

### **Exploration of Phytopharmacognostic study of *Alianthus excelsa* Roxb.(Simaroubaceae) Leaves**

#### **ABSTRACT**

Aim: To study the pharmacognostic and phytochemical parameters of *Alianthus excelsa* leaves. Material and Methods: *Alianthus excelsa* Roxb. is a tree belonging to family Simaroubaceae, which is indigenous to central and Southern India. The Microscopical, physicochemical, phytochemical investigation, isolation, characterization and anti-inflammatory activity of total methanolic extract of leaves of *Alianthus excelsa* was investigated. The total ash, water soluble, acid insoluble, alcohol soluble extractive, water soluble extractive, moisture content and fluorescence property of leaf powder was evaluated. Results: The leaf methanolic oven dried extract showed the presence of carbohydrates, phenolic, flavonoids, alkaloids and amino acids. Total phenolic and total flavonoid contents were estimated to quantify the presence of phenolic content in extracts. During the course of the experimental work the leaf parts showed the presence of phytoconstituents like flavonoids and saponin glycoside which was isolated by column chromatography characterization done by IR, NMR and Mass spectroscopy. The three flavonoids and one triterpenoid saponin isolated from leaf extract. These studies help in identification and authentication of the plant material. Conclusions: These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible.

**Keywords:** *Alianthus excelsa*, Physiochemical, Phytochemical, column chromatography.

## 1. INTRODUCTION

During the past decade, the indigenous or traditional system has gained importance in the field of medicine. In most of the developing countries, a large number of populations depend on the traditional practitioners, who are dependent on medicinal plants to meet their primary health care needs. Although, modern medicines are available, herbal medicine retained their image for historical and cultural reasons. Since the usage of these herbal medicines has increased, issues and moto regarding their quality, safety and efficacy in industrialized and developing countries are cropped up [1]. *Ailanthus excelsa* Roxb is a deciduous tree belonging to the family Simaroubaceae and is widely distributed in Asia. Its native origin is China and it is known as 'Tree of Heaven' and used in the Indian system of medicine for variety of purposes.<sup>1</sup> It is used in wounds, skin eruption, febrifuge, bronchitis, asthma and in conditions of diarrhoea and dysentery [2,3] *Ailanthus excelsa* Roxb is a large deciduous tree, 18-25 m tall; trunk straight, 60-80 cm in diameter; bark light grey and smooth, becoming grey-brown and rough on large trees, aromatic, slightly bitter. Leaves alternate, pinnately compound, large, 30-60 cm or more in length; leaflets 8-14 or more pairs, long stalked, ovate or broadly lance shaped from very unequal base, 6-10 cm long, 3-5 cm wide, often curved, long pointed, hairy gland; edges coarsely toothed and often lobed. *A. excelsa* extracts and some isolated compounds have demonstrated pharmacological properties such as significant antileukemic, [4] antifungal, antibacterial [5,6] and antimalarial [7] Phytochemically, it found to contains  $\beta$ - Sitosterol, Quassinoids, Ailantic Acid, vitexin; ailanthione, glalucarubinone, malanthine, excelsin, glaucarubol, 2-6 Dimethoxy-Benzquinone and Melanthin[8]. Authenticity, purity, and assay of crude drug must be crosschecked for standardization and quality assurance. In the present study, we have evaluated macroscopy, microscopy, physicochemical, and phytochemical parameters of leaves of *A. excelsa*. The main objective of this study is to supplement some information regarding correct identification and standardization of *Alianthus excelsa*.

## **2. MATERIAL AND METHODS**

### **2.1 Collection and Authentication of plant material**

The leaves of *Alianthus excelsa* was collected from local region of Nashik (Maharashtra, India) within the month of June 2017 and authenticated by Dr. Shimpi, Taxonomist, head of Botany department, G. E. Society's NSC Science College, Nashik Road, Nashik, India. Voucher specimen No. 160 was placed at the herbarium for future reference. One part of the leaves is conserved in Formalin (5ml): Acetic acid (5ml): 70% Alcohol (90ml) blend pertaining to histological research as well as the remaining part was shade dried, powdered and then sieved by using 20 mesh and as well, retained within an air tight container for long term use.

### **2.2 Macroscopic Study**

The macroscopic studies were carried out using organoleptic evaluation method. The arrangement, size, shape, base, texture, margin, apex, venation pattern, colour, odour, taste of leaves were observed [9] Macroscopic and microscopic characters were studied as described in quality control method. Photographs at different magnifications were taken by using digital camera.

### **2.3 Microscopic study**

Microscopic was carried out by preparing thin sections leaf. The thin sections were further washed with water, stained with safranin, haematoxylin, picric acid, dil. Iodine solution and mounted in glycerine for observation and confirm its magnifications (10X, 45X).

