Haryana Vet. (December, 2023) **62(2),** 122-128 ISSN 0033-4359 (P) **Research Article**

HIGH RESOLUTION LIQUID CHROMATOGRAPHY- MASS SPECTROMETRY ANALYSIS (Q-TOF-MS) AND FREE RADICAL SCAVENGING ACTIVITY OF EXTRACT OF *AZADIRACHTA INDICA* LEAVES

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ABSTRACT

An indigenous tree to the entire Indian subcontinent, *Azadirachta indica* (neem), a member of the Meliaceae family, is known for its ability to promote good health due to the abundance of antioxidants and flavonoids. The present study is focused on investigating the phytochemical composition of *Azadirachta indica* aqueous leaf extract using the high resolution liquid chromatography -mass spectrometry (HRLC-MS) method as well as the free radical scavenging potency that makes them beneficial for various therapeutic strategies. The first polyphenolic flavonoid quercetin and cynaroside were isolated from freshly harvested neem leaves and were recognized to have anti-oxidant, anti-inflammatory, antifungal and antibacterial properties. Other important phytoconstituents having similar medicinal properties such as Aurachin D, Gentisic acid, Allivicin, Lactapiperanol C, Bruceantin, Caulerpin, Copalliferol B were identified by HPLC-MS. The neem extract's ability to neutralise 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals *in-vitro* was evaluated. The findings of this study showed that leaf extracts of *A. indica* contains a variety of phytochemical compounds with therapeutic potential and can expertly protect the body against oxidative stress brought on by free radicals.

Keywords: Antioxidants, *Azadirachta indica*, Chromatography, Phytochemicals, Spectrometry

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Azadirachta indica, a native of the Indian subcontinent is a member of the "Meliaceae" family (Alzohairy, 2016). It is commonly referred to as the neem tree, is a tropical evergreen tree that is unique to the Indian subcontinent (Aneesa, 2016). The word "neem" derived from the Sanskrit word "nimba" is classified as "Village Pharmacy" or "Devine Tree" or "Nature's Drugstore" or "Medicinal Cabinet" or "Panacea" implying that it can treat all illnesses (Islas *et al.*, 2020). Further, it is worthy to mention that it contains glycoproteins, triterpenes, limonoids, flavonoids, phenols, tannins, nimbins, saponins, catechins, azadirachtin, and gallic acid are the main phytochemicals found in neem (Gupta *et al.*, 2017). The anti-inflammatory, antiarthritic, antipyretic, hypoglycemic, antigastric ulcer, antifungal, antibacterial, antiviral, antimalarial, and antitumor actions of these substances have been validated by earlier researchers (Srivastava *et al.*, 2020; Shewale, 2022). The medicinal value of neem has been recognized by the US National Academy of Science, which published a report in 1992 entitled, "Neem–A Tree of Solving Global Problem" (Joshi *et al.*, 2010). The golden plant established the fact that polyphenolic compounds in neem possess remarkable antioxidant properties (Vardhan and Sahoo, 2020). It modulates many cellular and molecular pathways, such as free radical scavenging, to exert its potentially beneficial benefits (Heyman *et al.*, 2017). The flavanoids and their derivatives like cynaroside, quercetin, gentisic acid and maritimetin were found among those screened antioxidant phytocompounds that could serve as a potential source of antioxidants and can be explored as therapeutic agents in free radical-induced diseases. Therefore, taking into account these previous reports that the *A. indica* has huge medical importance and this study was conducted to evaluate in detail phytochemical studies using most advanced phytoanalysis technique, HPLC-MS, for promising identification of the phytocompounds and to analyze antioxidant efficiency in the aqueous extract of *A. indica*.

MATERIAL AND METHOD

Preparation of *Azadirachta indica* leaf extract

Fresh leaves of *A. indica* were collected, washed under running tap water and then with distilled water to eradicate undesirable dirt and other foreign materials. The sample was air dried under shade until no moisture left. Approximately, 20g of *A. indica* leaves were minced properly and soaked overnight in 100 ml of sterile distilled water. The resulting mixture was heated at 60º C for 10 minutes. Subsequently, the mixture was centrifuged at 6000 rpm for 15 minutes and filtered using Whatman No. 1 filter paper. The pH of the extract was maintained at 7.3 and evaporated extract was stored at 4°C for further use (Roy *et al.*, 2017).

