Research Article

Alteration of Morphology and Constituents of *Azadirachta indica* Leaf due to Air Pollution and Exploiting the Phenomenon for Medicinal Usage on Gastric Ulcer through Molecular Docking

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ABSTRACT

The present study was attempted to know alteration in leaf morphology and phytochemicals in *Azadirachta indica A. Juss*., when exposed to air pollutants especially particulate matters and gases, and molecular docking approach to know necessary phytochemicals involved in gastric ulcer prevention as an anti-inflammation. The study areas were selected for industrial zone as experimental area viz. E1 (5.0 km distance from industry), E2 (2.0 km distance from industry but near roadside) and E3 (3.5 km distance from industry) compared to control area as C (10.0 km distance from industry), Durgapur, India. The present study was done for manual measurement of leaves as well as examining bioactive constituents in methanol extracts through Thin Layer Chromatography (TLC). Also molecular docking to know neem leaf phytochemicals interaction against MMP9 (matrix metalloproteinase-9). The results showed significant ($P<0.001$ and $P<0.01$) changes in morphological features as L (length), B (breadth) and L/B (length/ breadth) ratio. While screening of bioactive compounds, we found the appearance of new and absence of phytochemicals through the bands in TLC in the experimental leaves compared to control leaves. Molecular docking reveals lower energy binding values of kaempferol-3-O-β-rutinoside (-125.94 Kcal/mol) as competitive inhibitory potential against MMP9, which was absence in E1 and E2 area. It is concluded that *A. indica* leaves have capability of gastric ulcer prevention via MMP-9 mediated pathway if the active ingredient present in the leaves. Thus, prior to medicinal usage against gastric ulcer should be avoided leaves from air-polluted area.

Keywords: Air pollution; *Azadirachta indica*; Leaf morphology; Phytochemicals; Bioactive constituents, Preventive bio filter.

INTRODUCTION

Air pollution mainly arises from particulate matters (PM_{10} and $PM_{2.5}$) and gaseous pollutants that generate from industrial activities as well as from vehicular movements.[1-4] However, these pollutants have already been controlled by using several devices as Air Pollution Control (APC) equipment in industries as well as vehicular emission norms under Air (Prevention and Control of Pollution) Act, 1981 and Motor Vehicle Air Pollution Control Act, 1965. The routine surveillance is always done by State Pollution Control Board and local administration but generation at a low level through APC device and/or from vehicular exhaust may safe for human and other animals whereas how much effective for vegetation is still unclear. Generally, plants are used for air purifier by their detoxifying mechanism of major pollutants and other environmental factors.[4-7] However, it was observed in the previous study that particulates have been found higher at Durgapur industrial area when compared to the standard limit of 100 µg/m3 as per latest NAAQS 2009.[8]

Use of herbs, shrubs and trees are well documented by several reports for pollution remediation and disease prevention.[2,9-15] Moreover, bio monitoring of air pollution with special reference to trees are well known.[14-16] It was already established that few tree species are sensitive, moderately tolerant and tolerant to air pollutants when the study was carried out on Air Pollution Tolerant Index (APTI) in the Durgapur area.[16] The concept of greenbelt development by using broad leaved trees, trees having huge canopy cover, high chlorophyll content, presence of secondary metabolites, higher transpiration rate etc. as per CPCB (2000) guidelines are there. However, it has been well established to use the plant parts of many trees having potent phytochemicals viz. antioxidants, antimutagenic, antidiabetic, anti-inflammatory etc. for several diseasesprevention.[4,9]

Among, other medicinal tree species, *Azadirachta indica* A. Juss., commonly called neem tree belong to family Meliacae inhabited in any unfavourable environmental conditions. This tree is having medicinal properties as well as moderately pollutant tolerant capacity.[2] Major findings have been documented on medicinal properties from the different parts viz. leaf, bark, root, fruit, seed etc. of *A. indica* [9,17-19] and also leaf deformities for few plant species along with neem[20] and decrease in chlorophyll content[21] but no one has been studied before the impact of air pollutants on leaves in a combined approach that alteration of morphology with special reference to shape and visible injuries as well as the presence and/or absence of bioactive compounds along with inhibitory potential of disease prevention, when exposed to air pollutants from industries and vehicular emissions and this tree can be used as potent pollution control device as bio-filter because of moderately tolerant species.[16]

