities. Journal of Ethnopharmacology 1995; 46:153-159.
8. Rajbhandari M, Wegner U, Jülich M, Schöpke T, Mentel R.. Screening of Nepalese medicinal plants for antiviral activity. Journal of Ethnopharmacology 2001; 74: 251–255.
9. Taylor RSL, Towers GHN. Antibacterial constituents of Nepalese medicinal herb Centipedia minima. Phytochemistry 1998; 47(4): 631-634.
10. Parajuli, S, Chaudhary RP, Taylor RSL. Antibacterial activity of medicinal plants used to treat skin ailments in Kaski district, Nepal. In: P.K. Jha, S.R. Baral, S.B. Karmacharya, H.D. Lekhak, P. Lacoul and C.B. Baniya (Eds.). Environment and Agriculture: Biodiversity, Agriculture and Pollution in South Asia. Ecological Society, Kathmandu, Nepal, pp. 2001; 230-237.
11. Shrestha MP, Thapa A and Agrawal VP. Screening of the medicinal properties of some plants of Nepal. Biotech. Lett. 1999; 1: 33-36.
12. Bhattarai S and Bhuju DR. Antimicrobial Activity of Useful Parts of Woodfordia fruticosa (Linn.) Kurz. of Nepal . International Journal of Pharmaceutical & Biological Archives 2011; 2(2):756-761
13. Gyawali R and Kim KS. Volatile organic compounds of medicinal values from Nepalese *Acorus calamus* L. Kathmandu University of Journal of Science, Engineering and Technology 2009; 5(II):

51- 65.

14. Gyawali R, Jnawali D and Kim KS. Phytochemical screening of some species of Nepalese medicinal plants. Medicinal Plants in Nepal: An anthology of contemporary research. 2008; 43-49.
15. Gyawali R, Shrestha R, Tuladhar L, Shakya R, Shah S and Shrestha TM. Phytochemical studies and In vitro activity of *Wikstroemia canescens* Meisner, Journal of Tropical Medicinal Plants 2010; 11(2):205-206
16. Evans WC. Trease and Evans Pharmacognosy Elsevier publication,15th edition, 2005.
17. Meyer N, Ferrigni NR, Putnam JE. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med* 1982; 45: 31–32.
18. Ellis MD, Baxendale FP. Toxicity of seven monoterpenoids to tracheal mites (*Acari* : Tarsonemidae) and their honey bee (*Hymenoptera* : Apidae) hosts when applied as fumigants. *J Econ Entomol* 1997; 90: 1087-1091.
19. Déciga-Campos M, Rivero-Cruz I, Arriaga-Alba M, Castañeda-Corral G, Angeles-López GE, Navarrete A, Mata R: Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. J Ethnopharmacol 2007; 110: 334-342
20. Shao S, Zhou T, Tsao R. Antimicrobials from plants- food preservation and shelf-life extension. in: Comprehensive Biotechnology (Second Edition) 2011; 4: 645-658.
21. Quignard EL, Pohlit AM, Nunomura SM, Pinto AC, Santos EV, Morais SK, Alecrim AM, Pedroso AC, Cyrino BR, Melo CS, Finney EK, Gomes EO, Souza KS, Oliveira LC, Don LC, Silva LF, Queiroz MM, Henrique MC, Santos M, Pinto PS, Silva SG. Screening of plants found in Amazonas state for lethality towards brine shrimp. *Acta Amazon.* 2003; 33: 93–104.