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Phytochemical characterization of Aqueous Neem Extract

HPLC–MS analysis for antioxidant and other bioactive components was performed on a TOF/Q-TOF Mass Spectrometer (Agilent Technologies). Data acquisition and processing was accomplished using Agilent 6200 Series.

Specifications of LC-MS method metabolite_ESI_ +VE_MS/MS

The neem extract was characterized by HPLC–MS based on the method metabolite_ESI_+VE_MS/MS. This method had been carried out to permit the rapid qualitative analysis of Neem (*Azadirachta indica*) aqueous extract. HPLC–MS analysis of antibacterial and antioxidant components was performed on a TOF/Q-TOF Mass Spectrometer, (Agilent Technologies). Data acquisition and processing were accomplished using Agilent 6200 Series. The HPLC-MS method may tolerate less sample preparation and purification in addition to having great sensitivity, which significantly reduces overall analysis time. The injection volume of 10 µL, desorption liquid temperature 250°C, Threshold (Abs) 10000, Nebulizer (psig) 35, target (counts/spectrum) 25000.000, Purity Stringency (%) 100.000, Purity Cutoff (%) 30.000 was used. Full mass scan spectra were recorded in the negative ionization mode over the range of m/z 120-1200 (1 scan/s). High Pressure Limit was 200.00 bar, Flow 0.300 mL/min., Draw Speed 100.0 µL/min, Eject Speed 100.0 µL/min and Spectrum Range WL from 190 to 640nm. The phytochemicals were identified according to their retention time, pattern of mass spectra and its comparison with the KEGG, METLIN and CAS compound data bases. Compounds were identified by comparison of their retention time and UV and mass spectral data with test substances. According to injected absolute levels, the limit of detection for azadirachtin in extract samples was found to be (about) 1 ng/ml or 10 pg in SIM mode which is approximately 1000-times lower than values quoted in the literature for existing HPLC methods (approximately 200 ng/ml or 10 ng).

DPPH free radical scavenging assay

According to the method described in (Pokhrel *et al.*, 2015), with a small modification, the antioxidant activity of Neem was measured on the basis of the scavenging activity of the stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical. In brief, 100 L AgNPs received 0.1 mM of DPPH in methanol. Each extract was mixed with 3 ml of a methanolic solution containing DPPH radicals (0.2, 0.4, 0.6, 0.8, or 1 mg/mL). At 517 nm, the test tubes' absorbance was measured after 30 minutes of room temperature incubation. A lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The test solutions were prepared using the same amounts of ascorbic acid as the standard. The difference in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following standard equation. Scavenging effect (%) = (1-As/Ac)×100; whereas 'As' is the absorbance of the sample at t =0 min and 'Ac' is the absorbance of the control at t=30 min.

RESULT AND DISCUSSION

HPLC- MS analysis

The HPLC- MS analysis revealed several peaks in aqueous extract of A. indica (neem tree). The plant has demonstrated numerous health benefits, including immunomodulatory, antimalarial, antifungal, antioxidant, antibacterial, anticarcinogenic, antimutagenic, antiulcer and anti-inflammatory characteristics. Therefore, in this study, the phytochemical constituents determined by high performance liquid chromatography analysis and the antioxidant properties of aqueous extracts of *A. indica* leaves were investigated in both positive and negative ion modes to assess the potential protective benefits of this plant against degenerative reactions induced by free radicals.

From the chromatogram report in Fig. 1(a) and (b), the phytocompounds identified were of pharmacological importance. Saponins, flavonoids and alkaloids were only present in the aqueous extract, while steroids were only present in the ethanol extract (Hikaambo *et al.*, 2022). In Table 1, the phytochemical screening of the *A. indica* leaves revealed the presence of phenolics and tannins in aqueous extract. The LC-MS-based untargeted analysis in Table 2 showed the phytochemical profile of the leaves of *A. indica* in which 117 phytochemicals from different chemical classes were annotated, including organic acids, phenolic acids, flavonoids, and other compounds and in Fig. 2, ESI-MS fingerprint spectra of the above discussed phytochemicals from the leaves of *A. indica* with wide significance were postulated.