Generally, expression of MMP9 leads to inflammation as well as cancer indication.[22] On the other hand, neem leaf extracts prevent the ulceration in the intestine as a substitute of synthetic drugs.[23-27] It has been reported that the induction of protein matrix metalloproteinase-9 (MMP9) during gastric ulcer of animal and neem extracts can be suitable for the normalization of MMP9 activity along with ulcer improvement[27] as well as natural product like curcumin inhibited MMP9 activity when indomethacin is induced gastric ulceration.[28] The interaction study through molecular docking can be beneficial to detect protein activator or inhibitor when binds with ligand in relation to lower energy values and appropriate binding site.[29]

The present study was an attempt to detect alteration of morphological features with special reference to shape and visible injuries as well as bioactive compounds in the leaves of *A. indica*, inhabited in industrial zones thereby exposed to both industrial and vehicular emissions. Also to detect phytoconstituents alteration hampers in disease like gastric ulcer prevention through molecular docking approach as MMP9-ligands binding.

MATERIALS AND METHODS

Study area and selection of test species

The study areas were selected on the basis of industrial zone and vehicular emission as per Rana.[30] This study area is known as Durgapur industrial area (latitude = 23° 31' N and longitude = 87° 18' E), West Bengal, India and having chemical industries, cement plants, power plants, steel plants etc. The sampling was done for this study during summer season at 4 sampling stations viz. (i) C as control area (10.0 km from industries), (ii) E1 as experimental zone (5.0 km distance from industries), (iii) E2 as experimental zone (2.0 km distance from industries and near roadside) and (iv) E3 as experimental zone (3.5 km distance from industries) as per previous study of air dispersion modeling and isopleth mentioned during summer season.8 These four sampling stations were selected on the basis of air pollution mainly particulates dispersion and deposition and vehicular emitted particulates onto leaves. The plant species was selected *Azadirachta indica* A. Juss., because it is growing profusely in the study area and this species is known as air purifier as well as moderately air pollution tolerant.[16] The leaves were collected randomly from trees of same height, breadth etc. as per visual observation. The Google earth image of study area is exhibited in Fig. 1. The affected leaf shape and visible injuries onto leaf were determined by the study of shape as length (L), breadth (B) and L/B ratio and visible injuries onto leaves were observed manually without quantification. All the leaves randomly selected from 5 trees of above-mentioned area. The leaves sampling was done thrice during summer season for morphological analysis. In case of study of bioactive compounds, the extraction and analysis were carried out through TLC and mass spectroscopy. All the leaves were collected from above mentioned areas for the next step of study. All the leaves samples were collected thrice during summer season.

Morphological study of leaves

The 50 leaves were collected randomly from per tree of above-mentioned area. Individual leaf was cleaned properly in running tap water and soaked with blotting paper. The area of leaves especially L (Length), B (Breadth) and L/B (Length / Breadth) ratio of leaf (in cm), was measured manually.

Figure 1: Google earth image of the study area (N = North side)

Neem leaves extract preparation

The aqueous and methanolic extracts of leaves were done by the method of Prashanth and Krishnaiah[31] and Dutta[32] with some modification.

Aqueous extract preparation

All the leaves of *A. indica* were washed properly under tap water and blotted to remove water droplets, finally the leaves were dried under shed. All the leaves were ground to formed powder after shed drying. 10gm of dried powder was taken in a separate container. 50 ml of distilled water was added to it and kept for 48hours at room temperature followed by filtration and the filtrate was collected. The steps for extraction was repeated thrice and kept in sealed container for analysis.

