IN VITRO ANTIOXIDANT CAPACITY, ANTIMICROBIAL ACTIVITY AND CYTOTOXICITY OF THE ROOT OF *CINNAMONUM BEJOLGHOTA* (BUCH. -HAM.) SWEET (NA-LIN-GYAW)

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Abstract

The root of *Cinnamomum bejolghota* (Buch.-Ham.) traditional medicine uses Sweet, also known as Na-lin-gyaw, is extensively utilized in traditional medicine to treat various ailments. This study aimed to investigate some phytochemical constituents and assess biological activities, including antioxidant, antimicrobial, and cytotoxic properties. The antioxidant activity screening revealed IC_{50} values of 14.77 µg/mL for the ethanol extract and 59.57 µg/mL for the aqueous extract. The antimicrobial activity of different crude extracts (petroleum ether, ethyl acetate, ethanol, and water) was evaluated against eight strains of microorganisms using the agar well diffusion method, with the ethanol extract demonstrating significant antimicrobial efficacy. Additionally, the cytotoxicity of both the aqueous and ethanol extracts of *C. bejolghota* was assessed using the brine shrimp cytotoxicity bioassay, revealing that the ethanol extract exhibited greater cytotoxicity than the aqueous extract. The antioxidant, antimicrobial, and cytotoxic activities observed in the extracts may indicate potential therapeutic applications, including infections caused by microorganisms, oxidative stress-related conditions, and certain types of cancer.

Keywords*: C. bejolghota*, antioxidant, antimicrobial activities, cytotoxicity

Introduction

Cinnamon is one of the herbal spices obtained from inner bark that is commonly used in food flavouring, pharmaceuticals, and various industries. The Cinnamomum genus belongs to the Lauraceae family, comprising over 250 species worldwide. *Cinnamomum bejolghota* (Buch.-Ham.) Sweet is distributed in India, Bhutan, Bangladesh, Nepal, Madagascar, Myanmar, Laos, Vietnam, and Thailand. The bark of Thai cinnamon has various essential oils, including terpineol, linalool, p-cymene, cinnamaldehyde, and cinnamic acid derivatives. On the other hand, the bark has been used as a local medicine for the treatment of toothache, cough, cold, and diabetes (Atiphasaworn *et al*., 2017). Various biological activities have been reported that the bark extract of *C. bejolghota* possesses anti-diabetic, antioxidant, antibacterial, and antifungal activity (Gogoi *et al*. 2016). Rao et al. reported the anti-cancer activity of leaf and twig extract.

The aromatic and medicinal property of the plant contributes to its economic importance. *C.bejolghota* is widely used in traditional medicine and biological experiments. Many scientific publications have reported regarding pharmacological activities such as anti-virulence, antioxidant, antidiabetic, anticancer, anthelmintic, acaricidal, anti-allergenic, antimicrobial, and anti-haemorrhoid properties (Marinova *et al*, 2011) The current study screens preliminary photochemical constituents, antioxidant, antimicrobial, and cytotoxic activities of *C. bejolghota*.

Botanical Aspect of *Cinnamomum bejolghota* (Buch.-Ham.) Sweet (Na-lin-gyaw)

Family - *Lauraceae*

Botanical name - *Cinnamomum bejolghota (Buch.-Ham.) Sweet*

Myanmar name - Na-lin-gyaw

Part used - Root

Figure 1 Photograph of root of C. *bejolghota*

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Materials and Methods

Sample Collection and Preparation of Root of *C. bejolghota*

In June 2024, *C. bejolghota* was taken from the Kengtawng Township (Kengtawng Forest) Region, Myanmar. The plant was identified at the University of Yangon, Department of Botany. After the sample was cleaned and allowed to dry in the shade for a week, it was chopped into extremely small pieces and processed with an electric grinder to a fine powder. The sample in powder form was kept in an airtight container. Various crude extracts were prepared by using various solvents such as pet ether, ethyl acetate, chloroform, ethanol, methanol, and water.