Anti-oxidant Activity

The results of the HPLC-MS studies confirm the presence of a wide array of phytoconstituents with antioxidant properties. Antioxidants have an important role in the activation of antioxidant enzymes that play a part in the regulation of damage produced by free radicals/reactive oxygen species. Antioxidants can reduce oxidative damage indirectly by boosting the activity or expression of intracellular antioxidant enzymes or directly

Table 2. Structure and bioactivity of principle phytocomponents identified in the aqueous extracts of the Leaves of *A. indica*

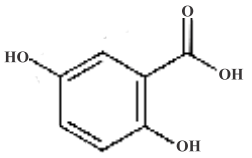
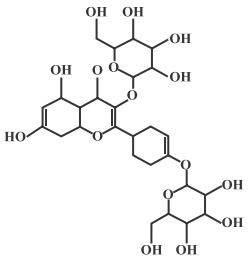
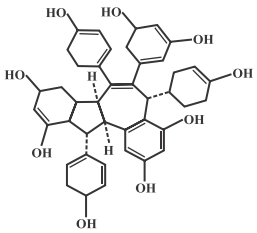
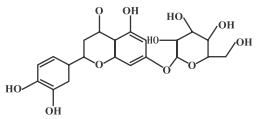
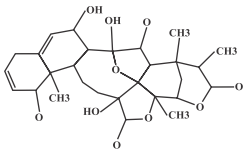
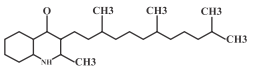
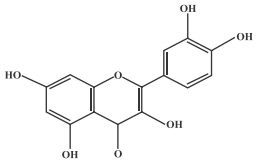
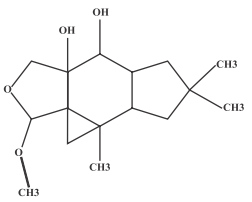
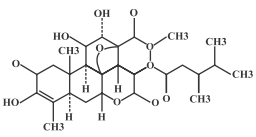
S.No.	Name of compound	Structure	Bioactivity with reference
1	Gentisic acid		anti-inflammatory, antirheumatic and antioxidant (Mardani-Ghahfarokhi and Farhoosh, 2020)
2	Allivicin		anti-bacterial (Geewananda *et al.*, 1986)
3	Copalliferol B		anti-bacterial (Geewananda *et al.*, 1986).
4	Cynaroside		antibacterial, antifungal and anticancer activities, antioxidant and anti-inflammatory (Rehfeldt *et al.*, 2022)
5	Physalin O		anti-inflammatory (Ji *et al.*, 2012)
6	Aurachin D		antibacterial and anti-inflammatory(Kruth *et al.*, 2022)
7	Quercetin		antioxidant, antibacterial and antifungal properties (Alzohairy, 2016)
8	Lactapiperanol C		antidiabetic (Daou *et al.*, 2022)
9	Bruceantin		antineoplastic, antiamoebic and antimalarial (National Cancer Institute)

Table 1. Chemical constituents and properties of the phyto-components identified in the aqueous extracts of the leaves of *A. indica* by LC–MS

