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Pharmacognostical studies of *Ricinus communis* under the influence of industrial effluent

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ABSTRACT

The aim of the present study is to evaluate the pharmacognostical markers of *Ricinus communis* L., an attempt has been made for a comparative study of plants growing near the vicinity of polluted, Atlas Cycles Industry, with those plants growing in non-polluted areas (ALTT Centre, Ghaziabad,India).It was noted that the industrial effluents not only changed the morphological and anatomical characters of plants, but also affected their chemical constituents. Analysis details contain data regarding colour, odour, BOD, COD, DO, pH, temperature, TS, TSS, TDS, oil and grease, heavy metals etc. which were found to have greater values as compared to standard values. Themorphological, anatomical parameters (xylem vessels,cambium and endodermis are in discontinuous manner, stomatal index, palisade ratio etc.) and physico-chemical parameters (number of spots in observation of TLC, water extractive and alcohol extractive values etc.) have shown a decreasing trends in those plants which were collected near the vicinity of Atlas cycle industry.

Key words: Industrial effluent, Pharmacognostical studies, *Ricinus communis*

Impact of industrial effluent on plants and human health are well known. Heavy metals and other pollutants contained in the industrial effluents and agricultural wastes enter into aquatic plants and roots of other plants, growing in their contacts, causing serious threats to them, because they do not degrade easily unlike other organic pollutants. In the vicinity of industries, often many medicinally important plants are found growing.*Ricinus communis* L. is an important ayurvedic medicinal plants which is also found growing near the vicinity of Atlas Cycle Industry in Ghaziabad. This plant species has been selected to study the impact of industrial effluent. The plant *Ricinus communis* is a monotypic genus and commonly known as Erandi. It belongs to family Euphorbiaceae.It is found throughout the country and widely cultivated in the tropics and warm regions of the country for its seeds, which yield well-known castor oil. Castor is one of the major oil seed crops of India and, in fact, India is the second largest producer of castor seed in the world. *Ricinus communis* is believed to be a native of tropical Africa. The plant contains alkaloids, ricinoleic acid, stearic, oleic, linoleic, dihydroxystearic acids, palmitic acid, β sitosterol, squalene (38 mg/100g), tocopherols and stearic acid (av. 45 µg/100 g made up mostly of g and d) (Chatterjee and Prakashi 1994). The plant also contain glyceride, tri-ricinolein, di-ricinoleins, oleo-diricinolein, linolco-diricinolein, monoricinoleins and non–ricinoleo glycerides. All parts of plant are used in various ailments. Roots of *Ricinus* is sweet in taste and used medicinally. It is also useful in inflammations, pains, ascites, fever, glands, asthma, eructations, bronchitis, leprosy, disease of rectum and the head.In Ayurveda, leaves are useful in "Vata" and "Kapha", instestinal worms, strangury, night blindness, earache and increased biliousness. The flowers are useful in glandular tumours, anal troubles and vaginal pain. Fruits are useful in "Vata", piles, diseases of the liver and spleen. Seeds are cathartic and aphrodisiac. Atlas Cycle Industry was selected for the study of *Ricinus communis* as they are growing near the vicinity of effluent discharged from this Industry. In this study various chemical tests were carried out with plant powder to find out the impact of Atlas Cycle Industry effluent on the selected plant species.

MATERIALS AND METHODS

The effluent discharged from Atlas Cycle Industry was analyzed by APHA (1998) and Trivedi and Goel (1986) methods. Fresh material of *Ricinus communis* was collected from both non-polluted (ALTT Centre) and polluted (Atlas Cycle Industry) areas of Ghaziabad, UP (India). Trease and Evans (1972) for the macroscopical studies, Metacalf (1980)

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for anatomical studies, Jackson and Snowdon (1968) for colour reaction test, I P (1966) for physical evaluation and Cromwell (1955) for the powder analysis were consulted. TLC was investigated by the method described by WHO (1998).

RESULTS AND DISCUSSION

Analysis of effluent

The effluent contains data regarding Colour, Odour, BOD, COD, pH, temperature, SS, TS, TSS, TDS, oil and grease, heavy metals etc. The result is given in Table 1.