### **2.4 Preparation of crude extract**

1 kg of powdered leaf sample was packed in a filter paper and made into thimbles. The sample filled thimbles were kept in the cylindrical sample holder present in the soxhlet apparatus and filled with organic solvents such as ethanol and methanol, individually. Plant samples were extracted with the said organic solvents. Organic solvents when mixed with plant sample produced coloured solution. Extraction was continued till the coloured solvent became transparent. During the extraction process, temperature of soxhlet apparatus set up was maintained at 65 °C for methanol extracts. The extraction process has taken approximately 10-12 hours for each sample. The methanol extract was distilled off under reduced pressure to obtained dry residue then it was dissolved in water and

partitioned with distilled water and n-butanol (1:1). Aqueous and butanol fraction were separated and aqueous fraction was again partitioned with n-butanol 2-3 times.

## **2.5 Fluorescence analysis**

Fluorescence study is an essential parameter for first line standardization of crude drugs. The powder drug of leaves and bark was treated separately with different reagents and exposed to visible and ultraviolet light to study their fluorescence behaviour [10].

## **2.6 Physicochemical and phytochemical analysis**

The physicochemical standards are important to check the quality, purity and adulteration of given crude drug. The foreign matter, LOD, ash, and extractive values were determined and summarized in table and the identification of phytoconstituents is done with the help of systematic preliminary phytochemical screening. It is also helpful in establishing a chemical profile of a crude drug for its proper evaluation. In addition, the total phenolic and flavonoid content of the extracts was determined by the method. The mean of three readings was used and the total phenol content and total flavonoid content was expressed in milligram of gallic acid equivalents/g extract and quercetin equivalents/g extract, respectively [11,12].

## **2.7 Isolation and characterization of phytoconstituents from *Alianthus excelsa* leaf**

### **2.7.1. Isolation and characterization of flavonoids**

The air-dried and powdered roots of *A. excelsa* (1.4 kg) were extracted with 70 % methanol (MeOH; 2×3 l) in a Soxhlet apparatus at 40°. After filtration the extract was evaporated under reduced pressure to render methanol extract (125 g). This extract was initially chromatographed over silica gel column under gradient conditions using benzene, chloroform, ethyl acetate and MeOH (100:0, 80:20, 60:40, 40:60, 20:80, 0:100). The eluted fractions were collected and monitored on TLC. Similar fractions were pooled together to obtain three major fractions (E<sub>1</sub>, E<sub>2</sub> and E<sub>3</sub>). Fraction E<sub>2</sub> (13.6 g) was re-chromatographed on silica gel column eluting with chloroform, ethyl acetate and MeOH mixture (10:0, 8:2, 6:4, 4:6, 2:8, 0:10, gradient) to yield three sub-fractions. These fractions were purified over Sephadex LH20 with 100 % MeOH to produce compounds 1 (17 mg, 0.0034 % w/w), 2 (13 mg, 0.0026 % w/w) and 3 (21 mg, 0.0042 % w/w), respectively. Structures of these isolated compounds were established on the basis of elemental analysis and UV, IR, <sup>1</sup>HNMR, <sup>13</sup>CNMR, and MS data as well as by comparison with published data [13].

### **2.7.2. Isolation and characterization of Saponin glycoside from *Alianthus excelsa* leaf:**

The methanol extract was distilled off under reduced pressure to obtain dry residue then it was dissolved in water and partitioned with distilled water and n-butanol (1:1). Aqueous and butanol fractions were separated and aqueous fraction was again partitioned with n-butanol 2-3 times. Combined n-butanol fraction was then evaporated under reduced pressure and dissolved in methanol. The methanolic solution was then drop wise added to solvent ether to yield crude saponin precipitates. The solvent was decanted off and precipitates were dried to constant weight at low temperature to yield crude saponins (10% w/w).

CS (crude saponin) was further subjected to column chromatography for separation & isolation of pure saponin glycoside. Total 220 fractions were collected from column chromatography by gradient elution using Ethyl acetate followed by methanol as more polar solvent in increasing proportion. The results of chemical test carried out for all the fractions indicated that the saponin present in the leaves of *Alianthus excelsa* include both triterpenoidal and steroidal moiety. Based on their similarity of TLC pattern, they were compiled to get final 18 fractions. The saponin compounds verified in HPTLC plate generated blue, pink, violet, violet black and yellowish brown saponin zones after spraying with Anisaldehyde sulphuric acid (AS) reagent. Eluted fraction 73 to 104 showed the presence of single spot on TLC with chloroform: methanol (80:20) as a solvent system and anisaldehyde sulphuric acid reagent as visualizing agent. The R<sub>f</sub> value of the same was found to be 0.36. After column chromatography, the fraction was evaporated and residue obtained was recrystallized from Ethyl acetate. AS reagent is suitable for the separation and detection of both, triterpene-saponins as well as steroidal saponins. The color of the saponin compounds observed before and after derivatization indicated that they include both triterpene and steroidal type of saponin [14].

