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COMPARATIVE PHYSICOCHEMICAL, PHYTOCHEMICAL AND HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY EVALUATION OF STEM BARK AND SMALL BRANCHES OF *AILANTHUS EXCELSA* ROXB.

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ABSTRACT: Over-harvesting of many traditional medicinal plants has become a threat to the country's species diversity and has resulted in the endangerment of certain medicinal plant species. Shortly, many plant species may be unavailable for the use of industry due to over-exploitation. The present study outlines the concept of plant part substitution. The studies were carried out in *Ailanthus excelsa* to evaluate the possibilities of using small branches in place of stem bark which will help sustainable utilization. Stem bark and small branches of *Ailanthus excelsa* are compared by the physicochemical analysis, phytochemical analysis, total phenolic contents, total flavonoid contents and High-Performance Thin Layer Chromatography (HPTLC). Phytochemical analysis and HPTLC of *n*-hexane, ethyl acetate, and ethanol extracts showed many similarities which suggest that small branches may have nearly similar active potency like stem bark and may provide the base for further study to use small branches as a substitute of stem bark of *Ailanthus excelsa.* The study will be helpful in identification and quality control of *Ailanthus excelsa* and can provide standard HPTLC profiles of *Ailanthus excelsa* with the selected solvent system for use as a reference for proper identification/ authentication of the drug.

INTRODUCTION: Medicinal plants comprise an effective source of conventional and modern medicine. In India, a large number of the population depends on medicinal plants and indigenous system of medicine for primary health care.

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Over-harvesting of many traditional medicinal plants has become a threat to the country's species diversity and has resulted in the endangerment of certain medicinal plant species. Substitution of the plant is, therefore, the need of the hour for medicinal plants becoming red listed. It will provide greater scope for the physician to utilize herbs that are easily available, cost-effective and most appropriate for the clinical condition. *Ailanthus excelsa* (Family: Simaroubaceae) commonly called aralu is a precious medicinal plant widely used in Ayurveda. As per Ayurvedic literature, stem bark of this plant is used in atisara,

krmi, arsa, sannipata jvara, bhrama, tvakaroga, chardi, kustha, pravahika, grahani, prmeha, svasa, gulma, and musaka visaja roga [1]. The stem bark is also reported for various pharmacological activities like antifertility, antifungal, antimalarial, anti-bacterial, anti-amoebic**,** hypoglycemic, insect feedent-deterrent, antipyretic [2], anti-amoebic [3], hepatoprotective [4], antiasthmatic [5]**,** bronchodilatory [6], antihistaminic [7] and management of asthma [8]. Several chemical constituents like C20-quassinoid: glaucarubin [9], 1,12-deoxy-13-formyl ailanthinol [10], C25-quassinoid: excelsin [11-13], 2-6 dimethoxy-benzoquinone, melanthin [14], 1,4-dihydroexcelsin, 2, 4-dihydroexcelsin [11], 3, 4-dihydroexcelsin, ailanthinone, glaucarubinone, glaucarubolone [13], 13, 18- dihydroexcelsin, glaucarubol [15], β sitosterol [14, 10], triacontane, hexatriacontane [16], ailanex A, ailanex B, and polyandrol [13, 17] *etc*. have identified and isolated from this plant.

Removal of stem bark from the trunk of this tree may affect the survival of this plant due to which availability of this plant may be difficult shortly for use in the Indian system of medicine. To protect the survival of this plant and to ensure the availability of stem bark as raw material to manufacturers and dealers of indigenous drugs, there is strong need to explore the possibility of substitution of stem bark with a suitable alternative. An approach which would satisfy the necessities of sustainable harvesting, yet simultaneously provide for health care needs, would be the substitution of stem bark with aerial part of the same plant. The present study is an attempt to evaluate the possibilities of using small branches in place of stem bark. Standard physicochemical parameters of small branches of *A. excelsa* have not been prepared yet. So work is also carried out to establish preliminary physicochemical standards of small branches.

