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Yabesh Bhitrikoty
Department of Horticulture, Sikkim University, 6th Mile, Tadong, Gangtok, Sikkim, India

Sujata Upadhyay
Department of Horticulture, Sikkim University, 6th Mile, Tadong, Gangtok, Sikkim, India

Corresponding Author:
Sujata Upadhyay
Department of Horticulture, Sikkim University, 6th Mile, Tadong, Gangtok, Sikkim, India

Phytochemical screening of Bokay timur (*Zanthoxylum alatum*) and Sill timur (*Lindera neesiana*) found in Sikkim Himalaya

Yabesh Bhitrikoty and Sujata Upadhyay

Abstract
Sikkim Himalayan range is characterized its richness in plant diversity and has been classified as an important ecological hotspot ecoregion of India. The present study was conducted by Dept. of Horticulture, Sikkim University, Gangtok during 2017-2019 to perform qualitative phytochemical analysis of *Zanthoxylum alatum* (Bokay timur) and *Lindera neesiana* (Sill timur). The fruits Bokay timur and Sill timur fruits were collected from seven and five different places of Kalimpong (W.B.) and Gangtok (Sikkim) respectively which had altitudinal variation. The fruits and seeds of these fruits are used in treatment of rheumatism, dysentry, stomach ache, caminative, anthelmintic, toothache, inflammation, headache, microbial infection and cancer. Besides this, seeds and bark are also used as an aromatic, tonic in fever, dyspepsia and in cholera. In present study, qualitative phytochemical analysis was done to test the presence or absence of alkaloids, flavanoids, glycoside, phenols, sterols, saponins, tannins and terpenoids in aqueous and ethanolic extracts. The result obtained from the study justifies that the phytochemicals like alkaloids, flavanoids, glycosides, sterols, tannins and terpenoids are found to be present in fruits of *Zanthoxylum alatum*, however saponins were completely absent in samples collected from Kalimpong (W.B.) and Gangtok (Sikkim). The *Lindera neesiana* (sill timur) fruits showed presence of phytochemicals in both extracts. The results of this study provides baseline for further research work for identification of its antioxidant properties and hepatoprotective potential of this plant. This study also emphasizes on further analysis on exploration, conservation and use of these plants for different medicinal purposes. The study also indicates correlation in quantity of phytochemical constituents with elevation.

Keywords: Phytochemical screening, *Zanthoxylum alatum*, *Lindera neesiana*, elevation

1. Introduction
India is known for its traditional medicinal system Ayurveda, Siddha and Unani. Indian flora serves as a land for medicinal plants and it is the largest producer and exporter of medicinal plants. According to World Health Organization, there are over 8000 plant species used in India for medicinal purpose. In present there are about 720.3 ha area has been engaged in cultivation of medicinal and aromatic plants with a total annual production of 866.4 MT in India (NHB, 2018). It is estimated that nearly US $ 53 million raw materials and drugs from medicinal plants are exported from India (Kumar, 2016) [11].

Kalimpong and Sikkim situated between $88^0$47 and $88^0$52 East longitude and, 27.06^0 and 27.53^0 North latitude in India have been included in Earth's biodiversity hotspot (Myers *et al*., 2000) [13] and several centers for plant diversity (WWF/IUCN 1995) [24].

'Bokay timur' (*Zanthoxylum alatum*) *Rutaceae and* 'Sill timur' (*Lindera nessiana*) Lauraceae are strong aromatic medicinal shrubs found in Kalimpong and Sikkim region and are used as commercial, medicinal, culinary, and wasteland crop. Fruits, leaves and seeds are eaten as vegetable. Besides this the leaves are also used as insecticides and an insect repellent. The fruit and seeds are also prescribed in treatment of rheumatism, dysentery, stomach ache, carminative, anthelmintic, toothache, inflammation, headache, microbial infections and cancer. The seeds and bark are also used as an aromatic, tonic in fever, dyspepsia and in cholera.