### **3. RESULTS AND DISCUSSION**

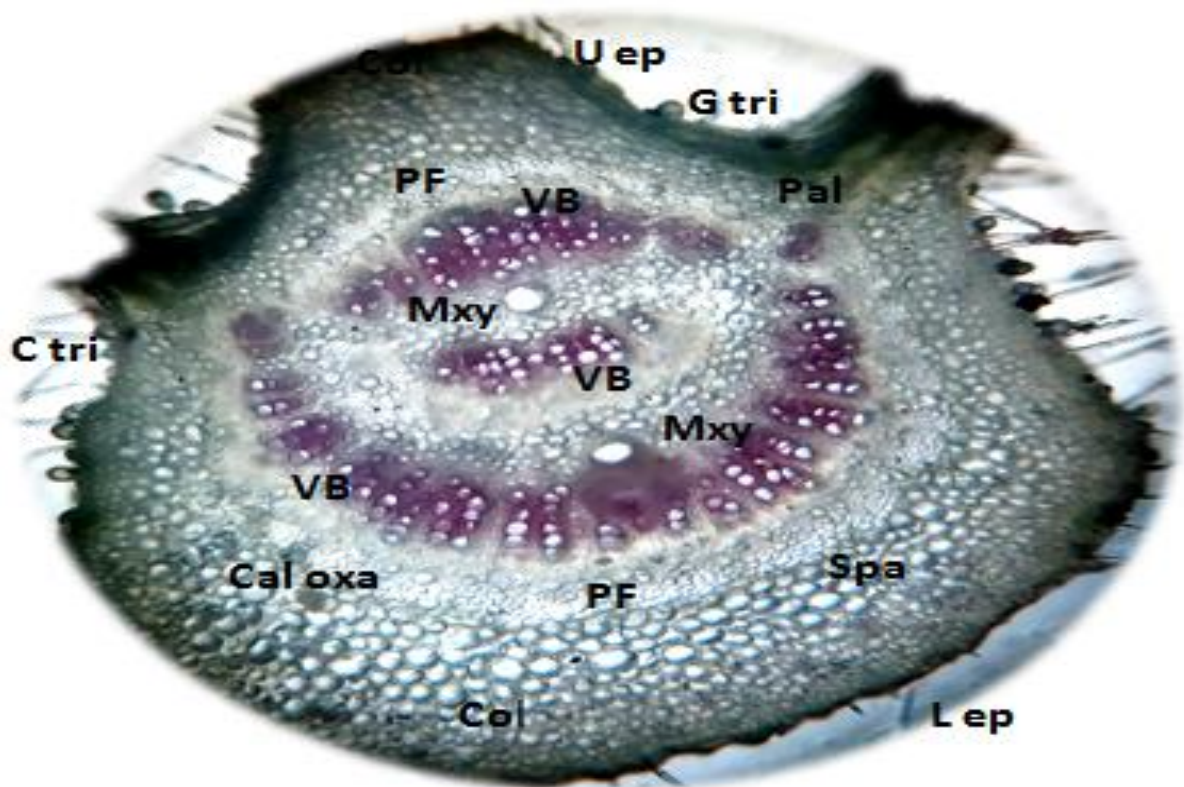
#### **3.1 Macroscopic feature**

A large deciduous tree grows up to 30 meters in height, with straight trunk. Leaves alternate, pinnately compound, large, 30-60 cm or more in length; leaflets 8-14 or more pairs, long stalked, ovate or broadly lance shaped from very unequal base, 6-10 cm long, 3-5 cm wide, often curved, long pointed, hairy gland, coarsely toothed and often lobed. Flower clusters droop at leaf bases, shorter than leaves, much branched; flowers many, mostly male and female on different trees, short stalked,

greenish-yellow; Fruit a 1-seeded samara, lance shaped, flat, pointed at ends, 5 cm long, 1 cm wide, copper, strongly veined and twisted at the base.

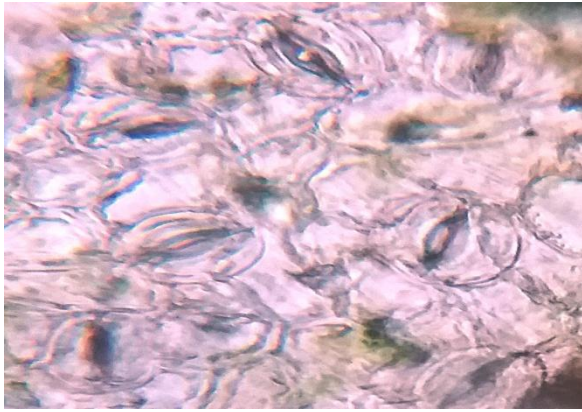
### 3.2 Microscopical study

Lamina of transverse section shows large, cuboidal cells of upper epidermis covered by a thin cuticle. Unicellular covering and glandular trichomes were present on both the epidermis, some of which are filled with cystolithic crystals and also paracytic stomata in both the epidermis. Underlying the upper epidermis is a bi-layered, compact, radially elongated palisade followed by spongy mesophyll composed of 5-8 layers of loosely arranged parenchymatous cells. Midrib consists of well-developed 6-7 layered thick wall collenchymas beneath the epidermis. Vascular bundles are bicollateral present at three sites and surrounded by continuous groups of non-lignified pericyclic fibres. Two small secondary vascular bundles are present above the primary vascular bundles. Ground tissue consists of loosely arranged polygonal parenchymatous cells filled with cluster and prism crystals of calcium oxalate and some cystolithic idioblasts (Figure 1 and Figure 2).