Methanolic extract preparation

The methanolic extract preparation was done as previously mentioned method. 50 ml of 100% methanol was added to it and kept for 48hours at room temperature followed by filtration and the filtrate was taken for study. The steps for extraction was repeated thrice and kept in sealed container for analysis.

Analysis by thin layer chromatography (TLC)

TLC screening for aqueous and methanolic extracts of leaves were followed by the method of Dutta[32] with some modification.

Study of aqueous extract

The aqueous extract was preceded for thin layer chromatographies (TLC) as per conventional one-dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary scissors. The TLC

plate markings were done with soft pencil. The glass capillaries were used to spot the sample for TLC, applied sample volume around 1 µl by using capillary at distance of 1 cm at 4 lanes. In the twin trough chamber with solvent system methanol: chloroform (2:8 ml) solvent system was used. After pre-saturation with mobile phase for 20 min for development were used. After the process was run, all the plates were dried and sprayed with freshly prepared iodine reagent to detect the bands on the TLC plates.

Study of methanolic extract

The study in TLC with methanolic extract preparation was done as previously mentioned method. The TLCs were carried out for both pure compound and crude extracts of the leaves of different study area.

Molecular docking and interaction study for MMP9 with neem leaf phytochemicals

Receptor selection

The crystal structural information of MMP9 (receptor) of human was obtained from Protein Data Bank (http://www.rcsb.org). The crystal structure was found complexed with five inhibitor molecules (PDB ID: 2OW0) was selected according to the wwPDB validation report.[33] The structure was based on X-ray Diffraction method of 2.0 åre solution and residue count of318 nos. The structure was modified without biasness, previously bounded ligands viz. zinc, calcium and chloride ions as well as 6MR for A chain were deleted by using Auto Dock Tools, Ver. 1.5.6.[34] In this process, the water molecules were deleted and the polar hydrogens were added. The crystal structure of MMP9 is depicted (Fig. 5A). The resultant structural complexes of the individual phytochemical and with MMP9 were finally analysed by using the Lig Plot software, Ver. 1.4 to determine some specific contacts between the atoms of the ligand and receptor.[35]

Ligands selection

From literature, 15 compounds (ligands) of *A. indica* were selected. These compounds were azadirachtinA, azadiradione, gallic acid, isomargolonone, kaempferol-3-*O*-β-rutinoside, margolone, nimbin, nimbinin, nimbolide, nimocinolide, quercetin, quercetin 3-rutinoside, quercetin-3-O-β-D-glucopyranoside, sulfonoquinovosyldia-cylglycerid and 1-O-(β-D-glucopyranosyl)-(2S,3S,4R,8Z)-2-[Q' R)-2'-hydroxy-tetracosanoilamino]-8(Z)-octadene-1,3,4-triol and obtained SMILES from Pub Chem database (https://pubchem.ncbi.nlm. nih.gov/compound/) to converted .pdb file by using CORINA online software (http://www.mol-net.de).

Receptor-ligands binding through molecular docking

The molecular docking was performed to know target-lead by using iGEMDOCK (Version 2.1) developed by Yang and Chen.[36] This software creates protein-compound interaction profiles the values of energy binding, electrostatic, hydrogen-bonding and van der Waals. Based on these profiles and compound structures, iGEMDOCK infers the pharmacological interactions and clusters the screening ligands during post-screening analysis. Ultimately, iGEMDOCK tool reveals ranking and visualizing the screening compounds by providing the pharmacological interactions and energy-based scoring function. The selected twelve ligands were subjected to accurate docking (genetic algorithm parameter) as per drug screening mode by setting population size of 200 along with 70 generations and 3 solutions. After the completion of the docking, the post docking analysis was performed to find the docking pose and its energy values.

Statistical Analysis

All the mean values of data were determined statistically significant differences between experimental and control leaf samples for morphological features by using Student's t-test (P<0.05 level). The statistical analysis was carried out by using software (Microsoft Ver. 8.1, Excel 2013 with add on statistical tool pack).