Organoleptic studies

Macroscopical: Stem was herbaceous, aerial, erect, cylindrical, branched, older portions were fistular, younger portions solid, glabrous, green purple, divided into node and internode. Leaf was simple, cauline, ramal, opposite, decussate in early stages but becomes alternate later. Petiole size 10-16 cm, hollow sometimes solids, glabrous, lamina, palmately lobbed, lobes 7-11 ovate to acute, margin serrate,dentate, dorsiventral and reticulate venation present.

Two nector secretry disc present at the base of joint of lamina and petiole. Plants growing in polluted stem as green purple in colour with large brown patches, less branched and glabrous. Leaves were light in colour, smaller in size with some brown patches, petiole size was 7-10 cm, lobes were 7-10 in numbers. However, flower was bracteate, pedicellate, incomplete, actinomorphic, unisexual, staminate,

Table 1 Physico-chemical characteristics of industrial effluent of Atlas Cycle Industry

Parameters	Characteristic of effluents	Maximum recommended concentration	Authority/ Reference
Colour	Yellowish	Should be absent	I.S.I. : 2490
Odour		Odourless	I.S.I. : 2490
pH	4-6	5.5-9.0	I.S.I. : 2296
Suspended solids (SS)	200 mg/l		
Total dissolved solids (TDS) (mg/l)	810 mg/l	2100.0	I.S.I. : 3307
Total suspended solids (TSS) (mg/l)	1010 mg/l	600.0	I.S.I. : 3306
Dissolved solids (DS)	720 mg/l		
Total solids (TS) (mg/l)	840 mg/l	2700.0	
BOD (mg/l)	16.0 mg/l	30.0	I.S.I. : 2490
COD (mg/l)	200 mg/l	250.0	I.S.I. : 2490, 1982
Oil and grease (mg/l)	Nil	10.0	I.S.I. : 2490
Chloride (mg/l)	Nil	600	I.S.I. : 2490
Chromium (Cr)	5 mg/l		
Nickel (Ni)	12 mg/l		
Zinc (Zn)	15 mg/l		
Cadmium (Cd)	4 mg/l		
Copper (Cu)	4 mg/l		
Temperature	50°C		

a b

Fig 1 Morphological differences of *Ricinus communis* growing in non-polluted (a) and polluted (b) areas

pentamerous and cyclic in both the cases.Fruit regma splitting into cocci and a caruncle was present at the apex of the seed and these were small in size in polluted plants. The main differences are in Fig 1 (a and b).

Anatomy/Histology stem: Stem of non-polluted showed single layer of epidermis covered by thin cuticle and both types of trichomes:non-glandular as well as glandular, 5-6 layers of collenchyma; 3-4 layers of parenchyma, single layered endodermis with casparion strip, pericycle in patches, cambium in continuous layer. Vascular bundles arranged in ring. Xylem vessels larger in size. Some micro and rosette crystals of calcium oxalate were also present in pith region.In case ofstem at polluted site showed the epidermis containing thick cuticle with both types of trichomes, 9-10 layers of collenchyma, 4-5 layers of parenchyma, cambium and discontinuous endodermis pericycle found absent.

Leaf: Transaction of leaf at control site showed single layer of epidermis containing thin cuticle and both types of trichomes glandular as well as nonglandular, randomly scattered anisocytic and anomocytic stomata on both surfaces with stiration. 10-14 layers of collenchyma below upper epidermis and 5-6 layers above lower epidermis in midrib region. Four vascular bundles present in midrib.