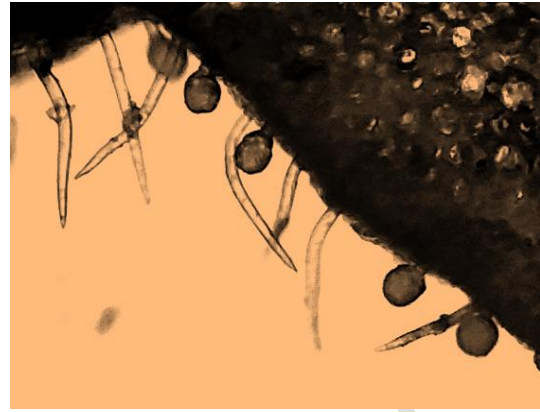


**Figure 1: Transverse section of leaf of *A. excelsa* leaf**

Uep: Upper epidermis, PF: Pericyclic fiber, G tri: Glandular trichome, Pal: Palisade tissue, VB: vascular bundle, Mxy: Metaxylem, Col: collenchyma, Cal oxa: Calcium oxalate crystals, C tri: covering trichome, Spa: Spongy parenchyma, Lep: Lower epidermis



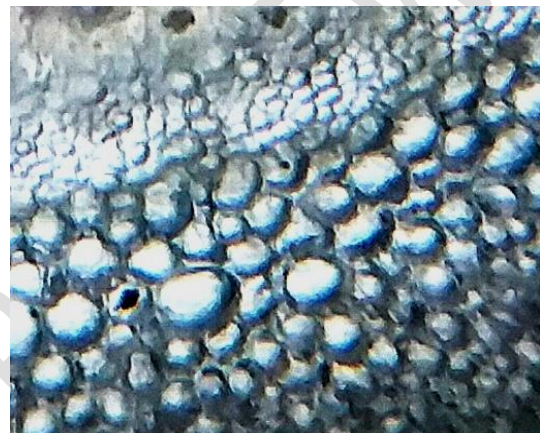
a. Paracytic stomata



b. Unicellular covering & glandular stomata



c. Xylem vessels along with phloem



d. Spongy mesophyll

**Figure 2: Magnified view of at 45 X**

### 3.3 Fluorescence analysis

The fluorescent colour is specific for each compound. Plant materials give different coloration when treated with various chemicals. Some plant constituents showed characteristic fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products which do not visibly fluoresce in daylight. If substance themselves are not fluorescent, addition of different reagents results in the conversion into fluorescent derivatives or decomposition products. Crude drugs are often assessed qualitatively in this way and fluorescence analysis was an important parameter for pharmacognostic evaluation of crude drugs (Table 1).

**Table 1 : Fluorescence analysis of *Alianthus excelsa* powder**

Reagents	Visible light	UV light (365nm)
Plant+1N NaOH Aqueous	Yellow	Bright yellow
Plant+1N NaOH Alcoholic	Light yellow	Bright yellow
Plant+1N Hcl	Light green	Dark green

Plant+H <sub>2</sub> SO <sub>4</sub> (1:1)	Light yellow	Green
Plant+HNO <sub>3</sub> (1:1)	Dark yellow	Yellow green
Plant+Ammonia	Yellow green	Yellow green
Plant+Iodine	Light green	Brown
Plant+5%FeCl <sub>3</sub>	Light green	Brown
Plant+Acetic acid	Light green	Green

The crude drug when viewed under UV light showed different fluorescence at different wavelengths. This is due to the presence of different phytochemical constituents in the drug [15]. Flavones which are light yellow in aqueous condition, under UV light, turns to bright yellow under alkaline conditions. Phytosterols, when treated with 50% H<sub>2</sub>SO<sub>4</sub> shows green fluorescence under UV light. Coumaric acid appears yellowish green in alkaline condition under UV radiation. Terpenoids, exhibits yellow green fluorescence under short UV light [16]. Berberin showed light yellow colour of fluorescence.

### 3.4 Physicochemical analysis

The total ash value represents both physiological and non-physiological ash. Physiological ash is the ash inherent in the plant due to biochemical processes and the non-physiological ash is the contaminants from the environment. These may be carbonates, phosphates, nitrates, sulphates, chlorides and silicates of various metals which were taken up from the soil. For the evaluation of purity of drugs, total ash value was particularly important. A high percentage of total ash value revealed the presence of inorganic constituents and very low value of acid insoluble ash indicated the presence of negligible amount of siliceous matter. The acid insoluble ash was a part of total ash that was insoluble in dilute HCl. Water soluble portion of the total ash constitutes the water soluble ash [17]. Water soluble ash can be used as an important indicator for the presence of exhausted material (Table 2)

**Table 2: Physicochemical evaluation of *Alianthus excelsa* leaves**

Sr. No.	Parameters	Percentage (w/w)
1.	Total ash	9.23
2.	Acid-insoluble ash	3.17
3.	Water soluble ash	4.93
4.	Moisture content	5.6
5.	Extractive values	
	i) Alcohol soluble	10.09

ii)	Water soluble	11.12
iii)	Chloroform soluble	2.9
iv)	Petroleum ether	1.2

### 3.5 Phytochemical analysis

Phytochemical analysis most of the chemical constituents of plant dissolved in aqueous and methanol, thus the percentage yield was increased tremendously than other solvents (Table 3).