MATERIAL AND METHODS:

Plant Material: Stem bark and small branches of *A. excelsa* were collected from the National Research Institute for Ayurveda Siddha Human Resource Development, Aamkho, Gwalior, (M.P), India. Identified and authenticated by Shree N. K. Pandey, Research Officer (Botany), NRIASHRD, Gwalior.

Instrumentation: CAMAG HPTLC system (Muttenz, Switzerland) equipped with semi-automatic TLC applicator Linomat IV, twin trough plate development chamber, Win CATS software version 1.4.2 and Hamilton (Reno, Nevada, USA) Syringe (100 µl).

Material and Reagents: All chemicals, reagents, and solvents used during the experiments were of analytical grade, and HPTLC plates were purchased from E. Merck Pvt. Ltd. (Mumbai, India).

Physicochemical Parameters: Stem bark & small branches were studied for various physicochemical standards like foreign matter, loss on drying at 105°C, total ash, acid-insoluble ash, alcohol-soluble extractive, water-soluble extractive and pH of 10% solution using standard methods [18, 19].

Preliminary Phytochemical Screening: *n*-hexane, ethyl acetate and ethanol extract of both stem bark and small branches were screened for the presence of phenols, tannins, carbohydrates, saponins, alkaloids, proteins, flavonoids, steroids, coumarins, quinone, furanoids, and terpenoids by the standard methods of Harbone [20] and Kokate [21].

Estimation of Total Phenolic and Flavonoid Content: Five grams of each of the shade-dried plant material was pulverized into coarse powder and subjected to ethanolic extraction using Soxhlet apparatus. Extracts were concentrated to dryness. Dried residues were then dissolved in 100 ml of 95% ethanol. The extracts were used for total phenolic and flavonoid assay.

Total phenolics content was determined by using Folin-ciocalteu assay [22]. An aliquot (1 ml) of extracts or standard solution of tannic acid (20, 40, 60, 80 and 100 µg/ml) was added to a 25 ml volumetric flask, containing 9 ml of distilled water. A reagent blank was prepared using distilled water. One milliliter of Folin-ciocalteu phenol reagent was added to the mixture and shaken.

After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. Volume was then made up to the mark. After incubation for 90 min at room temperature, absorbance against the reagent blank was determined at 550 nm with a UV/Vis spectrophotometer. Total phenolics content was expressed as µg tannic acid equivalents (TAE).

Total flavonoid content was measured by the aluminium chloride colorimetric assay [23]. An aliquot (1 ml) of extracts or standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) was added to a 10 ml volumetric flask containing 4 ml of distilled water. To the flask, 0.3 ml of 5% $NaNO_2$ was added, and after 5 min, 0.3 ml of 10% $AlCl_3$ was added. After 5 min, 2 ml of 1M NaOH was added, and the volume was made up to 10 ml with distilled water. The solution was mixed, and absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed as µg quercetin equivalents (QE).

HPTLC Profiles: HPTLC studies were carried out following the method of Sethi [24], Stahl [25] and Wagner et al [26]. Stem bark and small branches were powdered coarsely. Ten-gram powdered samples of each of stem bark and small offices were accurately weighed and exhaustively extracted by *n*-hexane, ethyl acetate and ethanol (each 100 ml) separately using Soxhlet apparatus. Extracts were filtered and concentrated under reduced pressure and made up to 10 ml in standard flasks separately. The mobile phase used for developing the *n*-hexane extract of stem bark and small branches were toluene: ethyl acetate (7:3 v/v) and for ethyl acetate and ethanol extracts of stem bark and small branches was toluene: ethyl acetate: formic acid (8: 2: 0.1v/v/v).