'Bokay timur' (*Zanthoxylum alatum*) belonging to family Rutaceae are found abundantly in the Eastern Himalayan region from Kashmir to Bhutan upto 2100 m and in Khasi hills upto 1350 m and is commercially important due to its edible fruits and leaves containing essential oils which are used in cosmetics and perfume industries. *Z. alatum* is synonymous to *Z. armatum.* In a study, Z. *alatum* was later named as *Z. armatum* and *Z. planispinum* but Flora Hupehensis considers both *Z. armatum* and *Z. planispinum* as distinct species (Gardener,

1995) [7]. The English name of the plant is 'Winged prickly ash' and commonly known as 'Toothache tree'. In its natural habitat, it grows up to 6 m in height with dense foliage and armed branched flattened prickles. Leaves are compound, 4 to 20 cm long, imparipinnate, rachis winged, serrate with gland dots and aromatic, containing a flavour like lime and mint. The ripe fruit follicles are usually reddish in colour and 4 to 5 mm in diameter. The dried fruit also contains an aroma that is present in brown fruit wall (pericarp-shell). It may be able to develop numbing or anaesthetic feeling on the tongue. Seeds are solitary, globose, shining and have bitter taste. Flowering occurs during the months of March and April. The green or yellow flowers are present in dense terminal and axillary sparse panicles. The bark of the plant is reported to contain a bitter crystalline principal identical with berberine and it also contains volatile oil, phenolic compounds and resin.

Similarly, 'Sill timur' (*Lindera neesiana*) family Lauraceae, is deciduous or evergreen tree or shrub attaining up to 9 meter height and grows in a temperate Himalayan region at different province of India, Nepal, China, Bhutan, and Myanmar. It is an aromatic and spicy plant with edible fruits having globose edible berry fruits with an alternate leaves and long petiolate, ovate base cuneate or cordate, entire, acute or acuminate, membranous, 7-20 cm long and 3-8 cm broad, nerves penni nerved, reticulate, aromatic, and glabrous and shoots are quite black when dry. Inflorescences are axillary, solitary or clustered umbels. Flowers are small, greenish yellow sweet scented, pedicel pubescent, Bracts 4, membranous, perianth orbicular, stamens 9; anther conspicuous. Fruits are globose black with minute membranous perianth. Many people use the powder of *L. neesiana* fruit to prepare pickles and it is used on daily basis as a food ingredient. The two different species i.e. *Lindera neesiana* and *Litsea citrate* are found in Kalimpong (W.B.) and Sikkim.

Literature survey reveals that the study of phytochemicals are important for the authentication of the quality of the plants and useful for pharmaceuticals and nutraceutical industries. The population of these plants is decreasing due to rapid urbanization and they need to be conserved. Also, there has been little work done on the phytochemical studies of these species. This study will furnish valuable information for the pharmacognostic evaluation as well as for pharmaceutical preparations from this plant and will also provide useful information for human health and contribute to the potential commercial use of the plant.

By keeping in view of the above mentioned points, the present study was undertaken with the objective to perform qualitative phytochemical analysis of *Zanthoxylum alatum* (bokay timur) and *Lindera neesiana* (Sill timur) in Kalimpong (W.B.) and Gangtok (Sikkim).

2. Materials and Methods

The research work was carried out during 2017-2019 at the laboratory, Dept. of Horticulture, Sikkim University, Gangtok and the methodology employed is being mentioned as follows:-

The fruits were grounded and extraction with aqueous and organic solvent (ethanol) was done for both the fruits (*Zanthoxylum alatum* and *Lindera neesiana*) and further phytochemical investigation was done.

Zanthoxylum alatum fruits were collected from seven different places in Kalimpong (W.B.) and Sikkim. *Lindera neesiana* fruits were collected from five different places in Kalimpong (W.B.) and Sikkim. The treatment details along with elevation (altitude) in metres have been presented in Table no. 2, 3, 4 and 5.

2.1 Drying of the samples

The collected plant sample (fruits) were thoroughly washed and cleaned with tap water twice followed by distilled water and kept in the shade for shade drying for 2-3 weeks and are ground into uniform powder using Philips Mixer grinder.

2.2 Preparation of plant extract

After drying the powdered specimens were subjected to aqueous extraction and ethanol extraction. Extraction of plant samples was carried out by maceration process. Each sample was weighed 10g on electronic balance and kept for 48 hours in a ratio of 1:7 at a room temperature for the extraction (Chettri *et al.*, 2017)[4]. For alkaloids, flavonoids, glycosides, sterols, saponins, tannins, and terpenoids, both the aqueous and ethanol extracts were filtered using funnel and filter paper Whatman no. 1. The extracts obtained were used for the phytochemical screening.