**Table 3: Phytochemical analysis of *Alianthus excelsa* extract**

Sr. No.	Chemical constituents	Aqueous extract	Methanol extract	Chloroform extract	Petroleum ether extract
1.	Test for carbohydrates				
	a) Molisch test	+++	+++	---	---
	b) Benedict's test	+++	+++	---	---
	c) Fehling's test	+++	+++	---	---
2.	Test for Alkaloids				
	a) Mayer's test	+++	+++	---	---
	b) Dragandroff's test	+++	+++	---	---
	c) Wagner's test	+++	+++	---	---
	d) Hager's test	+++	---	---	---
3.	Test for Glycoside				
	a) Modified borntreger's test	+++	+++	+++	+++
	b) Legal's test	+++	+++	+++	+++
4.	Test for saponin				
	a) Froth test	+++	+++	---	---
	b) Foam test	+++	+++	---	---
5.	Test for Phytosterol				
	a) Salkowski test	---	+++	---	+++
	b) Libermann burchard's test	---	+++	---	+++
6.	Test for Phenols				
	a) Ferric chloride test	---	+++	---	---
7.	Test for tannin				
	a) Gelatin test	---	+++	---	---
8.	Test for flavonoids				
	a) Alkaline test	---	++	---	---
	b) Lead acetate test	---	++	---	---
9.	Protein & Amino acids				

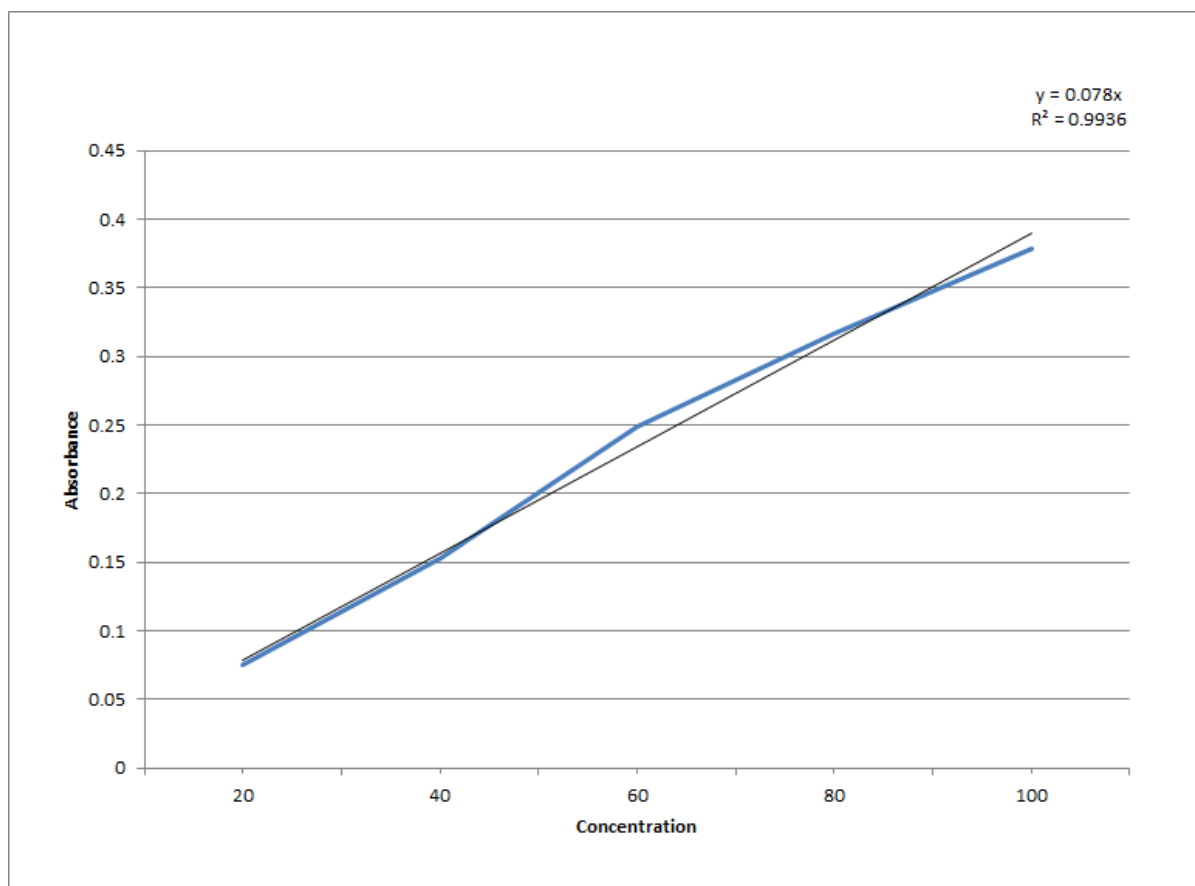
a)Xanthoprotein test	+++	+++	---	---
b)Ninhydrin test	+++	+++	---	---

### 3.6 Estimation of Total phenolic content:

The total concentration of phenolic compound was calculated on the basis of gallic acid calibration curve equation. Total Phenolic content of extract was measured by Folin- Ciocalteu reagent method. The total phenolic content of methanolic extract of *A. excelsa* leaves was found to 1.01% (Table 4 and figure 3).

