

**UHPLC-MS/SRM Method for Quantification of Neem Metabolites
from Leaf Extracts of Meliaceae Family Plants**

Supplementary Data

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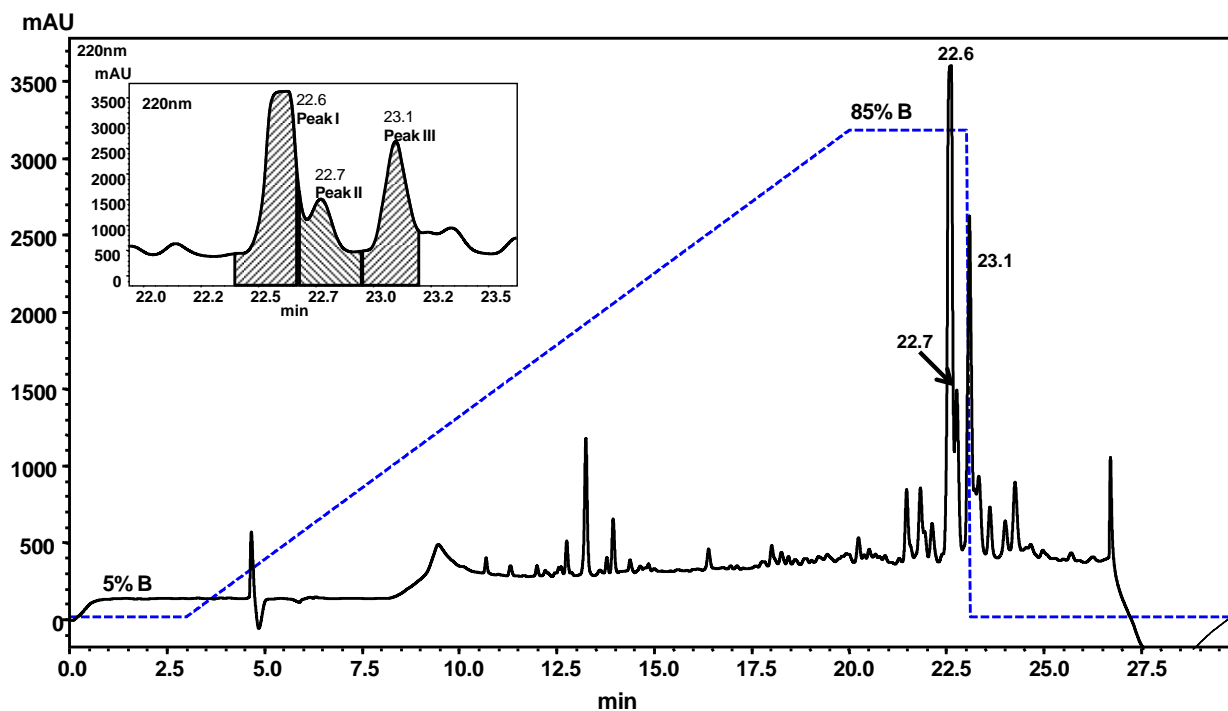
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Supplementary Table 1: Extraction of neem metabolites using different solvents.