Preliminary Phytochemical Tests

To classify the various organic constituents present in the powdered sample, including alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, and terpenoids, preliminary phytochemical tests were conducted using the reported methods (M-Tin Wa, 1972).

Investigation of *In Vitro* Antioxidant Activity of Crude Extracts of Root of *C. bejolghota* by DPPH Free Radical Scavenging Assay

(a) Preparation of DPPH solution and stock solution

DPPH (0.0609 g) was thoroughly dissolved in 95 % ethanol (100 mL). This solution was freshly prepared in the brown-coloured reagent bottle and stored in the fridge for no longer than 24 h. Each tested sample (0.02 g) and 20 mL of ethanol were thoroughly mixed by a shaker. The mixture solution was filtered, and the stock solution (100 µg/mL) was obtained. By adding ethanol, DPPH solutions with different concentrations of 50-6.25 µg/mL were prepared from the stock solution.

(b) Determination of *in vitro* antioxidant activity

The antioxidant activity of the root of *C. bejolghota* on the DPPH radical was determined by using the method of Marinova and Batchvarov (2011). The control solution was prepared by mixing 1.5 mL of 120 µM DPPH solution and 1.5 mL of 95 % ethanol using a shaker. The test sample solution was also prepared by mixing thoroughly 1.5 mL of 120 µM DPPH solution (Absorbance = 0.825 used for control) and 1.5 mL of each sample solution (100-6.25 µg/mL). The mixture solutions were allowed to stand at room temperature for 30 min. Then, the absorbance of these solutions was measured at 517 nm by using a UV-7504 spectrophotometer. Absorbance measurements were made in triplicate for each concentration, and then the mean values so obtained were used to calculate the percent inhibition of oxidation by the following equation:

$$\% \text{ RSA} = \frac{A_{DPPH-}(A_{smaple-}A_{blank})}{A_{DPPH}} \times 100$$

where, % RSA = % radical scavenging activity

A_{DPPH} = absorbance of DPPH in EtOH solution

A_{Sample} = absorbance of sample and DPPH solution

A_{blank} = absorbance of sample and EtOH solution

The experimental results were performed in triplicate. The data were recorded as mean ± standard deviation.

Screening of Antimicrobial Activity of the Samples by Agar Well Diffusion Method

By using the agar well diffusion method, the antimicrobial activity of four crude extracts (pet ether, ethyl acetate, ethanol, and water extracts) from the root of *C. bejolghota* was assessed against eight strains of microorganisms, including *Bacillus subtilis, Staphylococcus aureus, Pseudomonas fluorescens, Agrobacterium tumefaciens, Bacillus pumilus, Micrococcus luteus, and Candida albicans* (Anibijuwon and Udeze, 2009). These tests were examined at the Department of Chemistry, Pathein University.

Preparation of nutrient agar medium

Nutrient agar medium was created by adding 100 mL of sterile distilled water to a mixture of 0.5 g of meat extract, 0.5 g of peptone, 0.25 g of sodium chloride, and 1.5 g of agar powder that had been mixed in a sterilized conical flask. To dissolve the components, the resultant mixture was heated. This solution was then brought to a pH of 7.2 using a 0.1 M sodium hydroxide solution. The autoclave was used to sterilize it for 15 min at 121°C.

Determination of antimicrobial activity by agar well diffusion method

The antibacterial activity of the extracts against bacteria and fungi was assessed using the agar-well diffusion method. 1 mL of each solvent was used to dissolve 1 mg of each extract. There was a 15 min drying period following the inoculation. A sterile cork or tip was used to aseptically punch an 8 mm diameter hole, and 0.1 mL of the necessary concentration of extract solution was then added to the well. After that, depending on the microorganisms, agar plates were incubated under the right conditions. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. The plates were incubated for 24 h to allow the extract to diffuse through the agar media to form zones of inhibition. The extent of antimicrobial activity was measured by the diameter of the inhibition zone.