**Table 4: Absorbance observed in estimation of total phenolic content**

Sr. No.	Conc. (µg/ml)	Absorbance (nm)			Mean ± SEM
		I	II	III	
<b>Standard (Gallic acid)</b>					
1	20	0.0750	0.0744	0.0748	0.0746 ± 0.0002
2	40	0.1536	0.1532	0.1553	0.1534 ± 0.0002
3	60	0.2481	0.2484	0.2492	0.2486 ± 0.0001
4	80	0.3172	0.3170	0.3164	0.3168 ± 0.0002
5	100	0.3685	0.3790	0.3780	0.3787 ± 0.0001
<b>Samples</b>					
Methanolic extract	400	0.31740	0.31570	0.31876	0.3172 ± 0.0008



**Figure 3: Showing calibration curve of Gallic acid**

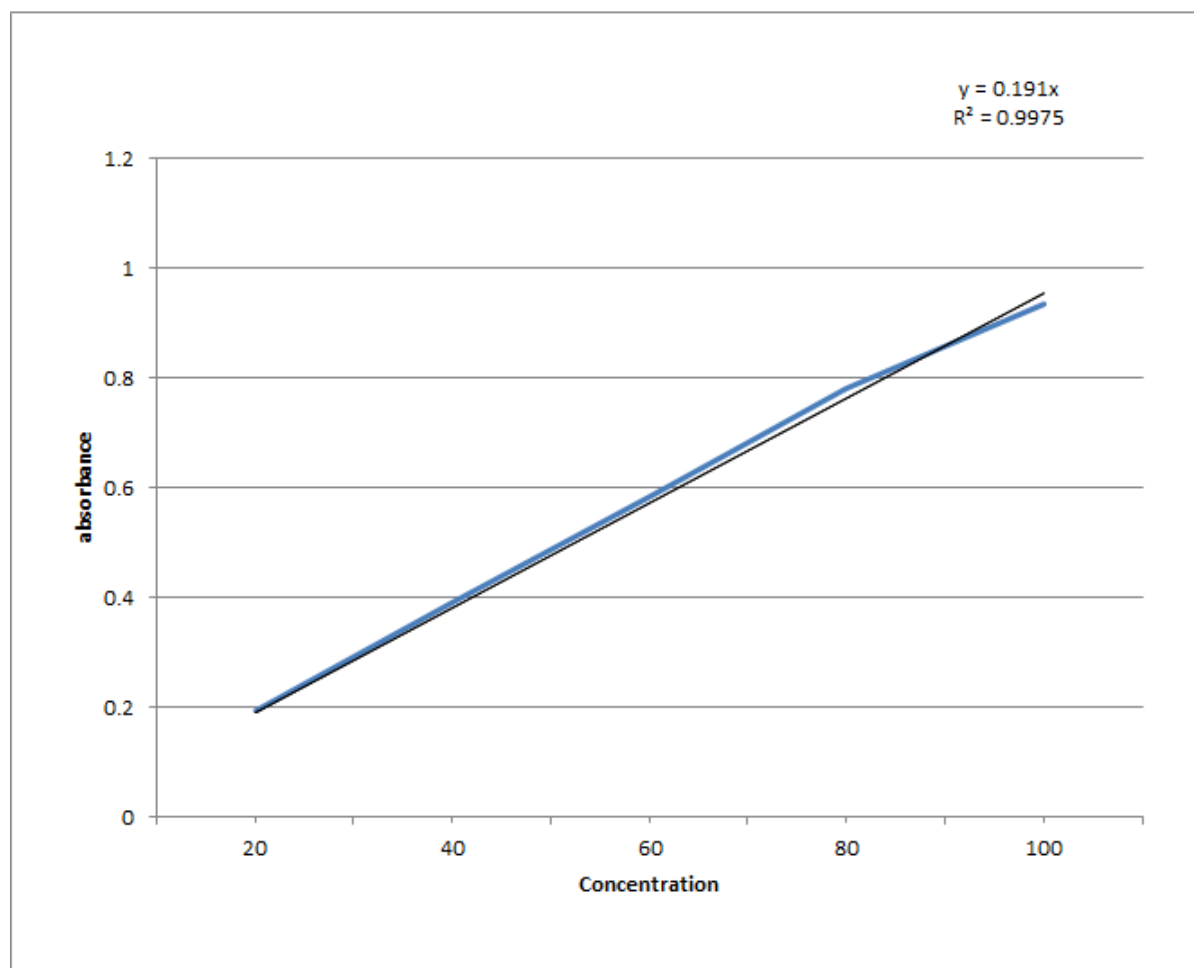
### 3.7 Estimation of Total flavonoid content

In above table absorbance of standard and test sample were mentioned. The total concentration of flavonoids was calculated on the basis of calibration Total flavonoids content of methanolic extract of *A. excelsa* was determined by aluminium chloride colorimetric method, by standard curve of rutin at various concentrations; and it was found to be 1.22% (Table 5 and figure 4).