Name	Formula	RT	Mass	m/z	Positive/ Negative ion mode
8-Hydroxy-2- chlorodibenzofuran	C12 H7 Cl O2	0.88	218.0129	241.0024	(M+Na)+
(+/-)-3-[(2-methyl-3- furyl) thio]-2-butanone	C9 H12 O2 S	0.904	184.0571	185.0643	(M+H)+
Anthranilic acid	C7 H7 N O2	1.219	137.047	138.0543	(M+H)+
Retronecine	C8 H13 N O2	1.243	155.0941	156.1011	(M+H)+
Adenine	C5 H5 N5	1.274	135.0538	136.0611	(M+H)+
8-Hydroxyadenine	C5 H5 N5 O	1.318	151.0486	152.0559	(M+H)+
2-Methylbenzaldehyde	C8 H8 O	1.411	120.0567	121.064	(M+H)+
Neuraminic acid	C9 H17 N O8	1.48	267.0937	268.1021	(M+H)+
Benzocaine	C9 H11 N O2	1.804	165.0783	166.0856	(M+H)+
2-Hexylbenzothiazole	C13 H17 N S	2.277	219.1095	220.1168	(M+H)+
N-Acetylserotonin	C12 H14 N2 O2	2.804	218.1048	219.1121	(M+H)+
3-Carboxy-4-methoxy-N- methyl-2-pyridone	C8 H9 N O4	2.996	183.0542	206.0432	(M+Na)+
Metipranolol	C17 H27 N O4	3.036	309.1903	310.1995	(M+H)+
Indoleacrylic acid	C11 H9 N O2	3.093	187.0622	188.0695	(M+H)+
L-Tryptophan	C11 H12 N2 O2	3.136	204.0887	205.0959	(M+H)+
Isocarbostyril	C9 H7 N O	3.14	145.0519	146.0591	(M+H)+
6-Methylquinoline	C10 H9 N	3.146	143.0726	144.0799	(M+H)+
D-Tryptophan	C11 H12 N2 O2	3.468	204.0874	205.0945	(M+H)+
Carbofuran	C12 H15 N O3	3.602	221.1068	222.114	(M+H)+
Methaphenilene	C15 H20 N2 S	3.687	260.1351	261.1431	(M+H)+
Venlafaxine	C17 H27 N O2	3.969	277.2011	300.1902	(M+Na)+
ibopamine	C17 H25 N O4	4.037	307.1766	308.1839	(M+H)+
Dihydrocorynantheine	C22 H28 N2 O3	4.199	368.2095	391.1986	(M+Na)+
Elaeokanine C	C12 H21 N O2	4.435	211.1559	212.1632	(M+H)+
Thesinine 4'-O-glucoside	C23 H31 N O8	4.555	449.1985	450.206	(M+H)+
2-Heptylfuran	C11 H18 O	5.093	166.1367	189.1259	(M+Na)+
Istamycin C1	C19 H37 N5 O6	5.274	431.27	432.2772	(M+H)+
Ascorbyl stearate	C24 H42 O7	5.365	442.287	443.2946	(M+H)+
Progeldanamycin	C27 H41 N O6	5.605	475.2959	476.3033	(M+H)+
(x)-1-Nonen-3-yl acetate	C11 H20 O2	5.709	184.1471	207.1363	(M+Na)+
Quercetin	C15 H10 O7	5.864	302.0404	394.7163	(M+H)+
Maritimetin	C15 H10 O6	6.167	286.0459	287.0532	(M+H)+
Chrysophanol 1-triglucoside	C33 H40 O19	6.172	740.2122	741.2195	(M+H)+
3-Oxo-12,18-ursadien-28-oic acid	C30 H44 O3	6.447	452.3328	453.3401	(M+H)+
Kuwanon Z	C34 H26 O10	6.521	594.1547	595.162	(M+H)+
6-C-Galactosylluteolin	C21 H20 O11	6.593	448.0974	449.1047	(M+H)+
Thalicsessine	C22 H27 N O4	6.727	369.1918	370.1988	(M+H)+
Menthanol	C10 H20 O	7.55	156.1525	179.1417	(M+Na)+
Bruceantin	C28 H36 O11	7.759	548.2312	549.2394	(M+H)+
Cucurbic acid	C12 H20 O3	7.817	212.1392	213.1473	(M+H)+
6-Methyl-2-methylene-6- octene-1,3,8-triol	C10 H18 O3	8.083	186.126	209.1153	(M+Na)+
6,6-Dimethoxy-2,5,5-trimethyl- 2-hexene	C11 H22 O2	8.329	186.1631	209.1523	(M+Na)+
2-(3-Phenylpropyl)tetrahydrofuran	C13 H18 O	8.665	190.1345	191.142	(M+H)+
(1R,2R)-1,2,7,7-Tetramethylbicyclo[2.2.1]hept an-2-ol	C11 H20 O	8.93	168.1523	191.1417	(M+Na)+
Geranyl 2-ethylbutyrate	C16 H28 O2	9.949	252.2096	275.1989	(M+Na)+
10,16-dihydroxy-palmitic acid	C16 H32 O4	10.863	288.2301	311.2194	(M+Na)+