Cytotoxicity Effects by Brine Shrimp Lethality Bioassay

The cytotoxicity of ethanol and aqueous extracts of *C. bejolghota* was assessed using a brine shrimp lethality bioassay, following the methodology outlined by Olowa *et al*. (2013). The brine shrimp (*Artemia salina*) was used in this bioassay (Ali *et al.,* 2013). 5 mg of the corresponding crude extract was dissolved in 5 mL of distilled water to create the sample solution. Sample solutions with concentrations of 800, 400, 200, 100, and 50 μg/mL were obtained by serially diluting the stock solution ten times with distilled water. Using a Pasteur pipette, 10 live brine shrimp nauplii were transferred to each chamber, which was then allowed to stand at room temperature for 24 h. Following a 24 h incubation period, the percentage of brine shrimp that survived was counted, and the 50 % lethal dosage (LC_{50}) was determined. Using the previously described method, the cytotoxic effect of control solutions ($K_2Cr_2O_7$ and caffeine) was also evaluated.

Results and Discussion

Phytoconstituents in the Root of *Cinnamomum bejolghota* (Buch.-Ham.) Sweet

Test tube techniques were initially used to conduct the phytochemical screening of *Cinnamomum bejolghota* (Buch.-Ham.). Alkaloids, α-amino acids, carbohydrates, glycosides, saponins, phenolic compounds, flavonoids, tannins, steroids, and terpenoids were found in the root of *C. bejolghota*, according to phytochemical tests; however, cyanogenic glycosides, starch, and reducing sugars were not present.

Table 1. Results of Preliminary Phytochemical Tests on C. *bejolghota*

No.	Test	Extracts	Test Reagent	Observation	Remark
1	Alkaloids	1% HCl	(i)Wagner's	reddish brown ppt	+
			(ii) Dragendorffs'	orange ppt	+
			(iii) Mayer's	white ppt	+
			(iv) Sodium picrate	yellow ppt	+
2	α-amino acids	H_2O	Ninhydrin	violet spot	+
3	Carbohydrates	H_2O	10 % α-naphthol & conc:H_2SO_4	red ring	+
4	Cyanogenic glycosides	H_2O	Sodium picrate solution	no brick red colour	-
5	Flavonoids	EtOH	Mg ribbon & conc: HCl	pink colour	+
6	Glycosides	H_2O	10 % lead acetate	white ppt	+
7	Phenolic compounds	EtOH	1 % $FeCl_3$	green colour	+
8	Reducing sugars	H_2O	Fehling's solution A & B	no brick red ppt	-
9	Saponins	H_2O	distilled water	frothing	+
10	Starch	H_2O	iodine solution	no blue colour	-
11	Steroids	PE	acetic anhydride & conc:H_2SO_4	green colour	+
12	Tannins	EtOH	1 % gelatin	white ppt	+
13	Terpenoids	$CHCl_3$	acetic anhydride & conc:H_2SO_4	pink colour	+

(+) = presence (-) = absence (ppt) = precipitation

***In vitro* antioxidant assay**

The free radical scavenging capacity of the extract was determined using the stable free radical containing DPPH. It can accept an electron or hydrogen radical. The odd electron in it makes the solution appear deep violet in colour. The absorption vanishes when DPPH accepts an electron, resulting in delocalization. An antioxidant's DPPH radical scavenging ability is supposed to be due to its hydrogen donating property (Soares *et al*., 1997). In the DPPH free radical scavenging assay, 6.25-100 µg/mL of water and ethanol extracts of the root of *C.bejolghota* were used. A decrease in absorbance exhibits an increase in radical scavenging activity. The radical scavenging activities of two crude extracts were expressed in terms of % RSA and IC_{50} (50 % inhibitory concentration). The IC_{50} values of ethanol extracts (14.77 µg/mL) showed higher radical scavenging activity than water extract compared to ascorbic acid (1.15 µg/mL). The results are shown in Table 1, 2 and 3, and Figure 2 and 3.