Table 5: Absorbance observed in estimation of total flavonoids content

Sr. No.	Conc. ( $\mu\text{g/ml}$ )	Absorbance (nm)			Mean $\pm$ SEM
		I	II	III	
<b>Standard (Rutin)</b>					
1	20	0.1945	0.1953	0.1949	0.195 $\pm$ 0.0002
2	40	0.3888	0.3893	0.3891	0.389 $\pm$ 0.0001
3	60	0.5825	0.5826	0.5831	0.583 $\pm$ 0.0002
4	80	0.7781	0.7786	0.7788	0.779 $\pm$ 0.0002

5	100	0.9327	0.9329	0.9321	0.933 ± 0.0002
<b>Samples</b>					
MeOH extract	400	0.9392	0.9393	0.9392	0.931 ± 0.0002



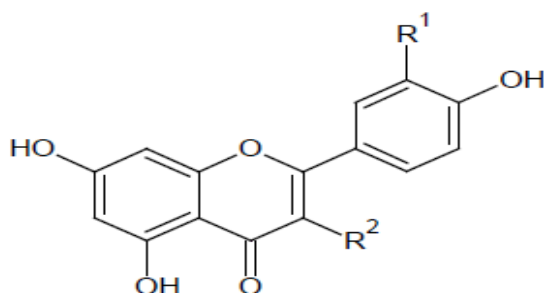
**Figure 4: Showing calibration curve of rutin**

### **3.8 Isolation and characterization of phytoconstituents from *Alianthus excelsa* leaf**

#### **3.8.1. Isolation and characterization of flavonoids**

Fraction E<sub>2</sub> (13.6 g) was re-chromatographed on silica gel column eluting with chloroform, ethyl acetate and MeOH mixture (10:0, 8:2, 6:4, 4:6, 2:8, 0:10, gradient) to yield three sub-fractions. These fractions were purified over Sephadex LH20 with 100 % MeOH to produce compounds 1 (17 mg, 0.0034 % w/w), 2 (13 mg, 0.0026 % w/w) and 3 (21 mg, 0.0042 % w/w), respectively. Structures of

these isolated compounds were established on the basis of elemental analysis and UV, IR,  $^1\text{H}$ NMR,  $^{13}\text{C}$ NMR, and MS data as well as by comparison with published data



**Figure 5: Structure of flavonoids isolated from *Alianthus excelsa***

Apigenin:  $\text{R}^1=\text{H}$ ,  $\text{R}^2=\text{H}$ ; Leuteolin:  $\text{R}^1=\text{OH}$ ,  $\text{R}^2=\text{H}$ ; Quercetin:  $\text{R}^1=\text{OH}$ ,  $\text{R}^2=\text{OH}$ .

Compound (1): elements %: (C 50.7, H 33.03, N<0.5)  $\text{C}_{15}\text{H}_{10}\text{O}_5$  (molecular weight: 270); MS/MS: m/z 269 (M-H); UV $_{\text{max}}$  (MeOH) nm: 270.4, 337; NaOAc nm: 275.2, 325.8, 391.6; NaOAc+ $\text{H}_3\text{BO}_3$  nm: 268.8, 341.2;  $\text{AlCl}_3$  nm: 276.8, 301.4, 343.6, 381;  $\text{AlCl}_3$ + HCl nm: 275.8, 301, 341.8; NaOH nm: 275.2, 325, 392.6; IR:  $3284\text{ cm}^{-1}$ : OH,  $1649\text{ cm}^{-1}$ : C=O, 1605, 1556 and  $1497\text{ cm}^{-1}$ : aromatic ring;  $^1\text{H}$  NMR (DMSO- $\text{d}_6$ , 400 MHz):  $\delta$  12.9, 10.9, 10.4 (1H, s, 5, 7, 4'-OH), 7.9 (2H, d, J 7.2 Hz, H-2'/H-6'), 6.9 (2H, d, J 7.2 Hz, H-3'/H-5'), 6.79 (1H, s, H-3), 6.49 (1H, d, J 2.1 Hz, H-8), 6.2 (1H, J 2.1 Hz, H-6);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $\text{d}_6$ ):  $\delta$  181.7 (s, C-4), 164.1 (s, C-2), 163.8 (s, C-7), 161.4 (s, C-5), 161.1 (s, C-4'), 157.4 (s, C-9), 128.5 (d, C-2'/C-6'), 121.1 (s, C-1'), 115.9 (d, C-3'/C-5'), 103.7 (s, C-10), 102.8 (d, C-3), 98.7 (s, C-6), 93.9 (d, C-8).