RESULTS AND DISCUSSION

Our data clearly indicates that both vehicular and industrial air pollution cause significantly changes in foliar morphology in relation to shape and visible injuries along with production and degradation of phytochemical in *A. indica* (Table 1 and Fig. 2, 3 and 4). Previous studies have emphasized that Durgapur (study area) is having exposure of particulates beyond the National Ambient Air Quality Standards (NAAQS) as a range of concentration of PM_{10} was reported 53.803–271.325 µg/m^3.[8,16,37]

Study of morphological alteration in leaves

In all experimental sites such as 5.0 km (E1), 2.0 km (E2) and 3.5 km (E3) from industrial zone and both industrial as well as roadside, abnormal shape and growth retardation in leaves were significantly (P<0.001 or 0.01) obtained when compared to control site (10.0 km (C) no direct industrial and automobile exposure) for L, B and L/B ratio (Table 1). The visible injuries like pigmentation (scattered chlorophyll loss) onto leaves as well as abnormal shape of margin of leaves were also found in all the experimental area but majorly were observed in E1 and E2 area compared to control area (C). All images for morphological deformities and visible injuries were depicted in Fig. 2.

Table 1 shows that the length (L) values significantly decreased (P<0.001) for both areas like 5.0 km (5.57 ± 0.83cm) and 2.0 km (5.56 ± 0.58cm) distance from industries while highly reduced value for 2.0 km distance that may due to both types of exposure of air pollutants. However, the leaves of 3.5 km (6.44 ± 1.29cm) were showed minimum reduction without significant differences when compared with control area (6.47 ± 0.52cm). In case of breadth (B) values of leaves, the experimental area showed decreased values significantly

(P<0.001) in5.0 km (1.51 ± 0.23cm) and 2.0 km (1.53 ± 0.16cm) distance from industries while decreased value without significant level at 3.5 km (1.85 ± 0.21cm) distance in comparison with control area (1.90 ± 0.21cm) at a distance of 10.0 km.

Table 1: Measurement of shape of leaves of *A. indica*

Sl. No.	Area	Parameters (n = 50; M ± SD)		
		L (cm)	B (cm)	L/B (cm)
1.	Control (C1) 10 km	6.47 ± 0.52	1.90 ± 0.21	3.44 ± 0.50
2.	Experimental (E1) 5 km	5.57 ± 0.83*	1.51 ± 0.23*	3.68 ± 0.37**
3.	Experimental (E2) 2 km	5.56 ± 0.58*	1.53 ± 0.16*	3.67 ± 0.32**
4.	Experimental (E3) 3.5 km	6.44 ±1.29	1.85 ± 0.21	3.45 ± 0.61

*P<0.001; **P<0.01

Interestingly, L/B ratio were increased for all the experimental area while significant (P<0.01) differences were observed only in 5.0 km (3.68 ± 0.37cm) and 2.0 km (3.67 ± 0.32cm) but increased value without significant at 3.5 km (3.45 ± 0.61cm) area when compared to control area at 10.0 km (3.44 ± 0.50cm) distance. In addition, the abnormal growth patterns were observed in all leaves of experimental area when compared to control area, suggesting particulate depositions restrict on 5.0 km distance and vehicular emission majorly at 2.0 km distance. In case of visible injuries like chlorosis, the whole leaves showed yellow colour and pigmentation as yellowish spots majorly onto the leaves of *A. indica* in experimental area as compared to control area. The abnormal shape of leaf margins was also observed in E2 area (Fig. 2).