Table 2 Radical Scavenging Activity and IC_{50} Values of Ethanol and Water Extracts of Root of *Cinnamomum bejolghota* (Buch.-Ham.) Sweet

Extracts	% RSA ± SD at different concentration (µg/mL)					IC_{50} (µg/mL)
	6.25	12.5	25	50	100	
Water extract	19.89 ±0.024	24.00 ±0.031	34.30 ±0.009	47.63 ±0.051	60.00 ±0.001	59.57
Ethanol extract	22.90 ±0.011	46.78 ±0.002	64.48 ±0.003	78.06 ±0.001	90.54 ±0.007	14.77

Table 3 Radical Scavenging Activity of Standard Ascorbic Acid

Sample	% RSA ± SD at different concentration (µg/mL)					IC_{50} (µg/mL)
	0.625	1.25	2.5	5	10	
Ascorbic acid	40.68 ± 0.041	50.85 ± 0.041	61.51 ± 0.005	78.30 ± 0.020	88.11 ± 0.001	1.15

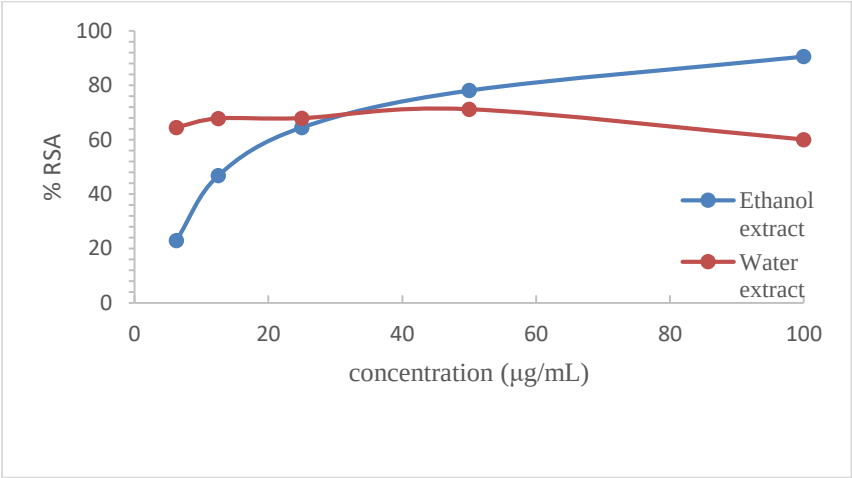

Figure 2 A plot of DPPH radical scavenging activities of ethanol and water extracts of *C. bejolghota* at different concentrations

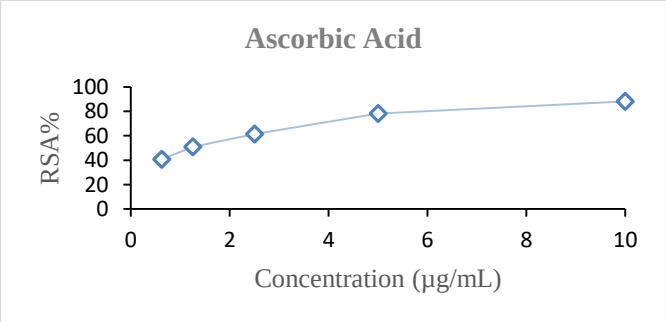

Figure 3 A plot of DPPH free radical scavenging activity of standard ascorbic acid

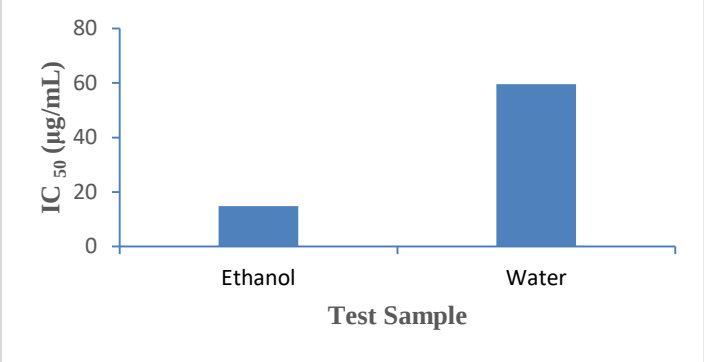

Figure 4 A bar graph comparing the IC_{50} value and % inhibition of ethanol and water extracts of *C. bejolghota*