Compound (2): elements %: (C 48.09, H 32.76, N<0.5)  $\text{C}_{15}\text{H}_{10}\text{O}_6$  (mol. wt. 286); MS/MS: m/z 285 (M-H); UV $_{\text{max}}$  (MeOH) nm: 254.4, 348.2; NaOAc nm: 268.2, 401; NaOAc+ $\text{H}_3\text{BO}_3$  nm: 259.8, 371.8;  $\text{AlCl}_3$  nm: 272.8, 419.8;  $\text{AlCl}_3$ +HCl nm: 262.8, 274.8, 296, 359.6296, 359.6; NaOH nm: 270.4, 407.8; IR:  $3208\text{ cm}^{-1}$ : -OH,  $1661\text{ cm}^{-1}$ : C=O, 1606, 1506 and  $1441\text{ cm}^{-1}$ : aromatic ring;  $^1\text{H}$  NMR (DMSO- $\text{d}_6$ , 400 MHz):  $\delta$  12.97 (1H, s, 5-OH), 7.43 (1H, dd, J 7.8 Hz, H-6'), 7.41 (1H, d, J 2.2 Hz, H-2'), 6.92 (1H, d, J 7.8 Hz, H-5'), 6.66 (1H, s, H-3), 6.47 (1H, d, J 2.2 Hz, H-8), 6.21 (1H, d, J 2.2 Hz, H-6).  $^{13}\text{C}$  NMR (100 MHz, DMSO  $\text{d}_6$ ):  $\delta$  181.5 (C-4), 163.9 (C-2), 163.8 (C-7), 161.1 (C-5), 157.3 (C-9), 149.4 (C-4'), 145.5 (C-3'), 121.5 (C-1'), 118.8 (C-6'), 115.8 (C-5'), 113.1 (C-2'), 103.6 (C-10), 102.8 (C-3), 98.6 (C-6), 93.7 (C-8).

Compound (3): elements %: (C 46.17, H 31.15, N<0.5) C<sub>15</sub>H<sub>10</sub>O<sub>7</sub> (mol. wt. 302); MS/MS: m/z 301 (M-H); UVmax (MeOH) nm: 256.2, 371.8; NaOAc nm: 279.4, 331.6, 395S; NaOAc+H<sub>3</sub>BO<sub>3</sub> nm: 272.8, 387; AlCl<sub>3</sub> nm: 278.5, 326, 438.3; AlCl<sub>3</sub>+HCl nm: 266.7, 372, 422S; NaOH nm: 329.2, 426.4; IR: 3277 cm<sup>-1</sup>: -OH, 1655 cm<sup>-1</sup>: C=O, 1610, 1522 and 1449 cm<sup>-1</sup>: aromatic ring; <sup>1</sup>HNMR (DMSO-d<sub>6</sub>, 400 MHz): δ 12.5 (1H, s, 5-OH), 9.4 (1H, s, 4'-OH), 7.68 (1H, d, J 2.1 Hz, H-2'), 7.55 (1H, dd, J 7.4 Hz, H-6'), 6.9 (1H, d, J 7.4 Hz, H-5'), 6.41 (1H, d, J 2.1 Hz, H-8), 6.19 (1H, d, J 2.1 Hz, H-6); <sup>13</sup>CNMR (100 MHz, DMSO-d<sub>6</sub>): δ 175.8 (C-4), 163.9 (C-7), 160.7 (C-5), 156.1 (C-9), 147.7 (C-3'), 146.7 (C-2), 145.0 (C-4'), 135.7 (C-3), 121.9 (C-1'), 119.9 (C-6'), 115.6 (C-5'), 115.0 (C-2'), 103.0 (C-10), 98.1 (C-6), 93.3 (C-8).

### **3.8.2 Isolation and characterization of Saponin glycoside from *Alianthus excelsa* leaf**

The isolated fraction CS-73(Compound 4) was subjected to UV analysis on Shimadzu 2010 instrument & absorption maxima (λ max.) of isolated compounds was found to be 349.5nm which resembles to λ max value of homoanular diene keto steroids. The melting point for the isolated compound CS-73 was determined in open capillaries in an electrothermal melting apparatus & was found to be 205° C respectively.

The results of chemical tests (libermann-Burchard, salkowski, Whitby and trichloroacetic acid test) performed for the isolated compound showed positive results indicating presence of steroidal saponin (Mohammad Ali)

White amorphous powder. m.p.: 271-273° C. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): 0.75, 0.77, 0.90, 0.91, 0.93, 0.98 (each 3H, s, CH<sub>3</sub> x6), 1.13 (3H, s, H-27), 2.82 (1H, dd, J= 3.6, 13.2 Hz, H-18), 3.23 (1H, dd, J= 11.2, 4.4 Hz, H-3), 5.27 (1H, t, J=3.5 Hz, H-12). <sup>13</sup>C-NMR (125 MHz, Pyridined<sub>5</sub>): δ C (from C-1 to C-30) 39.0, 28.2, 78.1, 39.4, 55.8, 18.8, 33.3, 39.8, 48.2, 37.4, 23.7, 122.6, 144.8, 42.2, 28.4, 23.8, 46.7, 42.0, 46.5, 31.0, 34.3, 33.2, 28.8, 16.6, 15.6, 17.5, 26.2, 180.2, 33.3, 23.8.

## **4. CONCLUSION**

Standardization is essential measure for quality, purity and sample identification. Preliminary phytochemical tests showed that methanolic extract of *Alianthus excelsa* has greater amount of phytoconstituents compared to other two extract i.e chloroform, petroleum and aqueous extract. The powder of drug exhibited different fluorescence character due to presence of different functional

groups. TLC could be helpful in the identification and authentication of crude drug. The present work is undertaken to obtain some pharmacognostical standards. The above studies provide information with respect to the identification, chemical constituents and physicochemical characters of *Ailanthus excelsa*. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible.

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