The present study is in agreement with other reports that besides root, stem, bark etc., alteration of leaf morphology and colours being an indicator of air pollutants viz. NO_x (oxides of nitrogen) and SO_x (oxides of sulphur), hydrocarbons, O_3 (ozone), particulate matters, HF (hydrogen fluoride), PAN (peroxyacetyl nitrate) etc.[20,39] On other hand, these pollutants may not affect animals and human buthave deleterious impact on plants at very low concentration(s).[38] It was also reported that reduction of chlorophyll content occurred in many plants such as *Azadirachta indica, Nerium oleander, Mangifera indica* and *Dalbergia sissoo* due to particulates pollution of cement industry,[21] thus supporting the present work for alteration of leaf colour as visible injuries like pigmentation in experimental area. In other words, it was observed that air pollution causes the reduction of chlorophyll content in the leaves of trees present in Durgapur,[16] which supports the results in which yellow coloured leaves obtained majorly 2.0 km and 5.0 km in the study area. It has already been declared that Durgapur is 7th polluted city of India.[37]

Study of alteration of phytoconstituents in leaves by TLC

The TLC of the aqueous extract results under UV visualization, showed a thick band (one number) of phytoconstituent in the lane of E1 and E2, which was fluorescedwith maximum intensity but less in E3 and absent in C while another band (one number) was observed in all the lanes (C, E1, E2 and E3) with a same distance. Furthermore, another band (one number) was also observed only in E1 and E2 lanes with a same distance, which was absent in C and E3 (Fig 3).

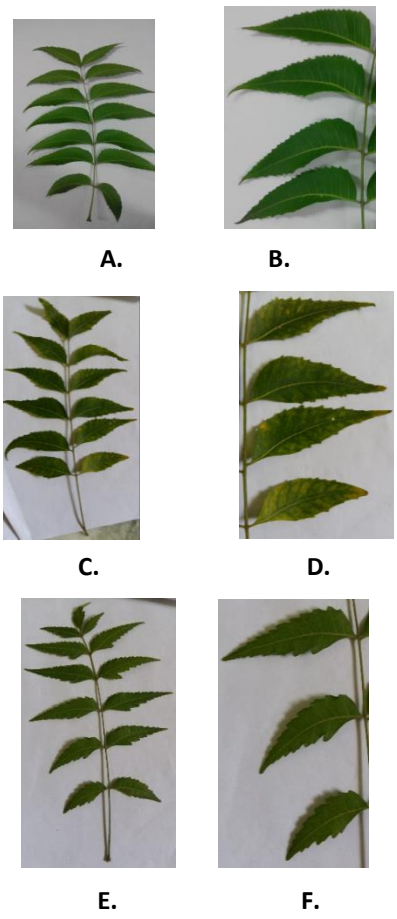


Figure 2: Alteration of leaf morphology and colour (A = Control (10 km) sample; B = Enlarge view; C = Experimental (5 km) sample; D = Enlarge view; E = Experimental (2 km) sample; F = Enlarge view)

It is interesting to note that major bands of bioactive compounds were observed in the methanolic extracts of leaves of *A. indica* through TLC screening. The day light visualization of TLC plate was not clear and did not consider in the present study but iodine vaporization showed different band colours with several numbers in each lane. In case of pure compound, distinct five bands