2.3 Qualitative analysis of phytochemicals:

The phytochemical test of Bokay timur (*Zanthoxylum alatum*) and Sill timur (*Lindera neesiana*) plants were carried out using aqueous solution and ethanol extracts on the powdered specimens using standard procedure to identify the various constituents (Ranganna, 2016) [21].

Table 1: Chemical tests for qualitative analysis of Bokay timur (*Zanthoxylum alatum*) and Sill timur (*Lindera neesiana*)

Chemical test	Procedure
Alkaloids	For qualitative analysis of alkaloids, 2 ml of plant extract was mixed with 2 ml of concentrated hydrochloric acid. Few drops of Mayer's reagent was added to this mixture. Appearance of green colour or white precipitate indicates the presence of alkaloids.
Flavonoids	This test was performed by using 1ml of each plant extract to which 2 ml of filtrate 10% aqueous sodium hydroxide was added. The change in colour from yellow to colourless in addition of dilute hydrochloric acid indicates presence of flavonoids.
Glycosides	This test was performed by taking 2 ml of each plant extracts, 3ml of chloroform and 10% ammonia solution was added. It forms a pink colour indicating presence of glycosides.
Phenol (Ferric chloride test)	This test was performed by taking 5 ml of each plant extract and addition of 5 ml of 5% ferric chloride solution. The formation of bluish green colour in the test tube confirms the presence of phenols.
Sterols (Salkowski test)	This test was carried out by taking 1ml of each plant extract mixed in 1ml of concentrated H_2SO_4. Formation of wine red colour indicates presence of sterols.
Saponins (foam test)	This test was carried out by taking 2 ml of each plant extract added with 5 ml of distilled water in a test tube and shaken vigorously formation of foam indicates the presence of saponins.
Tannins	The test was carried out by taking 2 ml of each plant extract, 2-3 drops of diluted solution of 5% $FeCl_3$ was added. The production of black or blue green colour indicates the presence of tannin.
Terpenoids	The test was carried out by taking 5 ml of each extract which was mixed with 2 ml of chloroform and 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colour of the interface was formed to show positive results for the presence of terpenoids.

2.4 Spearman's rank correlation coefficient

Since this study was on phytochemical screening was qualitative in nature, Spearman's correlation coefficient was used to find out relationship between elevation and quantity of phytochemical constituents. The formula for Spearman's rank correlation is being presented as follows:

$$\rho = 1 - \frac{6\Sigma d_i^2}{n(n^2-1)}$$

$\boldsymbol{\rho}$ = Spearman's rank correlation coefficient, di =Difference between ranks of each observation, n=number of observations, Spearman's rank correlation ranges between -1 to +1. A value of +1 means perfect association of rank, 0 means no association between ranks and -1 means negative association of rank.

3. Results and Discussion

Phytochemical screening was done to determine the presence of secondary metabolites in *Zanthoxylum alatum* and *Lindeera neesiana* fruits. Each of the fruit sample has two different polarities of crude extracts that make the total of eight crude extracts. The test was conducted to determine alkaloids, flavonoids, glycosides, phenols, sterols, saponins, tannins, and terpenoids. The ethanolic (E) and aqueous extracts (W) were studied.

It was evident from the above Table no. 2,3 that the abundant presence of terpenoids and tannins, were found in the treatments T_4, T_5 and T7 followed by moderate presence of alkaloids in treatment T_1, T_4, and T_5 whereas flavonoids and glycoside were found in all the treatments. However complete absence of saponins was observed in both the solutions in all the treatments collected from Kalimpong (W.B.). It was also noticed that the phenols were absent in the aqueous solution but present in ethanol solution. The preliminary phytochemical screening had showed the abundant presence of tannins in S2, S3 and S6, flavonoids are also found abundantly in S5 and S7 followed by moderate presence of alkaloids, phenols, sterols and terpenoids. Saponins were found completely absent in all the treatments.

Table 2: Phytochemical screening of *Zanthoxylum alatum* collected from Kalimpong (W.B.)