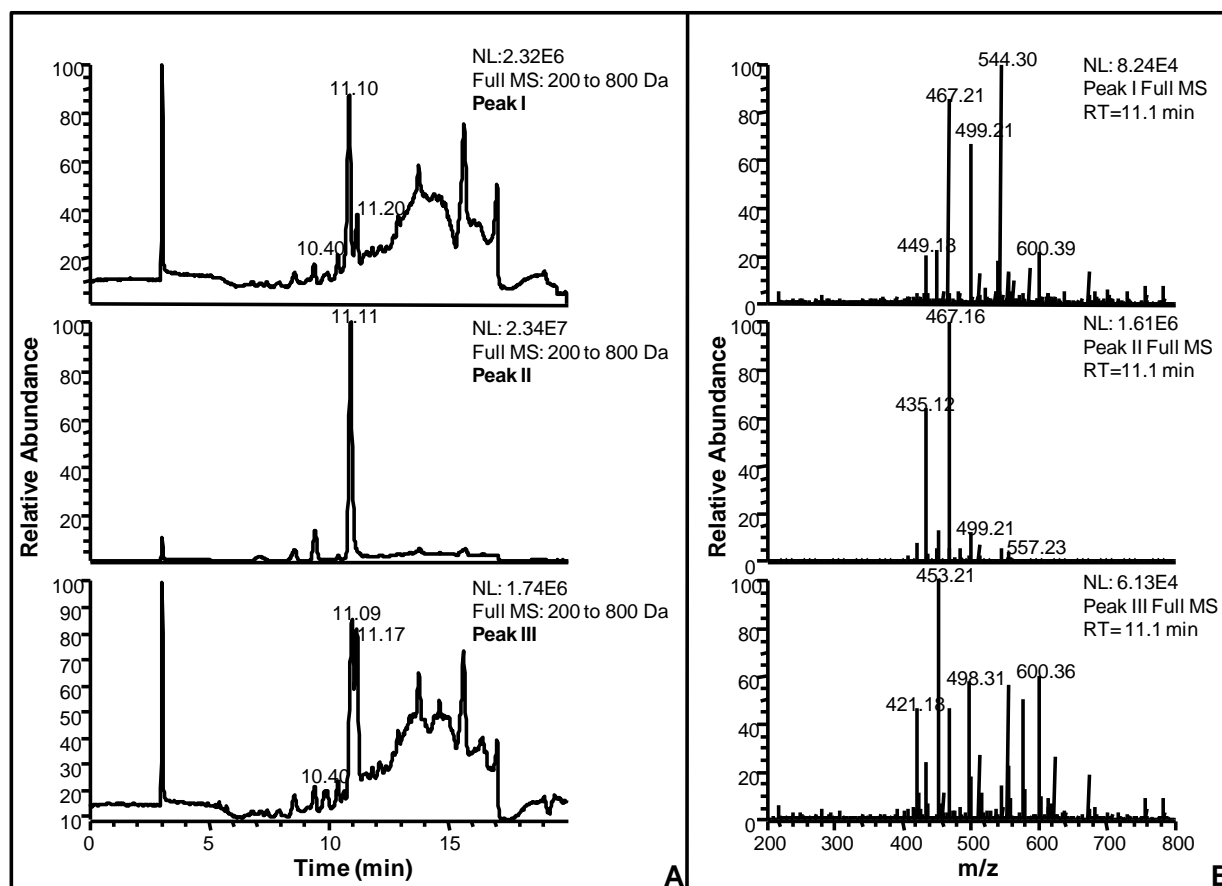
	Solvents	Aza A	Nimbin	Salanin	Azadiradione	Epoxy or hydroxy azadiradione
5 min Sonication	Methanol	2.83	95.01	199.48	125.24	226996.09
	Acetonitrile	2.77	2.20	28.37	25.14	56870.17
	Acetone	2.40	-6.67	9.00	13.10	41836.19
	Ethylacetate	2.16	20.87	57.27	39.13	98005.54
	Methyltert-butyl ether	2.95	71.80	159.53	92.42	207641.33
1 hr at 37 °C	Methanol	2.83	94.88	200.24	124.86	246959.23
	Acetonitrile	3.08	41.09	113.39	69.48	144373.88
	Acetone	2.75	38.14	106.45	58.98	151603.70
	EthylAcetate	2.30	53.40	128.04	80.91	170426.75
	Methyltert-butyl ether	2.53	65.87	155.37	94.73	203949.75

The amounts presented here are pg/μg of leaf extract

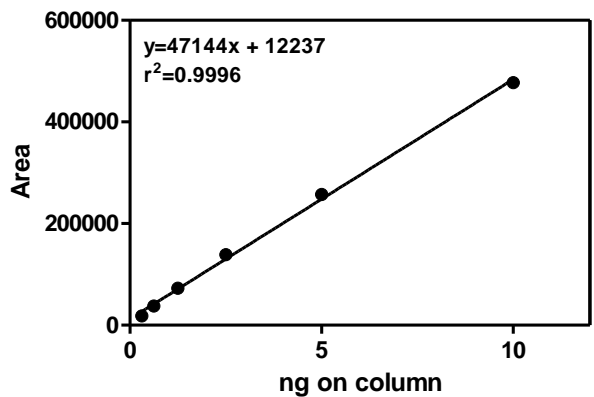
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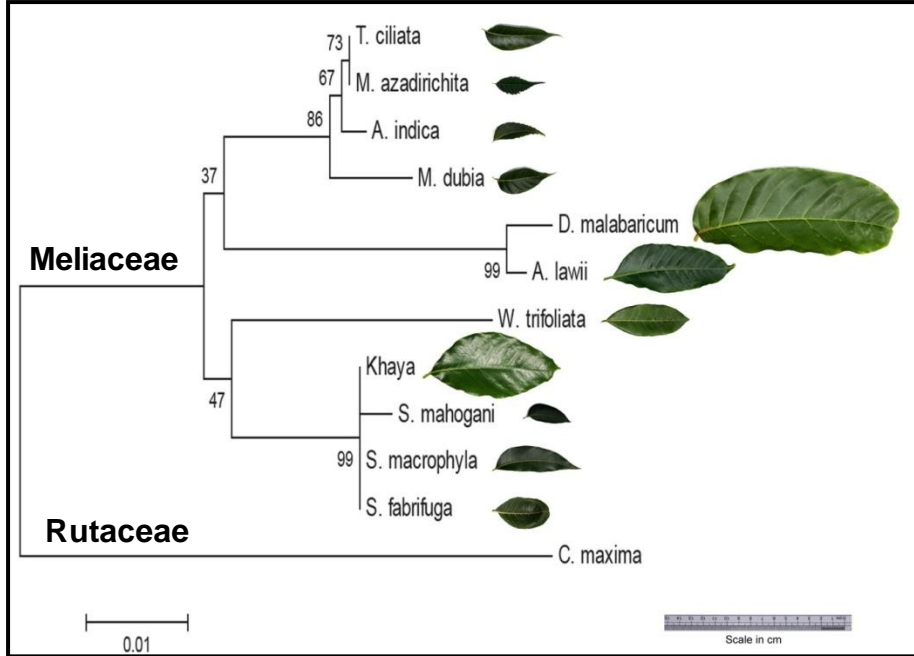
Supplementary Figure 1: UHPLC chromatogram of neem leaf extracts. Neem leaf extract was injected to semi preparative C-18 column with the gradient started from 5%-85% B in 3-20 min and 3 mL/min flow rate. The detection at 220 nm showed the major peaks retention time at 22.6, 22.7 and 23.1 min. The insert shows the fraction collected for the three peaks.