Samples were spotted in the form of bands of width 10 mm with a 100 µl Hamilton syringe on aluminum TLC plates pre-coated with Silica gel 60 F_{254} of 0.2 mm thickness with the help of TLC semi-automatic applicator Linomat IV attached to CAMAG HPTLC system, which was programmed through Win CATS software version 1.4.2. 10 µl of each extract of stem bark and small branches were applied in two tracks as 10 mm bands at a spraying rate of 10 seconds/µl. Track 1 was stem bark, and track 2 was small branches for each of the extracts applied.

Development of plate up to a migration distance of 80 mm was performed at 27 ± 2°C with mobile phase for each extract in a CAMAG HPTLC chamber previously saturated for 30 min. After development, the plate was dried at 60°C in an oven for 5 min and visualized under wavelength 254 nm and 366 nm for ultraviolet detection. Developed plate was then dipped in anisaldehyde sulphuric acid reagent for derivatization and dried at 105°C in hot air oven till the color of the band appears and visualized under white light. Images were captured by keeping the plates in photo documentation chamber, and R_f values were recorded by Win CATS software.

RESULTS AND DISCUSSION: Physicochemical parameters like foreign matter, loss on drying at 105 ºC, ash values, insoluble acid ash, extractive values, and pH are given in **Table 1**. These data can be used for identification of the drug. The approximately the same value of alcohol soluble extractives for both stem bark and small branches indicates the presence of approximately same amount of polar extractable compounds in stem bark and small branches.

TABLE 1: PHYSICOCHEMICAL PARAMETERS OF STEM BARK AND SMALL BRANCHES OF *A. EXCELSA*

S. no.	Parameters	Results	
		Stem bark	Small branches
1	Foreign matter (% w/w)	Nil	Nil
2	Loss on drying (% w/w)	8.31	8.79
3	Total ash (% w/w)	7.80	4.52
4	Acid insoluble ash (% w/w)	0.47	0.43
5	Alcohol soluble extractive value (% w/w)	2.11	1.62
6	Water soluble extractive value (% w/w)	9.00	16.11
7	pH of 10 % aqueous solution	5.90	6.64

Phytochemical analysis of different extracts of stem bark and small branches are shown in **Table 2**. Results reveal the presence of similar phyto-chemicals in stem bark, and small branches except for coumarins which were found present in ethyl acetate and ethanol extract of small branches and absent in stem bark and for terpenoids which were found present in ethanol extract of stem bark and absent in ethanol extract small branches.

The total amount of phenolics and flavonoids content of ethanolic extract of stem bark and small

branches of *A. excelsa* are summarized in **Table 3**. Results indicate that in comparison to stem bark, small branches had a high total phenolic and flavonoid contents.

TABLE 2: PHYTOCHEMICAL ANALYSIS OF EXTRACTS OF STEM BARK AND SMALL BRANCHES OF *A. EXCELSA*

Phytochemicals	Stem bark			Small branches		
	***n*-Hexane**	**Ethyl acetate**	**Ethanol**	***n*-Hexane**	**Ethyl acetate**	**Ethanol**
Phenols	-ve	+ve	+ve	-ve	+ve	+ve
Tannins	-ve	+ve	+ve	-ve	+ve	+ve
Alkaloids	-ve	+ve	+ve	-ve	+ve	+ve
Carbohydrates	-ve	+ve	+ve	-ve	+ve	+ve
Saponins	-ve	-ve	+ve	-ve	-ve	+ve
Proteins	+ve	+ve	+ve	+ve	+ve	+ve
Steroids	+ve	+ve	+ve	+ve	+ve	+ve
Flavonoids	-ve	-ve	-ve	-ve	-ve	-ve
Coumarins	-ve	-ve	-ve	-ve	+ve	+ve
Quinone	-ve	-ve	+ve	-ve	-ve	+ve
Furanoids	-ve	-ve	-ve	-ve	-ve	-ve
Terpenoids	+ve	+ve	+ve	+ve	+ve	-ve

TABLE 3: TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT OF ETHANOLIC EXTRACTS OF STEM BARK AND SMALL BRANCHES OF *A. EXCELSA*