Treatment	Alkaloids		Flavonoids		Glycosides		Phenols		Sterols		Saponins		Tannins		Terpenoids	
	E	W	E	W	E	W	E	W	E	W	E	W	E	W	E	W
Kalimpong (W.B.)The elevation (m) has been mentioned below place of collection (treatment detail)																
T_1 (Munsong) 1526m	+	++	+	-	+	+	+	-	+	+	-	-	+	+	+	+
T_2 (20th mile) 1481m	+	+	++	-	+	+	+	-	+	+	-	-	+	+	-	+
T_3 (18th mile) 1463m	+	+	++	+	+	+	++	-	++	+	-	-	++	+	-	-
T_4 (Bidyang) 1220m	+	++	+	+	-	+	+	-	+	-	-	-	+	+	++	+++
T_5 (Bhalukhop) 1615 m	+	++	+	-	+	+	+	-	++	+	-	-	+++	+	+++	+
T_6 (Rongchong) 1208m	+	+	+	-	-	+	+	-	+	+	-	-	++	+	+	++
T_7 (12th mile) 1247m	+	+	++	-	+	+	+	-	++	+	-	-	+++	++	+++	++

(Key= +++ Abundantly present, ++ Moderately present, + Lightly Present, - Absent)
E: Ethanolic extract, W: aqueous extract, as per visual scoring out of 10, +++ (8.5), ++ (5.5), +(2.5), -(0)

The result from the study reveals that the fruits of *Zanthoxylum alatum* of all the treatments collected from various places content various amount phytochemical and this varies from treatment to treatment this could be due to the altitudinal variation among the places and the climatic condition of the place of growing. These studies also confirm that the curative properties of these plants may be due to the presence of this various secondary metabolites.

In *Lindera neesiana*, phytochemical screening performed as per standardized procedure showed the presence of varied degree of phytoconstituents in both extracts. It is evident from the Table no. 4 that the sterols are abundantly present in treatment K1, K2, and K3. Phenols are also found abundantly in treatment K1 and K4. Terpenoids were found abundantly and moderately in K2, K1, K3, and in K4. Alkaloids, saponins, tannins and flavonoids are found to be lightly present in the all the treatment. It was also found that in all the treatments glycosides are completely absent except K1 and K5 in both extracts.

Table 3: Phytochemical screening of *Zanthoxylum alatum* collected from Sikkim

Treatment	Alkaloids		Flavonoids		Glycosides		Phenols		Sterols		Saponins		Tannins		Terpenoids	
	E	W	E	W	E	W	E	W	E	W	E	W	E	W	E	W
Sikkim: The elevation (m) has been mentioned below place of collection (Treatment detail)																
S1 (Assamlingzey) 1425 m	+	++	++	+	+	+	+	+	+	+	-	-	++	+	+	+
S2 (6th mile, Gangtok) 1527m	+	+	++	-	-	+	+	+	+	-	-	-	+++	+	+	-
S3(Burmaikpabon g) 1518m	+	+	+	+	+	+	+	+	+	++	-	-	+++	+	+	+
S4(Central Pendam) 1621m	+	+	++	++	-	+	+	+	+	-	-	-	++	+	++	+
S5 (Ranipool) 1001m	+	++	+++	+	+	+	++	+	++	+	-	-	+	+	++	+
S6 (Singtam) 925 m	+	+	++	+	-	+	++	+	++	+	-	-	+++	+	+	+
S7 (Namthang) 1311m	+	+	+++	-	-	+	++	+	++	+	-	-	+	++	+++	+

(Key= +++ Abundantly present, ++ Moderately present, + Lightly Present, - Absent)
E: Ethanolic extract, W: aqueous extract, as per visual scoring out of 10, +++ (8.5), ++ (5.5), +(2.5), -(0)

Table 4: Phytochemical screening of *Lindera neesiana* collected from Kalimpong, W.B.

Treatment details	Alkaloids		Flavonoids		Glycosides		Phenols		Sterols		Saponins		Tannins		Terpenoids	
	E	W	E	W	E	W	E	W	E	W	E	W	E	W	E	W
K1 (20th mile)1485 m	++	+++	+	+	+	-	+++	+	+++	+++	+	+	+	+	+	++
K2 (Mangwa)1687m	+	+	+	++	-	-	++	+	++	+++	+	+	+	++	+	+++
K3 (Rongchong) 1212 m	+	+	++	+	-	-	++	+	+++	++	+	+	+	++	+	++
K4 (Bhalukhop) 1619m	+	++	+	+	-	-	+++	++	++	++	+	+	+	+	-	++
K5 (Kamsi) 1228m	+	+	+	+	+	-	++	++	+	++	+	+	+	+	+	+