2-Tetradecylcyclobutanone	C18 H34 O	11.116	266.2617	289.251	(M+Na)+
Lactapiperanol C	C16 H26 O4	11.663	282.1813	283.1889	(M+H)+
L-Menthyl (R,S)-3- hydroxybutyrate	C14 H26 O3	11.725	242.1887	265.1779	(M+Na)+
Aurachin D	C25 H33 N O	12.176	363.2605	386.2517	(M+Na)+
5-Methylthioribose	C6 H12 O4 S	4.813	180.0441	179.0367	(M-H)-
Gentisic acid	C7 H6 O4	2.014	154.0281	153.0209	(M-H)-
2-Mercapto-3-pentanone	C5 H10 O S	4.09	118.0437	163.0417	(M-H)-
L-Lyxose	C5 H10 O5	1.145	150.0547	149.0475	(M-H)-
Caulerpin	C24 H18 N2 O4	5.686	398.1267	443.1249	(M+HCOO)-
Copalliferol B	C42 H32 O9	6.148	680.2045	739.2189	(M+HCOO)-
Allivicin	C27 H30 O16	6.149	610.1599	609.1526	(M-H)-
Aprepitant	C23 H21 F7 N4 O3	6.532	534.1448	593.1586	(M+CH3COO)-
CMP-N-glycoloylneuraminate	C20 H31 N4 O17 P	6.568	630.1409	629.1353	(M-H)-
Dopaxanthin quinone	C18 H16 N2 O8	7.099	388.086	447.0999	(M+CH3COO)-
Cynaroside	C21 H20 O11	7.838	448.1062	447.0989	(M-H)-
Physalin O	C28 H32 O10	8.784	528.1937	527.1868	(M-H)-
Corchorifatty acid F	C18 H32 O5	9.899	328.2302	327.2227	(M-H)-
Oxazepam	C15 H11 Cl N2 O2	9.931	286.0523	285.0449	(M-H)-
N-Desmethylclobazam	C15 H11 Cl N2 O2	10.312	286.0522	285.0448	(M-H)-
2R-hydroxy-stearic acid	C18 H36 O3	11.14	300.2708	359.2846	(M+CH3COO)-
Chloramphenicol succinate	C15 H16 Cl2 N2 O8	12.487	422.0334	481.047	(M+CH3COO)-
Lauryl hydrogen sulfate	C12 H26 O4 S	18.992	266.1583	265.151	(M-H)

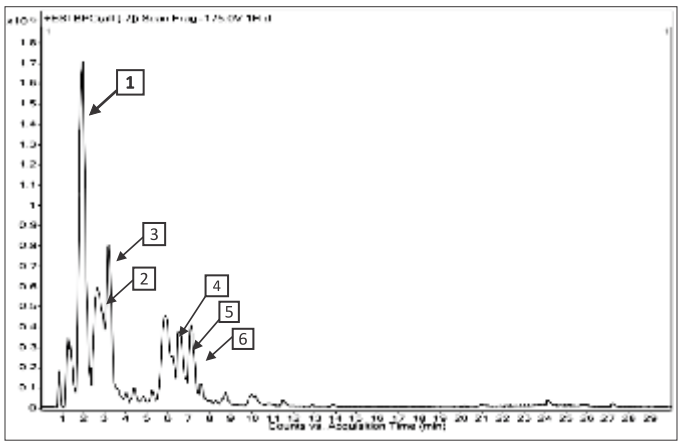


Fig. 1(a). HR-LC/MS spectrum peak of *A. indica* aqueous extract showing the chromatogram intensity against the acquisition time, (A) positive ion mode analysis. Numerical 1- Benzocaine; 2-Metipranolol; 3-Indoleacrylic acid; 4-Quercetin; 5-Maritimetin and 6-Menthanol

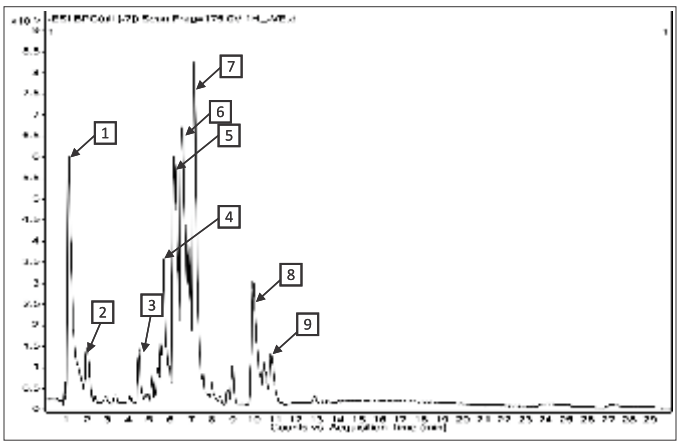


Fig. 1(b). HR-LC/MS spectrum peak of *A. indica* aqueous extract showing the chromatogram intensity against the acquisition time, negative ion mode analysis. Numerical 1-L-Lyxose; 2-Gentisic acid; 3-5-Methylthioribose; 4-Caulerpin; 5-Allivicin; 6-Copalliferol B; 7-Cynaroside; 8-N-Desmethylclobazam; 9-Corchorifatty acid