were identified under iodine vapourization such as sulfonoquinovosyldia-cylglyceride, quercetin-3-*O*-β-rutinoside, kaempferol-3-*O*-β rutinoside, quercetin-3-*O*-β-D-gluco-pyranoside and 1-O-(β-D-glucopyranosyl)(2S,3S,4R,8Z)-2-[Q'R)-2'-hydroxy-tetracosanoi-lamino]-8(Z)-octa-dene-1,3,4-triol and only single band was obtained under UV light namely 1-O-(β-D-glucopyranosyl)-(2S,3S,4R,8Z)-2-[Q′R)-2′-hydroxy-tetracosanoilamino]-8(Z)-octadene-1,3,4-triol (Fig. 4 A &C). In case of experimental TLC, the compound (sulfonoquinovosyldia-cylglyceride) was absent in all the area like C, E1, E2 and E3. The compound (quercetin-3-*O*-β-rutinoside) was absent in C and E3 but distinct band was found in E1 and E2. The compounds (kaempferol-3-*O*-β-rutinoside and quercetin-3-*O*-β-D-gluco-pyranoside) were absent in E1 and E2 but present in C and E3. The compound (1-O-(β-D-glucopyranosyl)(2S,3S,4R,8Z)-2-[Q′R)-2'-hydroxy-tetracosanoi-lamino]-8(Z) octadene-1,3,4-triol) was present in C, E1 and E2 but absent in E3 (Fig. 4B)while in Fig 4D, under UV light, the band was absent in the all experimental area when compared to pure compound. The numbers of bands were varied majorly in experimental leaves compared to control leaves but we unable to identified the exact compounds. Details of bands numbers and colours for both iodine vaporization and UV light were tabulated (Table-2). Several literatures suggested that neem leaf has potent bioactive compounds and these are biological active molecules with high medicinal properties as well as capable of prevent in phytotoxicity as defense mechanism within the plant body.[40-46] Several phytoconstituents e.g. m-toluylaldehyde, methyl 14-methylpenta-decanoate, lineoleoyl chloride and methyl isoheptade-canoate have been isolated by methanolic extract of neem leaves followed by GC-MS analysis and particular test for alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugars.[18,46]

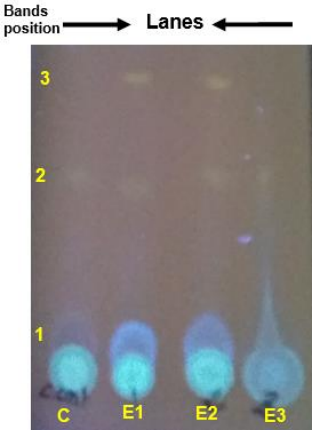


Figure 3: TLC of aqueous extract of bioactive compounds (UV visualization) [1-3 = band positions]

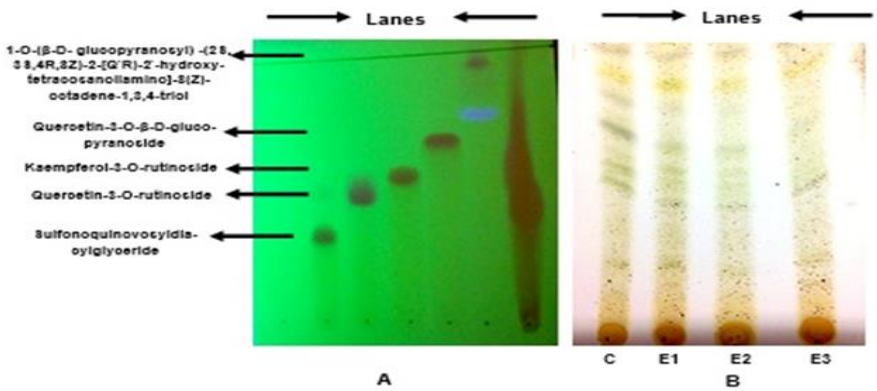
Susmitha et al.[18] have studied qualitative bioactive compounds in the leaves of *A. indica* and detected compounds such as alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugars. Usually phenolic compounds and flavonoids are potent bioactive compounds in *A. indica* for defending several environmental factors and azadirachtin and nimbin have been quantified as antioxidant.[17] It was already known that antioxidants can prevent the toxicity in living organisms. Reports on further characterization of bioactive compounds in the leaf samples of *A. indica* suggested that major phytochemicals are nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione and nimbiol.[19,46]