(Key= +++ Abundantly present, ++ Moderately present, + Lightly Present, - Absent)
E: Ethanolic extract, W: aqueous extract, as per visual scoring out of 10, +++ (8.5), ++ (5.5), +(2.5), -(0)

The phytochemical screening had showed the presence of all the phytochemicals in all the treatments and it was evident that abundant presence of sterols was found in treatment L1, L2 and L3 and abundant presence of flavonoids in treatment in L2 and L3. Moderate presence of alkaloids was observed in treatment L1 and L3 followed by moderate presence of phenols and terpenoids in treatment L4, L5 and L2 and L3 respectively. Saponins and tannins are found lightly present in all the treatments. The study reveals the presence of many important phytochemicals like alkaloids, flavonoids glycosides phenols, sterols, saponins, tannins and terpenoids. These secondary metabolites were produced in the plant and act as defense mechanism of their own, but this metabolite can also be utilized for the benefits of humans with anti-oxidant, antimicrobial, antidepressant, muscle relaxant and anti-inflammatory and many other activities (Briskin, 2017) [3].

Table 5: Phytochemical screening of *Lindera neesiana* collected from Sikkim

Treatment details	Alkaloids		Flavonoids		Glycosides		Phenols		Sterols		Saponins		Tannins		Terpenoids	
	E	W	E	W	E	W	E	W	E	W	E	W	E	W	E	W
L1(Assamli ngzey) 1426m	+	++	+	+	+	+	+	+	+	+++	+	+	+	+	+	+
L2(6th mile) 1527m	+	+	+++	+	+	+	+	+	+	+++	+	+	+	+	+	++
L3(Martam) 1491m	+	+	++	+++	+	+	+	+	+	+++	+	+	+	+	+	++
L4 (Kabigaon) 1553m	+	++	+	+	+	+	++	+	+	++	+	+	+	+	+	+
L5(Singtam Sumin) 1019m	+	+	+	+	+	+	++	+	+	++	+	+	+	+	+	+

(Key= +++ Abundantly present, ++ Moderately present, + Lightly Present, - Absent) E: Ethanolic extract, W: aqueous extract, as per visual scoring out of 10, +++ (8.5), ++ (5.5), +(2.5), -(0)

Phytochemical are present in the plants and are very important as this can be used for pharmacognostic, pharmaceuticals and is responsible for different medicinal properties. This study reveals that fruits of *Zanthoxylum alatum* are the rich source of phytochemicals like alkaloids, flavonoids, glycosides, phenols, sterols, tannins, terpenoids and thus can be used as potential health promoting source. Several workers have revealed that glycosides are responsible to lower blood pressure. Alkaloids have analgesic, antispasmodic and antibacterial properties. Different reports suggest that phenolic compounds exhibit to possess biological properties like apoptosis, anti-aging, anti-inflammation, reduced the chance of cardiac arrest. However, these phytoconstituents may vary from plant to plants due to many factors like plant physiology, environmental condition, soil conditions, geographical locations, elevations and biotic stress. Different studies also support that the secondary metabolites content is effected by seasonal and altitudinal variation too. Pandey *et al.*, (2018) [15] in their studies reported that berberine content of the *Thalictrum foliolosum* was varied inversely with the altitude, while it was also found that phenols and flavonoids contents increases at the higher altitude. Similar report was found by Rana *et al*., (2020) [20] in their study were they observed that the phenolic, flavonoid and terpenoids content of *Coleus forskohlii* root increases with the increase in altitude. Levels of secondary metabolites are both environmentally induced as well as genetically controlled (Makkar, 2007) [12].

Alkaloids are reported to exhibits many therapeutic effects like antimicrobial, sedatives, anti-allergic anti-inflammatory properties (Stray 1998; Okwu *et al*., 2004) [23, 18]. Several reports suggest that glycosides can improve the cardiac output and reduce heart disease like congestive heart failure and cardiac arrhythmia (Doss *et al*., 2011 [6]). It was also reported that flavonoids, tannins, and phenols acts as primary antioxidant and possesses antiviral, anti-allergic, anti-inflammatory and anti-cancer activities (Yun *et al*., 2015 & Polterait 1997) [25, 17]. This rich source of important secondary metabolites in this plants makes it a valuable source for different uses in medicinal uses as well as in the perfumery industries.