Mesophyll cells differentiated into palisade and spongy parenchyma; single layer palisade, 2-3 layers of spongy parenchyma; micro, prismatic and rosette crystal were present. But in case of polluted leaf 13-14 layers of collenchyma below the upper epidermis and 6-7 layers below the lower epidermis were noted, two vascular bundles present in midrib, single layer palisade and 3-4 layered spongy parenchyma; micro and rosette crystal of calcium oxalate were present in parenchymatous cells (Fig 2, a,b,c and d). The stomatal index in non- polluted leaves samples were obtained as 16.66–23.32 and 16.02–21.01 in upper and lower epidermis respectively.Whereas in polluted leaves it was 15.92– 20.00 and 13.28–16.32 in upper and lower epidermis respectively. The palisade ratio was found 10.50–13.50 in non-polluted samples and 6.55–8.75 in polluted samples. The vein islet number and vein termination number were obtained 12.00-16.00 and 44.00-48.00 in non-polluted samples respectively. In case of polluted plant samples both

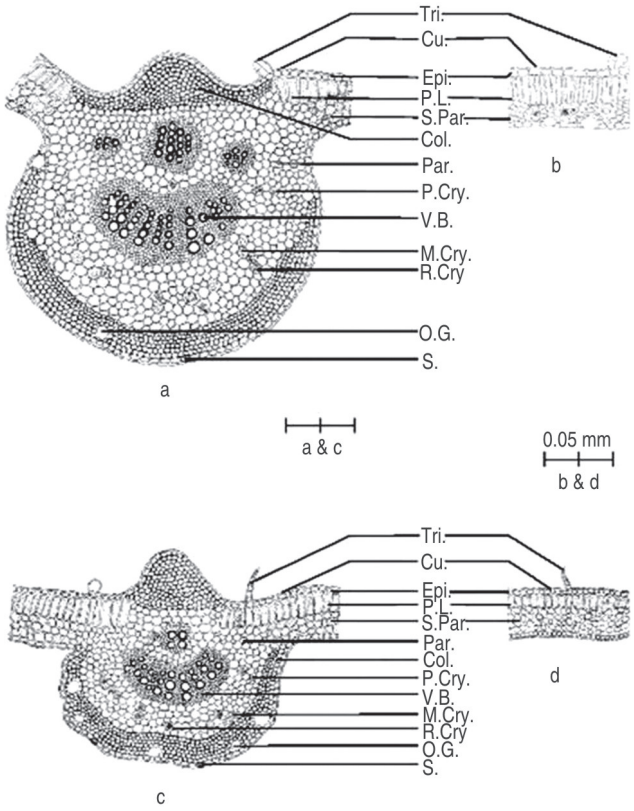

Fig 2 Anatomical differences in the leaves of *Ricinus communis* Linn. growing in non polluted (a through midrib) & (b through lamina) and polluted areas (c through midrib) & (d through lamina).

were obtained 12.00-24.00 and 68.00-76.00 respectively.

Powder analysis: The colour of the powder was green in non-polluted leaves and brown green in polluted leaves having strong unpleasant odour and bitter taste. Tubular shaped epidermis with numerous unicellular and multicellular trichomes were also noted. Stiration and anisocytic, anomocytic types of stomata were present in both the cases. Micro, rosettes and prismatic crystals of calcium oxalate were also present in parenchymatous cells.Glandular trichomes were present only in case of non-polluted samples.Vessels with annular thickening with simple perforation rims are measured in length and width. Tracheids are few, pitted and elongated with narrow tapering end their length and width were measured.Xylem fibres were also measured in both the cases.Differences in the cell size have been tabulated in Table 2.

Chemical analysis

Preliminary colour reaction tests: The result showed the presence of alkaloids, saponin, tannin, lignin, protein, carbohydrate, suberin, glucoside, oil, sugars, steroids and absence of flavanoids in both the cases. Degree of change in colour reaction tests are tabulated in Table 3.

TLC: Number of spots were 4-5 in non-polluted and 3-5 in polluted samples. There Rf values are tabulated in Table 4.

Physical evaluation

Fluorescence behaviour of plants: There were no significant results observed with fluorescence behaviour of plant powder and its extracts except some difference in colours only.

Extractive values and ash values: The percentage of water and alcoholic soluble extractives were lower in plants those collected from polluted sites, but LOD was higher in polluted samples. Total ash, acid insoluble and sulphated ash were higher in those samples which were collected from the polluted areas. The mean values are tabulated in Table 5.