Molecular docking between MMP9 and ligands

It was observed in molecular docking study that the ligand mainly kaempferol-3-*O*-β-rutinoside (-125.94) showed high binding affinity and lower energy binding values (Kcal/mol) compared to other compounds against MMP9 (Table 3). Among 12 chemicals, only one compound was inhibited the activity of MMP9 as competitive inhibitor (Fig. 5B and C). It was observed that the close contacts with hydrophobic residues such as Gly186, Pro421, Ala189, Met422, Gln402, Leu188 and Tyr420 while hydrogen bonding residues were found with Tyr423, His401, His411 and His405. It was found there is binding of ligand in the active site of MMP9 with a Zn^{2+} ion (Fig. 5C). This phytochemical is a nature of zinc-binding groups of inhibition for MMP9.[47] According to Eckhard et al.,[48] three histidine residues formed a bonding with catalytic zinc in active site pocket of MMP9, a similarity was obtained for the ligand kaempferol-3-*O*-β-rutinoside during docking and this ligand may be suitable inhibitor for MMP9 expression during gastric ulcer. The present docking study is revealed this compound of neem leaf may be prevented the wound healing with reduction of MMP9 activities during gastric ulcer, which supported the experimental and predictive results of other natural product like melatonin showed binding in the catalytic site of MMP9.[27] On the other hand, several reports have documented to inhibit MMP9 activity with the development of reversible hydroxamate-based peptidomimetic inhibitors (Batimastat, Marimastat), which possess a high affinity toward the catalytic site of the enzymes.[49-50] According to Tochowicz et al.,[33] potent MMP9 inhibitors like phosphinic acid and carboxylate inhibitors have an indole scaffold stated in the X-ray crystallographic structure. Other reports have documented that melatonin downregulates MMP-9 during prevention of non-steroidal anti-inflammatory drugs (NASIDs) induced gastric ulcer and alcohol-induced liver injury in experimental rodent models.[26,51] Moreover, this predictive study suggests the *in vivo* assay in the rat model with pure compound as well as crude extracts against MMP9 activity while in vivo assay with rat model has already been studied that neem leaf compounds have drug induced ulcer preventive capability.[52]

Table 2: Methanolic extracts of TLC bands for leaves of A. indica

Sl. No.	Area	Iodine vaporization		UV irradiation (365 nm)	
		Band Nos.	Colour	Band Nos.	Colour
1.	Control (C) 10 km	1	brown	**1**	bluish white
		2	absent	2	dark black
		3	brown	3	light black
		4	brown	4	light black
		5	black	5	Black
		6	brown	6	Absent
		7	yellow	7	dark black
		8	brown	8	bluish white
2.	Experimental (E1) 5 km	1	brown	1	bluish white
		2	black	2	light black
		3	brown	3	Absent
		4	brown	4	light black
		5	brown	5	light black
		6	black	6	dark black
		7	yellow	7	dark black
		8	black	8	bluish white
3.	Experimental (E2) 2 km	1	brown	1	bluish white
		2	black	2	light black
		3	brown	3	Absent
		4	brown	4	light black
		5	brown	5	light black
		6	absent	6	dark black
		7	yellow	7	dark black
		8	black	8	bluish white
4.	Experimental (E3) 3.5 km	1	brown	1	bluish white
		2	absent	2	light black
		3	black	3	Absent
		4	absent	4	Absent
		5	brown	5	light black
		6	absent	6	dark black
		7	yellow	7	dark black
		8	absent	8	bluish white



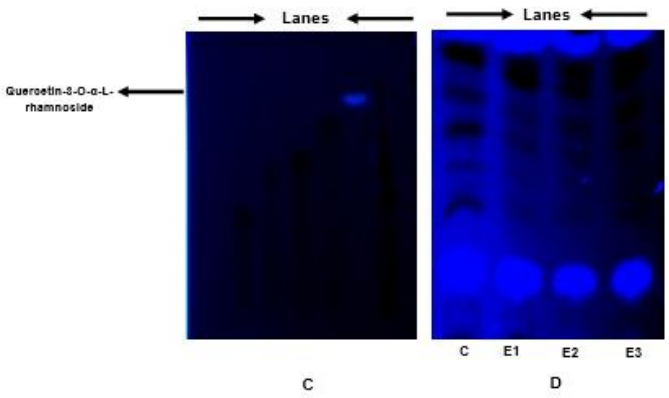


Figure 4: Thin layer chromatography of methanolic extract having different bioactive compounds from pure compound (A & C) and crude extract (B & D); (A & B = iodine vaporization visualization; C & D = UV visualization) (C= 10 km; E1 = 5 km; E2 = 2 km and E3 = 3.5 km)