3.1 Phytochemical analysis of *Zanthoxylum alatum* and *Lindera neesiana*

Both plant species were analysed qualitative (both in aqueous and ethanolic extract) to test the presence/absence of alkaloids, flavonoids, glycosides, phenols, sterols, saponins, tannins, and terpenoids in the present study.

3.1.1 Alkaloids and Flavanoids

In *Zathoxylum armatum* (Table no.1) preliminary phytochemical screening of all the treatments in both aqueous and ethanolic extract had showed the presence of alkaloids in all the treatments where T1, T4, T5, S1 and S5 show the moderate presence of alkaloids in aqueous solution. Similar result were also reported in the studies conducted in the different species of *Zanthoxylum* using different plant parts. Jothi *et al.*, (2019) [10] while conducting phytochemical analysis in bark of *Zanthoxylum armatum* also found the presence of alkaloids. Ayangla *et al.* (2016) [2] reported the presence of alkaloids in the leaves of *Zanthoxylum alatum*, *Zanthoxylum rhetsa* and seeds of *Zanthoxylum oxyphyllum* in

all the aqueous, ethanol and methanol solution. Flavonoids were present in both solutions collected from Gangtok, Sikkim where S5 and S7 shows the abundant presence of flavonoids in ethanol extract and T_2, T_3 and T_7 shows moderate presence of flavonoid collected from Kalimpong, W.B. In aqueous solution it was observed that some of the treatments T_1, T_2, T_5, T_6, T_7, S2, and S7 lack the presence of flavonoids. Similar results were also reported from Alam *et al.* (2018) who had also found abundant presence of flavonoids in the leaf, bark, and fruits of *Zanthoxylum armatum*. Pandey *et al.*, (2014) found abundant presence of flavonoids in ethanol, however flavonoids were absent in hot and warm water extraction.

In *Lindera neesiana* (Table no.4, 5) five different treatments were analzsed in both aqueous and ethanolic extracts to test the presence or absence of the alkaloids and it was found that alkaloids are present in both ethanol and aqueous extracts. Where in aqueous extracts treatment K1 shows the abundant presence of alkaloids followed by moderate presence in K4, L1 and L2. Chettri *et al.* (2017) [4] reported the presence of alkaloids which also supports the findings in present study. However, Devika *et al.*, (2016) [8] while conducting phytochemicals and antioxidative analysis reported that alkaloids were completely absent in the leaves and bark of *Litsea floribunda*. It was evident from the Table no.3 and 4 that flavonoids were found present in both the extracts where L2 and L3 flavonoids were found abundantly. Similar result was reported by Devi. *et al.* (2015) [5] while conducting phytochemical analysis of *Cinnamomum tamala* (Lauraceae) found in Thoubnal district of Manipur they have found the presence of flavonoids. Pandey *et al.*, (2014) [14] also claimed the presence of flavonoids in the bark of *Cinnamon zeylanicum* found in Uttarakhand (India) and her findings support the results in present study.

3.1.2 Glycosides and Phenols

In *Zanthoxylum armatum*, it is evident from Table no.2 that all the treatments show the fair presence of glycosides in most of all the treatments in both solutions. The aqueous solution S2, S4, S6 and S7 lack presence of glycosides. Similar result was reported in the leaves of *Zanthoxylum armatum* by Ayangla (2016) [2]. Phenols were present in all the treatments collected from Gangtok, Sikkim where treatments S5, S6, S7 show the moderate presence among all the treatments. All the treatments collected from Kalimpong, W.B. show negative result in aqueous extracts but not in ethanolic extract as it was found that T3 shows moderate presence and rest show fair presence of phenols. The variation in the expression of the phytonutrient could be due difference in the polarity of the solvents or might be due to the different factors like altitudinal variation between these two places or might be the physiological factors. Rajkumar *et al.* (2014) [19] reported that methanolic and hexane solvents had more phytonutrients being extracted as and when compared to ethanolic and aqueous solvent. This is similar to the result obtained in the present study.