S. no.	Plant parts	Total phenolics µg of TAE/ g dry weight*	Total flavonoids µg of QE/ g dry weight*
1.	Stem bark	50.1±0.067	45.2±0.0918
2.	Small branches	91.9±0.149	224.8.±0.376

*Values are expressed as Mean ± SD

Comparative HPTLC profile of *n*-hexane, ethyl acetate and ethanol extracts of stem bark and small branches of *A. excelsa* were recorded to reveal the chemical pattern of each extract. HPTLC profile of *n*-hexane extract of both stem bark and small branches **Fig. 1** and **Table 4** showed no bands when visualized under UV at 254 nm. At UV 366 nm, stem bark and small branches showed five and seven bands, respectively out of which all the five bands at R_f 0.08 (green), 0.43(green), 0.71 (fluorescent green), 0.87 (red), 0.90 (red) were found similar. Visualization under white light after derivatization with anisaldehyde sulphuric acid reagent stem bark and small branches showed five and nine bands, respectively out of which five bands at R_f 0.21(blue), 0.31(blue), 0.38(blue), 0.46(blue), 0.84(grey) were found similar.

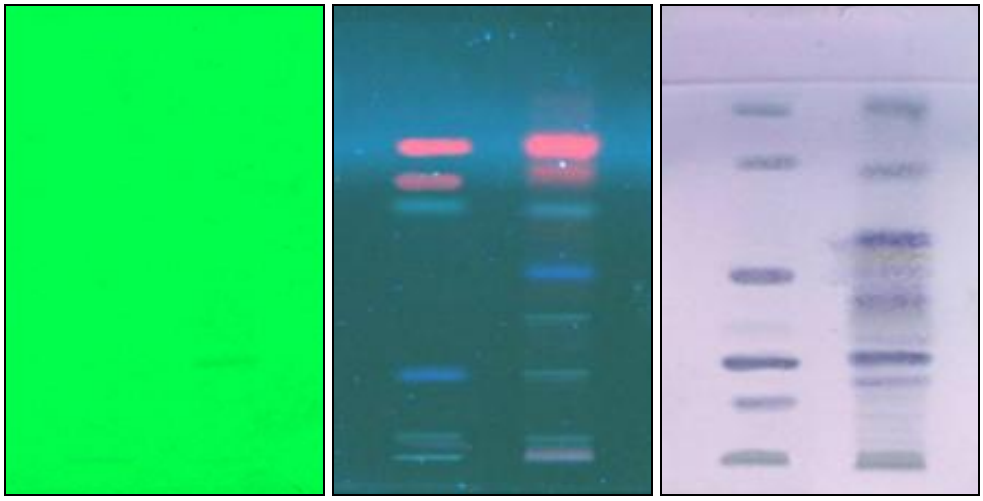

FIG. 1: HPTLC PROFILE OF *n*-HEXANE EXTRACTS OF STEM BARK AND SMALL BRANCHES OF *A. EXCELSA* (TRACK 1: STEM BARK, TRACK 2: SMALL BRANCHES)

TABLE 4: R_f VALUE OF n-HEXANE EXTRACT OF *A. EXCELSA*

S. no.	Wavelength	R_f value	
		Stem bark	Small branches
1	254 nm	No band	No band
2	366 nm	0.08, 0.43, 0.71, 0.87, 0.90.	0.08, 0. 43, 0.58, 0.65, 0.71, 0.87, 0.90.
3	Visible light after derivatization	0.21, 0.31, 0.38, 0.46, 0.84.	0.21, 0.26, 0.31, 0.38, 0.46, 0.49, 0.53, 0.68, 0.84.

It is interesting that all the bands of stem bark were found present in the small branches. This denotes that in hexane extract all the compounds which are present in stem bark are also present in small branches and a number of bands in small branches indicate the additional number of compounds in this.