The effluent samples collected from the selected industry was analysed for different physico-chemical parameters which showed higher values than the recommended values by ISI. Similar results were also obtained by Kumar *et al*. (1991).The critical observations on the data clearly indicate that the plants growing in polluted sites were badly affected and there were a significant reduction in number of parameters those studied as compared to the control plants. Morphological characters were found to be decreased in the selected plant collected from polluted areas. Similar observations are recorded by Palaniswamy *et al*. (1995). Angadi and Mathad (1998) have studied the effect of copper, cadmium and mercury on the morphological, physiological and biochemical characteristics of *Scenedesmus quadricauada* (Turp) de Breb. and found maximum inhibition in the growth, chlorophylls, total DNA, total RNA and protein contents of cells at the higher metal concentrations. Therefore it is observed from various studies that the same species respond differently under different conditions.

Thick cuticle observed in the transverse sections of the stem collected from the polluted area also matched with the findings of Percy *et al.* 1992. Cuticle is the first point of attack of pollutants; our results indicated an increase in the thickness of cuticle at the polluted sites which indicate that the plants have an effective barrier for the pollutants entry. Trivedi and Singh (1989, 1990) studied the epidermal features (stomatal density and index) of different plants (*Boerhaavia diffusa* and *Amaranthus viridis*) under the impact of air pollution. Significant reduction in cell size of the pollution effected plants was also reported by Ansari and Iqbal (1992). Ogunkunle *et al*. (2013) had observed several modifications in anatomical structures, as found in the leaf epidermis, conducting elements in roots and stems were damaged, when the plant treated with different concentrations of effluent . It is evidently clear from the study that low concentration of efluent can be non-toxic for growth of vegetables but increasing the concentration can be covertly toxic to the vegetable which possibly may translate to low biomass production. The reduced length of vessel elements coupled with their augmented frequency appears to be a significant adaptations to the stress of pollution. In contrary to the above reporters more number of parameters (xylem vessels) observed in the plant samples collected from polluted area over to control population in

Table 2 Powder analysis of *Ricinus communis* growing in non-polluted and polluted areas