In *Lindera neesiana* (Table no. 4) while analyzing the presence or absence of glycosides it was found that glycoside was fairly present in all the treatments collected from Gangtok, Sikkim in both extracts. However, it is found that in aqueous extract glycoside is totally absent in treatments collected from Kalimpong, W.B. Pandey *et al.*, (2014) [14] found the absence of glycosides in the bark of *Cinnamon zeylanicum*. It was evident from the Table no 4 and 5 that phenol was found in both treatments however treatments collected from Kalimpong, W.B. show the abundant presence of phenols in K1 and K4 followed by the moderate presence in K2, K3 and K5. Previous studies by Pant *et al.* (2018) [15] confirm the presence of phenols in the leaf extract of *Persea odoratissima.*

3.1.3 Sterols and Saponins

In *Zanthoxylum armatum* (Table no.2, 3) sterols were found present in all the treatments collected from both the places i.e. Gangtok, Sikkim and Kalimpong, W.B., however T4, S2 and S4 show absence of sterols in aqueous extracts. The present study found the absence of saponins in all the treatments collected from both the places. Previous studied also claimed the presence of saponins as evident by findings of Jothi *et al.* (2019) [10] who found the presence of saponins in the bark of *Zanthoxylum armatum*. Alam *et al.* (2018) [1] also found abundant presence of saponins in the leaf, bark, and fruits of *Zanthoxylum armatum.*

In *Lindera neesina* (Table no.4, 5) phytochemical screening of the both treatments it was found that sterols were present abundantly in both extracts. K1, K2, K3, S1, S2 and S3 shows the abundant presence of sterols and rest show the moderate presence of sterols. It was evident from the Table no. 5 that all the treatments in both aqueous and ethanolic extract had showed the presence of saponins in all the treatments.

3.1.4 Tannins and Terpenoids

In *Zanthoxylum armatum*, overall, all the treatments under the present study exhibited varying level of tannins. However, tannins were found abundantly in the treatment T_5, T_7, S2, S3, and S6 and was moderately present in T_3, T_6, S1, S4 in ethanolic extract. In aqueous solution it is found that tannins were fairly present in all the treatments. Z. Nur *et al.*, (2019) [26] conducted phytochemical screening of *Citrus microcarpasp*. (Rutaceae) and found positive result for tannins in hexane and ethyl acetate crude extracts. Terpenoids was found abundantly present in T_5 T_7 and S7, moderately and fairly in T_4 S4 and S5. Over all the treatments (T1-T7), it was found that terpenoids were absent in treatments T3. This could be due to the polarity of the solution. Previous studies confirmed the presence of terpenoids in leaves, stem and barks of *Zanthoxylum armatum* collected from the different parts of Nepal. Shrestha *et al.*, (2015) [22] while conducting phytochemical screening of different plants parts of *Zanthoxylum armatum* (Found in different parts of Nepal) found the presence of terpenoids in leaves and stem.

In *Lindera neesiana*, while analysing the presence or absence of tannins it was evident from the Table no. 5 that tannins were present in all the treatments where K2 and K3 showed the moderate presence. Pant *et al.* (2018) [16] in their study revealed the presence of tannins in the leaf extract of *Persea odoratissima* which supported the present findings of the study. Terpenoids were present in both extracts in all the treatments however K2 shows the abundant presence in all treatments followed by K1, K3, K4, L2 and L3 show moderate presence of terpenoids in aqueous extracts. While investigating pharmacognostic and preliminary phytochemical evaluation of *Cinnamomum bejolghota*, sweet bark Gogoi *et al.*, (2016) [9] reported presence of terpenoids in the petroleum ether extract and chloroform extract however terpenoids were absent in ethyl acetate and methanolic extract this could be due to the difference in the polarity of different solutions.