HPTLC profile of ethyl acetate extract of stem bark and small branches **Fig. 2** and **Table 5** showed four and no bands at UV at 254 nm. At UV 366 nm stem bark and small branches showed seven and six bands, respectively out of which four bands at R_f 0.08 (red), 0.36 (dark blue), 0.51(fluorescent blue), 0.74 (fluorescent blue) were found similar. Visualization under white light after derivatization with anisaldehyde sulphuric acid reagent, both stem bark and small branches showed seven bands out of which three bands at R_f 0.17 (purple), 0.20 (blue), 0.89 (dark green) were found similar. This indicates the presence of many similar compounds also in ethyl acetate extract of stem bark and small branches.

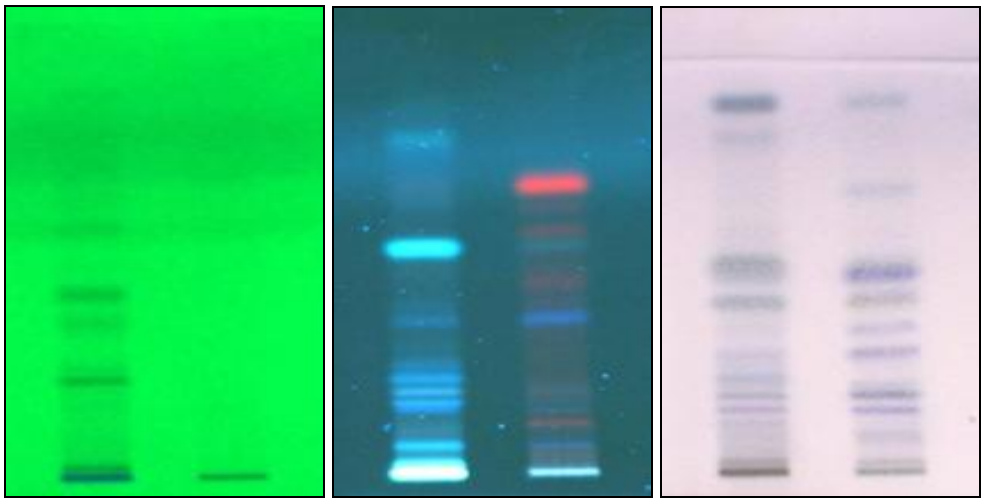

FIG. 2: HPTLC PROFILE OF ETHYL ACETATE EXTRACTS OF STEM BARK AND SMALL BRANCHES OF *A. EXCELSA* (TRACK 1: STEM BARK, TRACK 2: SMALL BRANCHES

TABLE 5: R_f VALUE OF ETHYL ACETATE EXTRACT OF *A. EXCELSA*

S. no.	Wavelength	R_f value	
		Stem bark	Small branches
1	254 nm	0.22, 0.35, 0.41, 0.55.	No band
2	366 nm	0.08, 0.17, 0.20, 0.23, 0.36, 0.51, 0.74.	0.08, 0.13, 0.36, 0.43, 0.51, 0.74.
3	Visible light after derivatization	0.17, 0.20, 0.24, 0.30, 0.50, 0.82, 0.89.	0.17, 0.20, 0.34, 0.41, 0.48, 0.67, 0.89.

HPTLC profile of ethanol extract of both stem bark and small branches **Fig. 3** and **Table 6** showed no bands when visualized under UV at 254 nm. At UV 366 nm stem bark and small branches showed seven and eight bands, respectively out of which five bands at R_f 0.10 (fluorescent blue), 0.17 (blue), 0.22 (blue), 0.38 (dark blue), 0.65 (red) found similar. Visualization under white light after derivatization with anisaldehyde sulphuric acid reagent stem bark and small branches showed five and eight bands, respectively out of which one band at R_f 0.47 (purple) found similar. This indicates the presence of many similar compounds in ethanol extract of stem bark and small branches also.