Antimicrobial Activity

The antimicrobial activity of four crude extracts (PE, EtOH, EtOAc, and water) of *C. bejolghota* was tested on bacteria (*B. subtilis, E. coli, P. fluorescens, A. tumefaciens, B. pumilus, M. luteus, and S. aureus*) and the *C. albicans* fungus, which was determined by the agar well diffusion method. The measurable zone diameter, including the well diameter, shows the degree of completeness of the antimicrobial activity. In this experiment, the well diameter was set at 8 mm. The ethanol extract demonstrated the highest antimicrobial activity among other extracts. These extracts showed nearly the same inhibition zone diameters as standard chloramphenicol and nystatin. The results are shown in Table 4, and Figures 4 and 5.

Table 4 Results of Antimicrobial Activity of Crude Extracts of the Root of *C. bejolghota*

No	Type of Microorganisms	Inhibition zone diameter (mm)				
		Water	EtOH	EtOAc	PE	STD
1	*Bacillus pumilus*	13	25	25	14	27
2	*Bacillus subtilis*	13	25	24	13	27
3	*Candida albicans*	13	26	24	14	27
4	*Escherichia coli*	13	25	25	14	27
5	*Micrococcus luteus*	13	26	24	13	28
6	*Pseudomonas fluorescens*	12	25	25	13	28
7	*Staphylococcus aureus*	13	25	25	13	28
8	*Agrobacterium tumefaciens*	14	26	24	14	27

Agar well diameter = 8 mm

10 mm-14 mm = low activity
15 mm- 20 mm = medium activity
20 mm above = very high activity

*STD for bacterial = chloramphenicol
for fungus = Nystatin

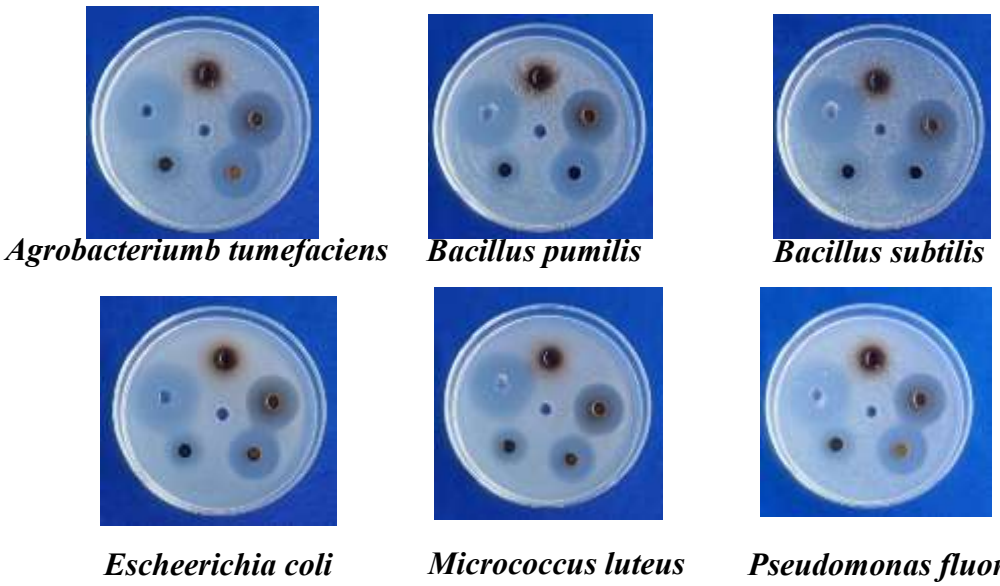

Figure 5 Photograph of screening of antimicrobial activity of various crude extract of root of *C. bejolghota*