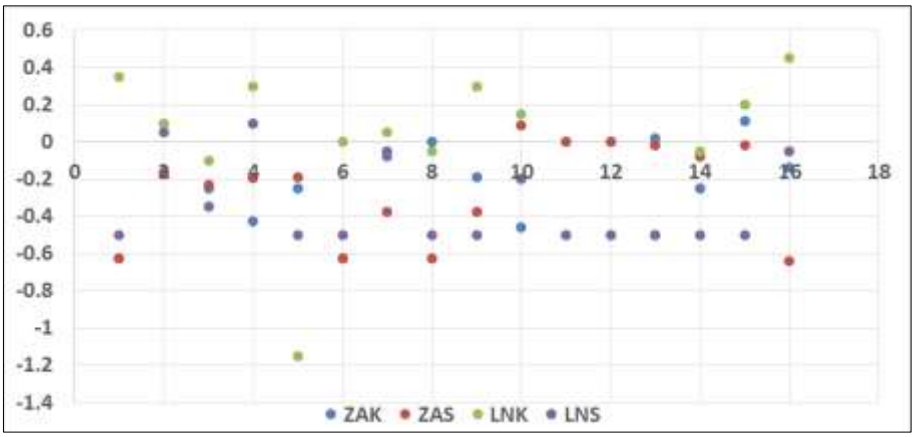

Fig 1: Scatter diagram showing Spearman rank correlation between elevation and phtochemicals

Table 6: Spearman's rank correlation between elevation and various phytochemical constituents

	Alkaloids		Flavonoids		Glycosides		Phenols	
	E	W	E	W	E	W	E	W
Zanthoxylum alatum (Kalimpong (W.B.)	-0.625	-0.161	-0.25	-0.429	-0.25	-0.625	-0.08	0
Zanthoxylum alatum (Sikkim)	-0.625	-0.17	-0.23	-0.196	-0.19	-0.625	-0.375	-0.625
Lindera neesiana (Kalimpong, W.B.)	0.35	0.1	-0.1	0.3	-1.15	0	0.05	-0.05
Lindera neesiana (Sikkim)	-0.5	0.05	-0.35	0.1	-0.5	-0.5	-0.05	-0.5

	Sterols		Saponins		Tannins		Terpenoids	
	E	W	E	W	E	W	E	W
Zanthoxylum alatum (Kalimpong (W.B.)	-0.19	-0.46	0	0	0.02	-0.25	0.11	-0.14
Zanthoxylum alatum (Sikkim)	-0.375	0.09	0	0	-0.02	-0.08	-0.017	-0.64
Lindera neesiana (Kalimpong, W.B.)	0.3	0.15	-0.5	-0.5	-0.5	-0.05	0.2	0.45
Lindera neesiana (Sikkim)	-0.5	-0.2	-0.5	-0.5	-0.5	-0.5	-0.5	-0.05

The Spearman's rank correlation between altitude and phytochemical constituents was calculated. As the study was qualitative in nature, scores were given for abundant presence (8.5), moderate presence (5.5) and slight presence (3.5).

Negative Spearman's rank correlation was observed in *Zanthoxylum alatum* (Kalimpong, W.B. and Sikkim) alkaloids (E, W), flavonoids (E,W), glycosides (E,W), phenols (E,W), sterols (E,W), tannins (W), terpenoids (W). Negative Spearman's rank correlation was observed in *Lindera neesiana* (Kalimpong, W.B.)- glycosides (E). phenol (W), saponins (E,W), tannins (E,W); *Lindera neesiana* (Sikkim)- alkaloids(E), flavonoids (E), glycosides (E,W), phenols (E,W), sterols (E,W), saponins (E,W), tannins (E,W) and terpenoids (E,W). The rest of the values in Spearman's rank correlation were found to be positive. Spearman's rank correlation was found to be better in indicating negative correlation between majority of phytochemicals and elevation. It was observed that there exists some relationship between the elevation and quantity of phytochemical constituents. As the study was qualitative in nature, it indicates and paves way for further studies on quantification of phytochemical constituents in *Zanthoxylum alatum* and *Linsera neesiana.*

4. Conclusion

Zanthoxylum alatum and *Lindera neesiana* are important and commonly found fruit plant species and Sikkim Himalayan region in India. In the present study, dry fruit powdered of these plants was test for different phytochemicals like alkaloids, flavonoids, glycosides, sterols, saponins, tannins, and terpenoids in aqueous and ethanol extracts. The obtained result justifies that the phytochemicals like alkaloids, flavonoids, glycosides, sterols, tannins, and terpenoids are found to be present in the fruits of *Zanthoxylum alatum.* It was also found that saponins was completely absent in the samples collected from Sikkim and Kalimpong (W.B.) however previous study claims the presence of saponins. Similarly fruits of *Lindera neesiana* showed the presence of all the phytochemicals in both extracts. It was observed that for majority of phytochemical constituents, their quantity decreases as elevation increases. Further research work is required to be done for the identification of antioxidant, hepatoprotective potentials of these species.

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