Table 3: Binding energies for selected ligands

Sl. No.	Ligands	Total Binding Energy	Vander waals Forces	H-Bond	Electrostatic bonding
1.	Kaempferol-3-O-β-rutinoside	-125.94	-86.22	-39.72	0
2.	Quercetin	-114.06	-99.46	-14.61	0
3.	Quercetin 3-O-rutinoside	-112.41	-74.22	-38.19	0
4.	Azadiradione	-100.01	-88.62	-11.39	0
5.	Azadirachtin A	-95.67	-70.89	-24.77	0
6.	Nimbolide	-94.65	-76.50	-18.16	0
7.	Nimocinolide	-94.82	-84.87	-8.09	-1.85
8.	Margolone	-92.98	-89.31	-1.38	-2.30
9.	Margolone	-92.98	-89.31	-1.37	-2.30
10.	Nimbinin	-91.84	-85.26	-6.58	0
11.	Nimbin	-91.06	-80.47	-10.58	0
12.	Isomargolonone	-88.65	-79.90	-7.48	-88.65
13.	Gallic acid	-77.75	-56.16	-21.58	0

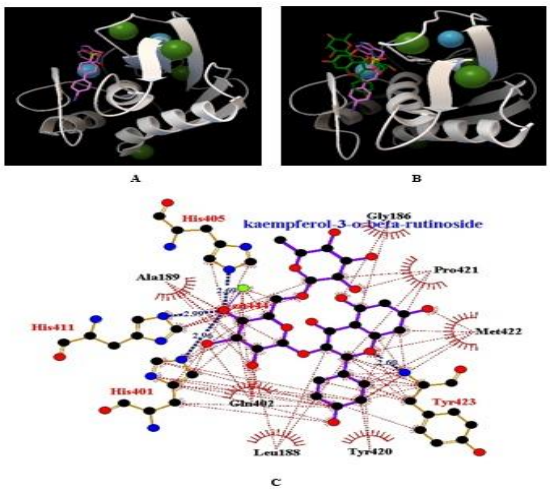


Figure 5: A. Crystal structure of MMP9 (PDB ID: 2OW0). Ribbon representation is showing Cyan spheres = zinc ions; Green spheres = calcium ions; Ball and stick structure = 6MR (N-[(4'idobiphenyl-4-yl)sulfonyl]-D-tryptophan). B. Three-dimensional structure of docking visualized under AutoDock Tools (MMP9 and kaempferol-3-O-β-rutinoside docked pose). C. Two-dimensional binding interaction of MMP9- kaempferol-3-O-β-rutinoside by using LigPlot.

CONCLUSION

It is concluded that air pollution induced alteration in leaf morphology with special reference to shape and colour as well as bioactive constituents of *A. indica* as judged through TLC using aqueous and methanol extracts of experimental leaves. The alteration mainly loss of compound due to air pollution may not be suitable for disease prevention. Moreover, several studies revealed the antioxidant property of both azadirachtin and nimbin phytochemicals in *A. indica* leaves.[17] Beside these phytochemicals, alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugars were also documented in neem leaves.[18,46] According to Bhajoni et al.,[52] phytochemicals containing neem leaf have showed antiulcer activity.

This present work may be beneficial to explore the biosynthesis of new phytoconstituent(s) or absence of specific constituent(s)in the leaves of *A. indica* from air polluted area. From this study, we hypothesize that the ligand mainly kaempferol-3-O-β-rutinoside, the active phytochemical of *A. indica* is more effective in inhibiting MMP9 activity than other compounds. In other way, MMP9 down regulation helps in gastric ulcer prevention with the help of melatonin.[26,51] It is suggesting that prior to medicinal usage from the extract of leaves, it should be checked the qualitative and quantitative variations in secondary metabolites content due to air pollution.

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