by interacting with free radicals and reducing oxidative damage (Nita and Grzybowski, 2016). These compounds stabilize or deactivate free radicals, frequently before they attack targets in biological cells and have reportedly been found in medicinal plants (Rahmani *et al.*, 2015). Flavonoids have been present in plants for more than a billion years and have a wide range of biological functions, but antioxidants are one of their most important biological functions (Anand David *et al.*, 2016). According to Prashanth *et al.* (2015), these polyphenolic chemicals are present in neem leaf extracts that are both aqueous and ethanolic. The most notable characteristic of quercetin is its antioxidant activity. It seems to be the most powerful flavonoid for protecting the body against reactive oxygen species, produced during the normal oxygen. The reactivity of the extracts prepared from *A. indica* was analyzed with 2, 2-Diphenyl-1-Picryl hydrazyl, a stable free radical that reduced by accepting hydrogen or electron from the donor molecule. The extent of DPPH radical scavenging (Fig. 3) at different concentrations (0.2-1.0 mg/ml) of *A. indica* extract was measured with ascorbic acid as the standard. In comparison with ascorbic acid, the plant extract had a lower level of inhibitory activity. The

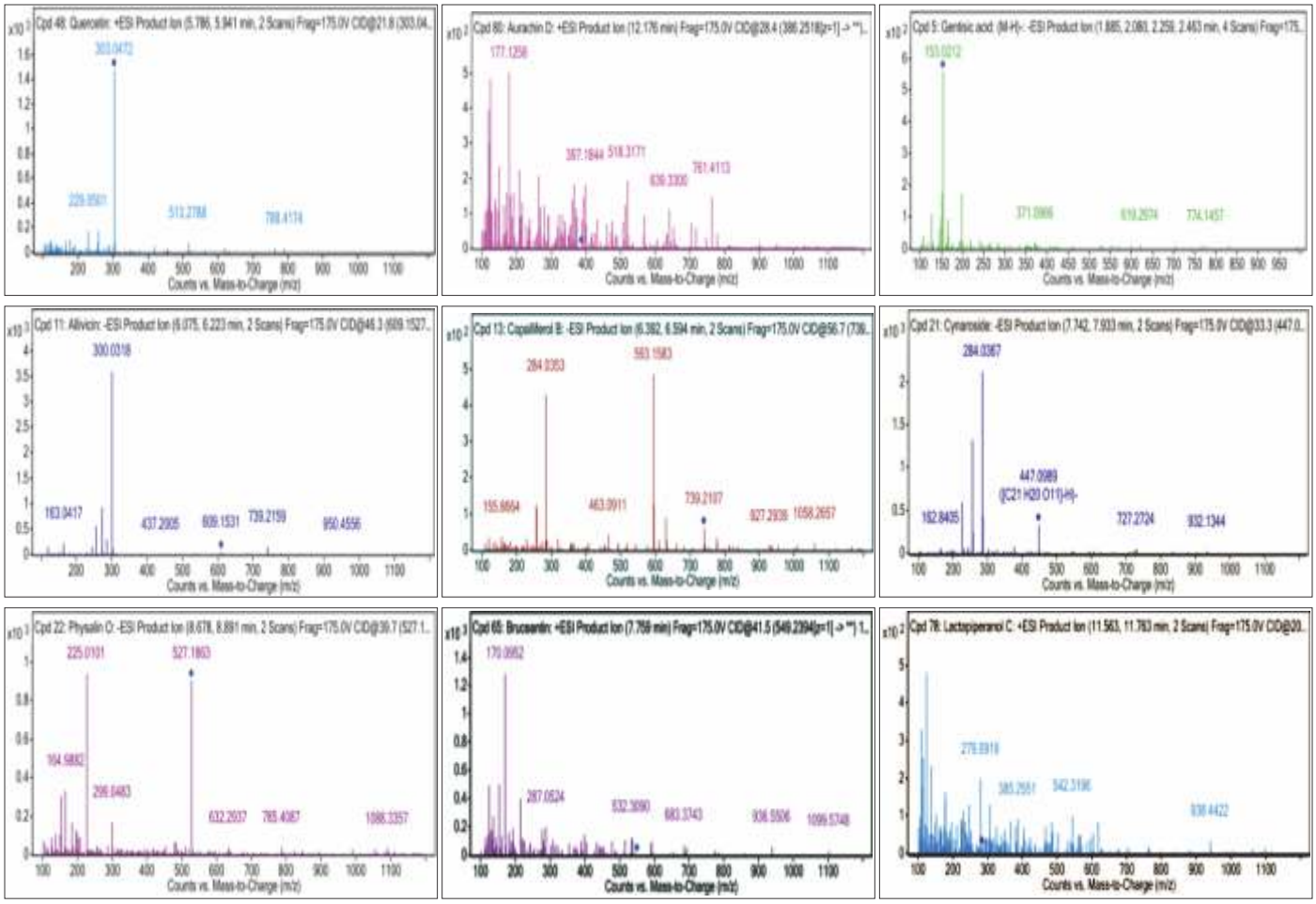


Fig. 2. Typical direct flow injection analysis ESI-MS fingerprint spectra of above discussed phytochemicals from the leaves of *A. indica*

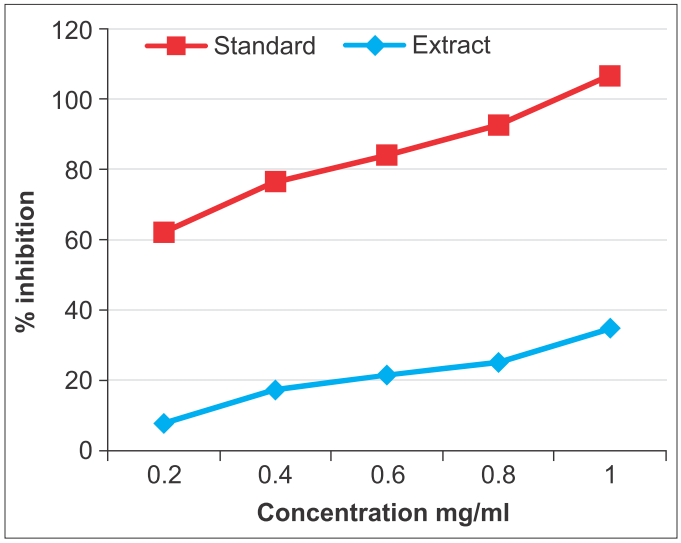


Fig. 3. DPPH radical scavenging activity of different concentrations of aqueous leaf extract, of *A. indica* and ascorbic acid (standard)

results showed that the greatest degree of inhibition activity was seen at higher concentrations of plant extract, followed by decreasing inhibition activity at lower concentrations. Neem tree leaf extracts had free radical scavenging properties that were found to be effective against the DPPH radical.