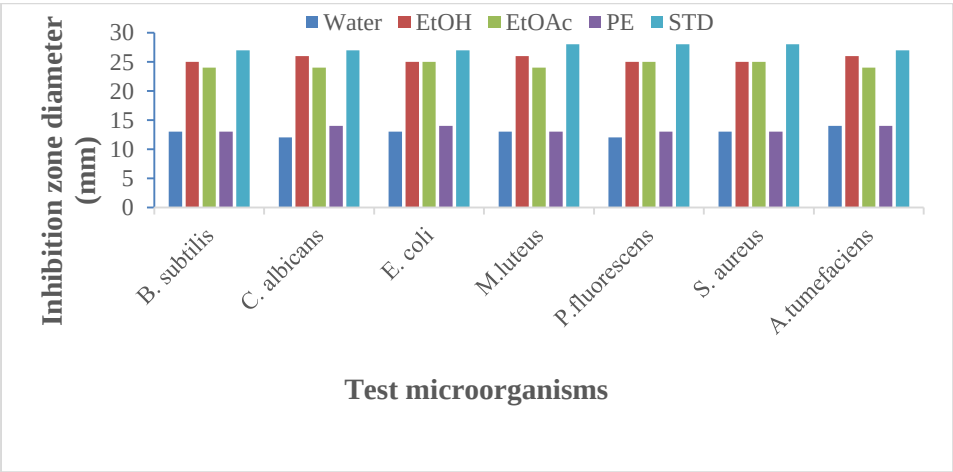

Figure 6 A bar graph of Inhibition Zone diameters for various crude extracts of root of *C. bejolghota*

Cytotoxicity Effect of Crude Extracts of the Root of *Cinnamomum bejolghota* (Buch.-Ham.) Sweet

Most toxicity studies that use the brine shrimp assay determine the toxicity by counting the survived nauplii after 24 h of exposure to the test. The brine shrimp lethality bioassay was used to assess the cytotoxicity activity of ethanol and water extracts of the root of *C. bejolghota*. The cytotoxic effect was expressed as LC_{50} values (50 % lethality concentration). Standard potassium dichromate ($K_2Cr_2O_7$) and caffeine were chosen because $K_2Cr_2O_7$ is a well-known toxic agent in this assay and caffeine is a natural product. The results are shown in Table 5 and Figure 7. The ethanol extracts exhibited more cytotoxic effects than the water extract. The results showed the LC_{50} values of ethanol and water extracts (319.7 µg/mL and 512.86 µg/mL), respectively. The standard $K_2Cr_2O_7$ was 89.98 µg/mL, and caffeine was > 1000 µg/mL. The resulting LC_{50} values were compared with the Deciga-Campos criteria. The ethanol extracts showed medium toxicity due to LC_{50} values between 500 and 1000 µg/mL.

Table 5 Cytotoxicity of Ethanol and Watery Crude Extracts of Root of *C. bejolghota*

Test Sample	% Mortality of Brine Shrimp Concentration ± SD					IC_{50}
	50	100	200	400	800	(µg/mL)
water extract	13.4 ±0.557	20.00 ±1.00	33.4 ±0.557	43.4 ±1.00	66.7 ±1.00	512.86
ethanol extract	20.00 ±1.00	30.00 ±1.00	40.00 ±1.00	56.70 ±1.00	60.00 ±0.557	319.7
*patassium dichromate	36.67 ± 0.53	53.34 ± 0.58	72.00 ± 1.00	85.47 ± 1.15	100.00 ±0.00	89.98
** Caffeine	0.00 ± 0.00	0.00 ± 0.00	6.67 ± 1.53	16.67 ± 0.58	23.34 ± 1.53	>1000