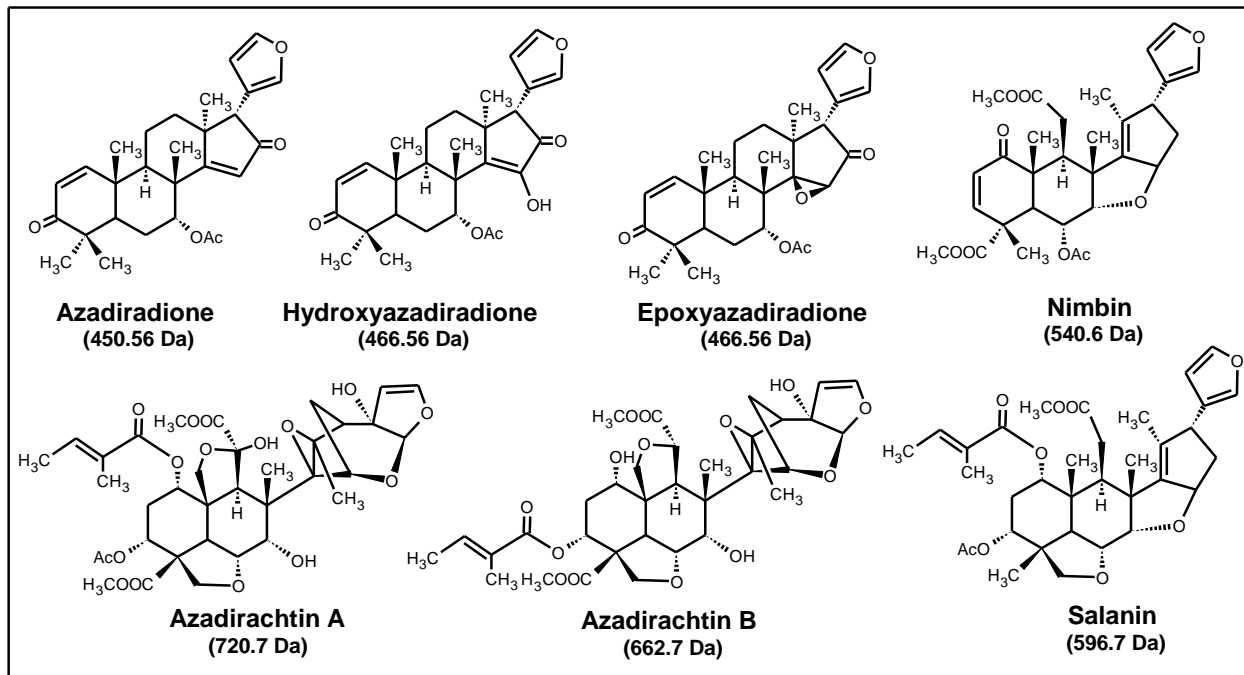
Supplementary Figure 2: UHPLC-MS chromatogram of three peaks collected from neem leaf extract. (A) UHPLC-MS chromatogram of all three peaks collected from neem leaf extracts, (B) the corresponding mass spectra of the peaks retention time at 11.1 min.



Supplementary Figure 3: Estimation of E/H-azadi concentration. Different concentration of azadi from 0.312 to 10 ng on column was injected and analysed in the UHPLC-MS/SRM method for both the transitions [Azadi (451→391) and E/H-azadi (467→435)]. Concentration versus area under the curve was considered to plot the standard curve. Based on this the calculated concentration of peak I (0.012 mg/mL), peak II (0.66 mg/mL) and peak III (0.009 mg/mL).



Supplementary Figure 4: Phylogenetic analysis of Meliaceae family plants based on RuBisCo small subunit. The eleven plants belong to Meliaceae family and one plant (Citrus maxima) belongs to Rutaceae family.



Supplementary Figure 5: Chemical structure of neem metabolites.