CONCLUSION

The present chromatographic study provides abundant information for structural elucidation of the chemical compounds present in *A. indica* aqueous leaf extract especially when tandem mass spectrometry (MS) is applied. Consequently, the combination of HRLC and MS provides a quick and precise method for phytochemical identification and points out that extracts of *A. indica* leaves comprise a variety of compounds in appreciable quantity that have preventive and therapeutic efficacy against different pathological conditions and protect the body against the harmful effects of oxidative stress caused by free radicals, therefore, they can be used as a source of potent natural antioxidant.

REFERENCES

Alzohairy, M.A. (2016). Therapeutic role of *Azadirachta indica* (Neem) and their active constituents in disease prevention and treatment. *Evid. Based Complement. Altern. Med.* 7382506.

Anand David, A.V., Arulmoli, R. and Parasuraman, S. (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. *Phcog Rev.* **10(20)**: 84-89.

Aneesa, N. and Gayathri, G. (2016). Beneficial effects of neem oil- An updated review. *J. Pharm. Sci. Res.* **8(8)**: 756-758.

Daou, M., Elnaker, N.A., Ochsenkühn, M.A., Amin, S.A., Yousef, A.F. and Yousef, L.F. (2022). *In vitro* -glucosidase inhibitory activity of *Tamarix nilotica* shoot extracts and fractions. *PloS one.* **17(3)**: e0264969.

Geewananda, Y.A., Gunawardena, P., Sotheeswaran, S., Uvais, M., Sultanbawa, S. and Surendrakumar, S. (1986). Another antibacterial polyphenol, copalliferol B, from *Vateria copallifera* (dipterocarpaceae). *Phytochem.* **25(6)**: 1498-1500.

Gupta, S.C., Prasad, S., Tyagi, A.K., Kunnumakkara, A.B., Aggarwal, B.B. (2017). Neem (*Azadirachta indica*): an Indian traditional panacea with modern molecular basis. *Phytomedicine.* **34**: 14-20.