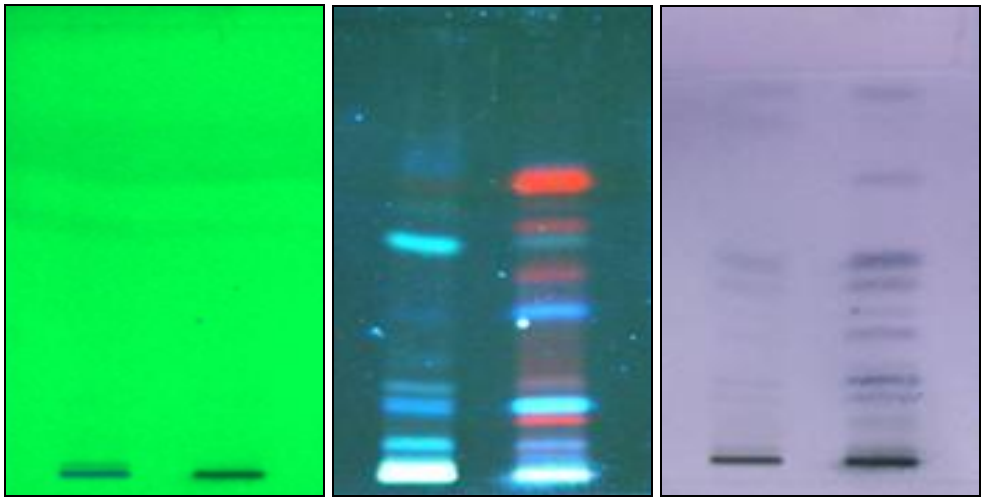

FIG. 3: HPTLC PROFILE OF ETHANOL EXTRACTS OF STEM BARK AND SMALL BRANCHES OF *A. EXCELSA* (TRACK 1: STEM BARK, TRACK 2: SMALL BRANCHES)

TABLE 6: R_f VALUE OF ETHANOL EXTRACT OF *A. EXCELSA*

S. no.	Wavelength	R_f value	
		Stem bark	Small branches
1	254 nm	No band	No band
2	366 nm	0.10, 0.17, 0.22, 0.38, 052, 0.65, 0.70.	0.10, 0.14, 0.17, 0.22, 0.38, 0.46, 0.53, 0.65.
3	Visible light after derivatization	0.19, 0.40, 0.47, 0.78, 0.86.	0.10, 0.17, 0.20, 0.30, 0.41, 0.47, 0.65, 0.84.

CONCLUSION: Many similarities in HPTLC profiles and phytochemical analysis of *n*-hexane, ethyl acetate and ethanol extracts of stem bark and small branches suggests that small branches may have nearly similar active potency like stem bark. The study provides the base for further study to recommend small branches in place of stem bark which will help sustainable utilization. The study will also be useful in identification and quality control of drug and can provide standard HPTLC profiles with a selected solvent system for proper identification/ authentication of the drug.

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CONFLICT OF INTEREST: Nil

REFERENCES:

1. The Ayurvedic Pharmacopoeia of India: Ministry of Health and Family Welfare, Department of Indian System of Medicine and Homeopathy. New Delhi: Government of India. 1st Edition, Part I, Vol. III 1999.
2. Lavhale MS and Mishra SH: Nutritional and therapeutic potential of *Ailanthus excelsa*: A review. Pharmacogn Rev 2007; 1(1): 105-113.
3. Yoganandam GP, Periyanayagam K, Ilango K, Biswas D and Gowri R: *In-vitro* anti-amoebic activity of stem bark of *Ailanthus excelsa* Roxb. (Simaroubaceae). News lett Pharmacologyonline 2009; 1: 27-34.
4. Yoganandam, GP, Periyanayagam K and Ilango K: Protective effect of ethanolic extract of stem bark of *Ailanthus excelsa* Roxb. against CCl_4 induced hepato-toxicity in rats. Drug Invention Today 2009; 1(1): 28-31.
5. Kumar D, Bhujbal SS, Deoda RS and Mudgade SC: *In-vitro* and *in-vivo* antiasthmatic studies of *Ailanthus excelsa* Roxb. on guinea pigs. J Sci Res 2010; 2(1): 196-202.
6. Kumar D, Bhujbal SS, Deoda RS and Mudgade SC: Bronchodilator activity of aqueous extract of stem bark of *Ailanthus excelsa* Roxb. Pharmacognosy Res 2010; 2(2): 102-106.
7. Kumar D, Bhat ZA, Singh P, Bhujbal SS and Deoda RS: Antihistaminic activity of aqueous extract of stem bark of *Ailanthus excelsa* Roxb. Pharmacognosy Res 2011; 3(3): 220-224.
8. Kumar D, Bhujbal SS, Patil PS and Buge PV: *In-vitro* and *in-vivo* activities of stem bark of methanolic extract of *Ailanthus excelsa* Roxb. in the management of asthma. Int J Pharmacol 2010; 6(3): 284-289.
9. Khan SA and Shamsuddin KM: Quassinoids from *Ailanthus excelsa*. Indian J Chem 1978; 16B:1045-1046.
10. Bhatia N, Mohan Y and Khosa RL: Chemical studies on *Ailanthus excelsa* Roxb. Bark. Indian Drugs 1983; 20: 240.
11. Khan SA, Zuberi SS and Shamsuddin KM: Isolation and structure of excelsin, a new quassinoid from *Ailanthus excelsa*. Indian J Chem 1980; 19B:183-184.
12. Tripathi AK and Jain DC: Excelsin: an insect feeding-deterrent isolated from *Ailanthus excelsa* (Simaroubaceae). Phytother Research 1993; 7(4): 323-325.

13. Joshi BC, Pandey A, Sharma RP and Khare A: Quassinoids from *Ailanthus excelsa*. Phytochemistry 2003; 62(4): 579-584.
14. Jain MK: Chemical examination of *Ailanthus excelsa* DC. Indian J Chem 1964; 2B: 40-41. 15.
15. Khan SA and Shamsuddin K: Isolation and structure of 13, 18-dehydroexcelsin, a quassinoid, and glaucarubol from *Ailanthus excelsa*. Phytochemistry 1980; 19(11): 2484-2485.
16. Mehta CR and Patel CN: Chemical examination of the bark of *Ailanthus excelsa* Roxb. Part I. Indian J Pharm 1959; 21: 143-145.
17. Pandey A, Joshi BC, Sharma RP and Khare A: New quassinoids from *Ailanthus excelsa*. Med Chem Res 2004; 13(8): 781-789.
18. Mukharjee PK: Quality Control of Herbal Drugs. New Delhi: Business Horizons Pharmaceutical Publishers; 2008: 186.
19. Indian Herbal Pharmacopoeia. Indian Drug Manufacturers' Association. Revised edition. Mumbai: Indian Herbal Pharmacopoeia, 2002: 493 494.
20. Harborne JB: Phytochemical methods. A guide to modern techniques of plant analysis. Chapman and Hall Co., New York, Edition 3rd, 1998.
21. Kokate CK, Purohit AP and Gokhale SB: Pharmacognosy. Nirali Prakashan, Pune, India, Edition 39th, 2007.
22. Singleton VL and Rossi Jr. JA: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Viticult 1965; 16: 144-158.
23. Zhishen J, Mengcheng T and Jianming W: The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999; 64: 555-559.
24. Sethi PD: High-Performance Thin Layer Chromatography. CBS Publishers and Distributers, New Delhi, Student, Edition 1st, Vol. X, 1996.
25. Stahl I: Thin Layer Chromatography. A laboratory handbook. Springer- Verlag Berlin, 1969.
26. Wagner H and Bladt S: Plant drug analysis a thin layer chromatography. Atlas, Springer-Verlag, Germany, Edition 2nd, 1984.

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