Characters	Powder analysis	
	Non-polluted	Polluted
Xylem vessel (mm)	L=1.18 + 0.11; CV = 9.31 W=0.11+ 0.04; CV=36.11	L=0.50 + 0.14*; CV = 27.29 W=0.11+ 0.02; CV = 17.59
Diameter of xylem pore (mm)	D= 0.15 +0.04; CV=24.84	D=0.13+ 0.04**; CV = 32.00
Xylem tracheid (mm)	L=1.28 + 0.29; CV=22.76 W=0.11+ 0.03; CV=26.42	L=1.15 + 0.19**; CV = 12.26 W=0.06 + 0.01*; CV = 19.67
Xylem fibre (mm)	L=0.98+ 0.17; CV=17.13 W=0.04 + 0.01; CV=21.95	L= 0.64+ 0.06*; CV = 8.72 W=0.04+0.015*; CV=13.33
Stomata		
Guard cell (mm)	L=0.04+ 0.01; CV = 18.42 W = 0.03 + 0.01; CV = 16.13	L = 0.03+ 0.01*; CV = 17.24 W=0.02 + 0.01*; CV = 25.00
Stomatal pore (mm)	L = 0.02+0.003; CV = 20.00 W = 0.03+0.001; CV = 3.33	L=0.01+0.002*; CV = 18.18 W=0.03+0.001***; CV= 3.57
Stomatal index		
Upper surface	R = 19.38– 23.32 SD=18.51 + 1.99; CV=10.78	R= 16.66 – 21.42 SD=14.85 + 1.41; CV=9.49
Lower surface	R=16.03 – 21.02 SD=17.46 + 1.62; CV=9.27	R=14.39 – 16.32 SD=15.23+1.20 ; CV = 7.8778
Trichomes		
Unicellular and warty (mm)	L=0.19+0.004; CV=2.11 W=0.03+0.01; CV = 20.69	L= 0.10 + 0.01; CV = 11.76 W=0.03+0.003*; CV= 11.54
Multicellular (mm)	L=0.12 + 0.02; CV = 19.67 W=0.04 + 0.003; CV = 8.57	L = 0.11 + 0.01; CV = 4.72 W=0.03+ 0.001; CV = 3.33
Glandular (mm)	L=0.06 + 0.01; CV = 8.62 W=0.05 + 0.002; CV = 4.08	L=0.05 + 0.004*; CV = 8.69 W=0.04 + 0.002; CV = 5.26
Rosette crystals (mm)	L = 0.090 + 0.002; CV = 2.22 W = 0.06 + 0.01; CV = 13.79	L=0.10 + 0.03**; CV = 24.27 W=0.08 + 0.002; CV = 2.56
Prismatic crystals (mm)	L=0.09 + 0.02; CV = 22.47 W=0.03 + 0.002; CV = 7.14	L=0.08+0.003***; CV=3.75 W=0.03+0.004*; CV=2.90
Xylem vessel (mm)	L=1.18 + 0.11; CV = 9.31 W=0.11 + 0.04; CV = 36.11	L=0.50+0.14*; CV=27.29 W=0.11+0.02; CV=17.59
Diameter of xylem pore (mm)	D= 0.153 +0.04; CV = 24.84	D=0.13+0.04**; CV=32.00
Xylem tracheid (mm)	L=1.28 +0.29; CV = 22.76 W=0.11 + 0.03; CV = 26.42	L=1.15 + 0.19**; CV=12.26 W=0.06+ 0.01*; CV= 19.67
Xylem fibre (mm)	L=0.98+0.17; CV=17.13 W=0.04 + 0.01; CV=21.95	L= 0.64 + 0.06*; CV = 8.72 W=0.04+0.005*; CV=13.33
Palisade cell (mm)	L=0.14+0.01; CV=6.57 W=0.04+0.003; CV=7.32	L=0.12 + 0.003*; CV=2.61 W=0.04 + 0.003*; CV=7.06
Palisade ratio	R = 10.50 – 13.5 SD=11.93+1.32; CV=11.09	R = 6.55– 8.75*** SD=7.58 + 1.04**; CV=13.75
Vein islets number	R= 12.00 -16.00 SD=12.67 +2.49; CV=19.67	R = 12.00 - 24.00 SD=18.67 +4.99 CV=26.72
Vein termination number	R=44.00–48.00 SD=44.00 +3.26; CV=7.41	R= 68.00– 72.00 SD=73.30+2.77***; CV=3.78

Significant * at 0.1%, ** at 1.0%, *** at 5.0%

Datura inoxia by Iqbal *et al*. (1986). Chaudhari and Patil (2001) has also observed the inhibition and stimulation in xylem and phloem in pith region when grown under the stress condition caused by polluted water. In the present findings less secondary growth was observed in most of the selected plants collected from polluted areas. Jabeen and Abraham (1998) also showed less secondary tissue in *Largerstroemia reginae* and *Alstonia scholaris* trees exposed to air pollutants. Uaboi-Egbenni *et al*. (2009) had similar observations on *Abelmoschus esculentus* under the influence of industrial effluents.

Our result indicates reduced trichomes frequency, more number of stomata, presence of collenchyma layers, reduced layers of spongy parenchyma with smaller cell size, less layered ground tissue, decreased ratio of stomatal index and palisade layers; more numbers of crystals with bigger

Table 3 Colour reaction tests of *Ricinus communis* growing in non-polluted (NP) and polluted (P) areas.