Heyman, L., Houri-Haddad, Y., Heyman, S.N., Ginsburg, I., Gleitman, Y. and Feuerstein, O. (2017). Combined antioxidant effects of Neem extract, bacteria, red blood cells, and Lysozyme: possible relation to periodontal disease. *BMC Complementary and Altern. Medicine.* **17(1)**: 399.

Hikaambo, C., Kaacha, L., Mudenda, S., Nyambe, M., Chabalenge, B., Phiri, M., Biete, L., Akapelwa, T., Mufwambi, W., Chulu, M. and Kampamba, M. (2022). Phytochemical analysis and antibacterial activity of *Azadirachta indica* leaf extracts against *Escherichia coli*. *Pharmacology and Pharmacy.* **13**: 1-10.

Islas, J.F., Acosta, E., G-Buentello, Z., Delgado-Gallegos, J.L., Moreno-Trevino, M.G., Escalante, B. and Moreno-Cuevas, J.E. (2020). An overview of Neem (*Azadirachta indica*) and its potential impact on health. *J. Funct. Foods.* **74**: 104171.

Ji, L., Yuan, Y., Luo, L., Chen, Z., Ma, X., Ma, Z. and Cheng, L. (2012). Physalins with anti-inflammatory activity are present in *Physalis alkekengi* var. franchetii and can function as Michael reaction acceptors. *Steroids.* **77(5)**: 441-447.

Joshi, M., Purwar, R., Wazed Ali, S. and Rajendran, S. (2010). Antimicrobial textiles for health and hygiene applications based on eco-friendly natural products. In woodhead publishing series in textiles, Medical and Healthcare Textiles. 84-92. Woodhead Publishing.

Kruth, S., Schibajew, L. and Nett, M. (2022). Biocatalytic production of the antibiotic aurachin D in *Escherichia coli*. *AMB Expr.* **12(1)**: 138.

Mardani-Ghahfarokhi, A. and Farhoosh, R. (2020). Antioxidant activity and mechanism of inhibitory action of gentisic and -resorcylic acids. *Sci. Rep.* **10(1)**: 19487.

Nita, M. and Grzybowski, A. (2016). The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. *Oxid. Med. Cell. Longev.* 3164734.

Pokhrel, B., Rijal, S., Raut, S. and Pandeya, A. (2015). Investigations of antioxidant and antibacterial activity of leaf extracts of *Azadirachta indica*. *Afr. J. Biotech.* **14(46)**: 3159-3163.

Prashanth, G.K., Prashanth, P.A., Bora, U., Gadewar, M., Nagabhushana, B.M., Ananda, S., Krishnaiah, G.M. and Sathyananda, H.M. (2015). *In vitro* antibacterial and cytotoxicity studies of ZnO nanopowders prepared by combustion-assisted facile green synthesis. *Karbala Int. J. Mod. Sci.* **1(2)**: 67-77.

Rahmani, A.H. and Aly, S.M. (2015). Nigella sativa and its active constituents thymoquinone shows pivotal role in the diseases prevention and treatment. *Asian J. Pharm. Clin. Res.* **8(1)**: 48-53.

Rehfeldt, S.C.H., Silva, J., Alves, C., Pinteus, S., Pedrosa, R., Laufer, S. and Goettert, M.I. (2022). Neuroprotective effect of luteolin-7-O-glucoside against 6-OHDA-induced damage in undifferentiated and RA-differentiated SH-SY5Y Cells. *Int. J. Mol. Sci.* **23(6)**: 2914.

Roy, P., Das, B., Mohanty, A. and Mohapatra, S. (2017). Green synthesis of silver nanoparticles using *Azadirachta indica* leaf extract and its antimicrobial study. *Appl Nanosci.* **7**: 843-850.

Shewale, S.P., Panadare, D. and Rathod, V.K. (2022). Extraction of total antioxidants from *Azadirachta indica* (neem) using three phase partitioning and its process intensification using ultrasound. *Prep. Biochem. Biotechnol.* **52(5)**: 534-539.

Srivastava, S.K., Agrawal, Babita, Kumar, A. and Pandey, A. (2020). Phytochemicals of *Azadirachta indica* source of active medicinal constituent used for cure of various diseases: A Review. *J. Sci. Res- BHU*, **64(1)**: 385-390.

Vardhan, S. and Sahoo, S.K. (2020). In silico ADMET and molecular docking study on searching potential inhibitors from limonoids and triterpenoids for COVID-19. *Comput. Biol. Med.* **124**: 103936.