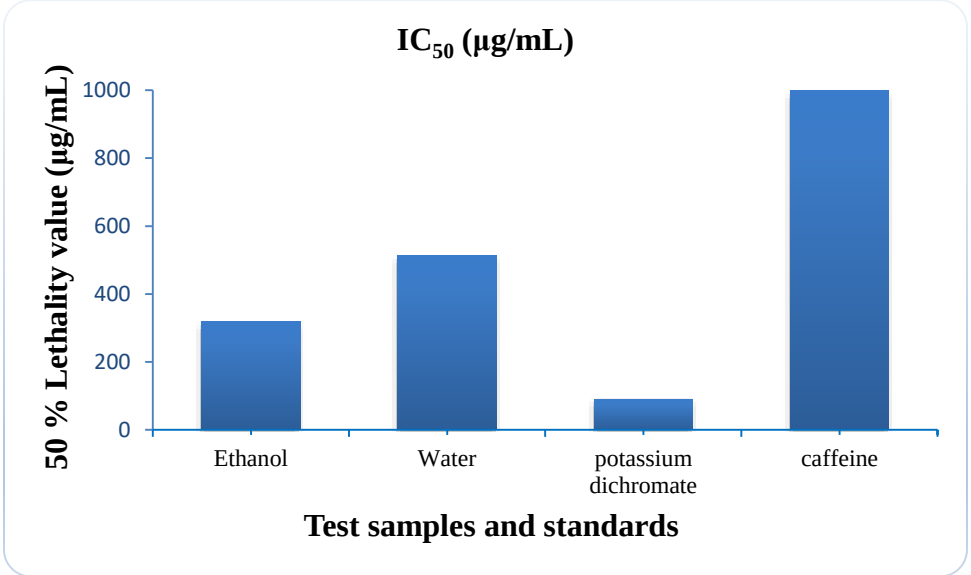

Figure 7 The cytotoxic effect of crude extracts and standard

Conclusion

This study shows that the root extracts of *C. bejolghota* have antimicrobial and antioxidant properties. This study validates *Cinnamomum bejolghota* as potentially leading to the development of new therapeutic agents or nutraceuticals and contributing to the scientific advancement of traditional medicine in Myanmar. Further studies are essential to substantiate its traditional applications in managing cancer, inflammatory diseases, cardiovascular conditions, and neurological disorders.

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References

Ali, N., U. Alien, S. W. A. Shaha, M. Junaid, G. Ahmed, W. Ali, and M. Ghias. (2013). "Acute Toxicity, Brine Shrimp Cytotoxicity, Anthelmintic and Relaxant Potentials of Fruits of *Rubus fruiticosis Agg"*. *BMC Complement Altern. Med*., **13**, pp.136-143.

Anriphasaworn, P., S. Monggoot and P. Pripdeevech. (2017). "Chemical Composition, Antibacterial and Antifungal Activity of *Cinnamomun bejolghota* Bark Oil from Thailand". *Journal of Applied Pharmaceutical Science*, **7**(4), pp. 067-073.

Anibijuwon, I. I., and A.O. Udeze. (2009). "Antimicrobial Activity of *Carica Papaya* (Pawpaw Leaf) on Some Pathogenic Organisms of Clinical Origin from South-Western Nigeria". *Ethnobotanical Leaflets*,**13**, 850-864

Gogoi, B., B. Kakoti, S. Bora, and N.S. Borah. (2016). "Pharmacognostic and Preliminary Phytochemical Evaluation of *Cinnamomum bejolghota* (Buch.-Ham.) Sweet Bark". *Indian Journal of Natural Products Resour*ces, **7** (13), pp. 59–64.

Marinova, G. and V. Batchvarov. (2011). "Evaluation of the Methods for Determination of the Free Radical Scavenging Activity by DPPH". *Bulgarian Journal of Agricultural Science*, **17**, pp. 11-24.

M-Tin Wa. (1972). "Phytochemical Screening Methods and Procedures". *Phytochemical Bulletin of Botanical Society of America*, **5** (3), pp. 4-10.

Olowa L.F. and M. N. Olga. (2013). "Brine Shrimp Lethality Assay of the Ethanolic Extract of Three Selected Species of Medicinal Plants from Iligan City, Philippines". *Int Res J Biol Sci*., **2**, pp., 4-77.

Soares, J. R., T. C. P. Dinis, A. P. Cunha and L. Almeida. (1997). "Antioxidant Activity of Some Extracts of *Thumus Zygis*". *J. Free Radic. Res*., **26**, pp. 469-478.