Reagent	Test for	Nature of colour	Degree of changes	
			NP	P
Dragenorff's Reagent {Cromwell (1955)}	Alkaloid	Orange ppt	++++	+++
Mayer's Reagent	Alkaloid	Brown	++	+
Wagner's Reagent (Trease and Evans (1983)	Alkaloid	Brown	+++	++
Tannic Acid	Alkaloid	Turbidity	+++	+
Hager's Reagent	Alkaloid	Yellow	+++	++
Phloroglucinol + HCl	Lignin	Negative		
$FeCl_3$	Tannin	Black	+++	++
Molisch Test	Carbohydrates	Red	++++	++
Millon's Reagent	Protein	Red ppt	+++	++
Xanthoproteic Test	Protein	Yellow	++++	+++
Bendict's Reagent after heating	Sugars	Red violet	+++	+
Sample + Heating with strong KOH + H_2SO_4	Suberin	Red black	++++	++
Molisch Test after hydrolysis	Glucoside	Dark yellow	+++	+++
Plant powder + H_2O + Shake	Saponin	Froth (W)	++++	++
Mg powder + Conc. HCl	Flavin	Red	++	+
Libermann's Buchard Reagent	Steroids	Violet	+++	+
Sudan IV	Oils	Red violet	+++	++

Table 4 Rf values of *Ricinus communis* growing in non-polluted and polluted areas

Wave lengths	Non-polluted Rf-values	Polluted Rf-values
Sunlight (Visible)	0.32, 0.34, 0.49, 0.88	0.32, 0.34, 0.49, 0.89
UV light (254 nm)	0.32, 0.34, 0.49, 0.80, 0.89	0.32, 0.34, 0.49, 0.80, 0.89
UV light (365 nm)	0.32, 0.49, 0.80, 0.89	0.32, 0.49, 0.89

size in leaves of polluted plant samples. Similar observations were noted by Faroqui and Singh (1990). Low stomatal frequency were observed in the plants grown in polluted areas, which may be an adaptation of ecotypic significance regulating entry of harmful gaseous pollutants into the plants tissues in a limited and controlled, especially when the plant grown in polluted area. Physical evaluation included fluorescence behaviour, extractive and total ash values. The plant samples collected from polluted areas showed quick differentiations to fluorescence behaviour. Water and alcohol extractive values were found lowered in plants collected from polluted areas. Ash values were comparatively higher in polluted plant samples. Same

Table 5 Extractive and ash values of *R. communis* growing in non-polluted and polluted areas.

Extractive values and ash values (%)		
Parameters	Non-polluted	Polluted
Water soluble	18.895 + 0.561; CV = 2.969	17.852 + 0.061; CV = 0.342
Alcohol soluble	21.340 + 0.600; CV = 2.811	38.615 + 0.790**; CV = 2.046
LOD	10.124 + 0.020; CV = 0.197	8.900 + 0.446**; CV = 5.010
Total ash value	14.712 + 0.251; CV = 2.731	11.950 + 0.395**; CV= 0.794
Acid insoluble	1.942 + 0.053; CV = 2.731	1.951 + 0.011**; CV = 5.689
Sulphated ash	21.392 + 0.412; CV=1.925	26.920 + 0.531**; CV=1.972

Significant 0.1%, ** at 1.0%, *** at 5.0 %

observations were made by Sharma and Habib (1995). The percentage of ash contents was higher in the plant samples collected from polluted areas as compared to control, because ash content of the plants is the direct manifestation of bioaccumulation of minerals absorbed as macronutrient and micronutrients which take up different functions.

From the observation of TLC, it was seen that the number of spots were decreased in the samples of plants collected from the polluted areas. Similar observations were noted by Mashaly (1988).

In order to determine the quality of medicinal plants with regard to its authenticity pharmacognostical characters, viz. macroscopical, anatomical, powder analysis, chemical analysis, TLC, fluorescence behaviour, extractive values and ash values are very important. Anatomy often proves very useful for individual identification of plants so microscopical methods are of great value towards their identification and authenticity of the plant drugs. They provide evidences concerning relationship of groups such as families or help to establish the affinities of genera of uncertain taxonomic status. The number of stomata and epidermal cells, vein-islets and vein termination number per unit area, palisade ratio, stomatal index etc. give constant structure for different species of plants. Moreover, different types of stomata, crystals, fibers, trichomes etc. present in powdered drug help in identification of plants or differentiation in comparison of same plant, which are collected from the control/industrial area.

It is concluded that the plant under the pollution stress have suffered in its drug quality. These changes might be due to the presence of heavy metals in the effluent discharged from Atlas Cycle industry.

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