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HISTO-PHARMACOGNOSTICAL STUDY OF *SOLANUM XANTHOCARPUM* SCHRAD& WENDL. A DASHMOOL PLANT UNDER THE IMPACT OF PRAGATI PAPER MILL, INDUSTRY EFFLUENT

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ABSTRACT

To carried out the histo-pharmacognosy of *Solanum xanthocarpum* Schrad & Wendl. a dashmool under the impact of Pragati Paper Mill, industry effluent. The effluent of Pragati Paper Mill was analyzed by Trivedi & Goel, 1986 method. Metacalf and Chalk, 1950 was consulted for anatomical studies of selected plant; for chemical analysis Johanson, 1940, Cromwell, 1955 & Trease and Evans, 1983 were followed. TLC was investigated by WHO, 1998. The physico-chemical parameters of Pragati Paper Mill effluent were found higher values as compared to standard values. The morphological & anatomical parameters were showed declining trend in those plants which were collected from polluted sites. The colour reaction tests showed only degrees of changes. The number of spots in observation of TLC, stomatal index, palisade ratio, water extractive and alcohol extractive values were decreased in those plants which were collected near the vicinity of Pragati Paper Mill whereas vein Islet & vein termination number, ash values were comparatively greater in same samples. The conclusion of this study is that the plants should not be collected form polluted areas (near the vicinity of any industry) for the preparations of medicines, since majority of parameters reflect decreasing data in those plants which were taken from polluted area.

KEYWORDS: Histo-pharmacognosy, *Solanum xanthocarpum,* Effluent analysis

INTRODUCTION

The rapid industrialization and the growth in the population substantially alter the nature of inter-action of man and his environment. The soil, water and air are polluted by industrial and domestic wastes.*Solanum xanthocarpum* Schrad. & Wendl. is selected to study the impact of industrialeffluent (Pragati Paper Mill, Ghaziabad) for the present study. Bio-monitors are of the view that the polluted water which is an intricate system of living and nonliving substances like acids, alkalis, chlorides, heavy metals, dissolved solids, nitrates, sulfates, aquatic life bacteria, fungal forms *etc*. is responsible for environmental hazards and destroying many plants species and also for including a variety of changes in the morphology, anatomy,

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MATERIALS AND METHODS

The samples of *Solanum xanthocarpum* were collected from the area near to Pragati Paper Mill, Sahibabad industrial area, Ghaziabad to investigate the effect of industrial pollution. The effluent was analyzed by using the standard method of Trivedi & Goel (1986). Fresh and matured samples of both the plants are collected from polluted (Pragati Paper Mill) and non-

polluted areas (village Bayana, Ghaziabad) to their macro morphological andanatomical studies. For anatomical studies Metacalf (1980) were consulted. TLC was evaluatedaccording to WHO, Geneva, 1998. For chemical analysis Johanson, 1940, Cromwell, 1955 & Trease and Evans, 1983 were followed.

RESULTS

Analysis of effluent: The physio-chemical analysis of the effluents discharged by the industry has been done,result shown in table 1.

Table 1. Physico-chemical Characteristics of industrial effluent of Pragati Paper Mill.

S. No.	Characteristics	Effluent value of Pragati Paper Mill.	Maximum Recommended Concentration	Authority/ Reference
1.	BOD (mg/l)	110.0	30.0	I.S.I. : 2490
2.	COD (mg/l)	568.0	250.0	I.S.I. : 2490
3.	COLOUR	Milky	Should be absent	I.S.I. : 2490
4.	ODOUR	Pleasant	Odourless	I.S.I. : 2490
5.	Oil and Grease (mg/l)	ABSENT	10.0	I.S.I. : 2490
6.	pH	3.0	5.5-9.0	I.S.I. : 2296
7.	Total Dissolved Solids (mg/l)	620.0	2100.0	I.S.I. : 3307
8.	Total Solids (mg/l)	732.0	2700.0	----------
9.	Total Suspended Solids (mg/l)	112.0	600.0	I.S.I. : 3306

Histo – Pharmacognostic Studies

a. Organoleptic Studies :

Macroscopical Studies : Non-polluted stem was numerous branches and zigzag with, dense stellate tomentum, prickles compressed, straight trichomes. Leaves were ovate to elliptical, 3-5 cm long, sinuate to sub- pinnatifid, obtuse to subacute, stellately hairy on both sides. Lamina is 5-10 cm long and 2.5- 5, cm broad. Whereas in the plants grown in polluted areas stem was less branched, yellow green in colour and 1.3-1.5 cm long. Leaves were less green in colour, more stellate hairs on both the sides, lamina 4.5- 8.5 cm long and 2.0-5.0 cm broad (**Plate-1**). Odour waspungent in both the plant samples. The results are shown in **table-2.**

a **b**

Plate:1 Morphological differences of ***Solanum xanthocarpum*** **Schrad. & Wendl.** growing in non polluted (a) and polluted (b) areas.

Table 2. Macroscopical differences of *S. xanthocarpum* Schrad. & Wendl. plant grown in non-polluted and polluted areas.

S. No.	Parameters	Non Polluted	Polluted
1.	Height of plant (cm)	R = 03.000 – 05.000	R = 01.300 – 01.500
2.	No. of leaves per plant	SD = 19.630 ± 01.620; cv = 08.253	SD = 12.520 ± 01.890*; cv = 15.096
3.	Leaf Colour	Dark green	Yellow green
4.	Lamina Size (cm)	L = 09.000 ± 00.816; cv = 09.067 W = 05.330 ± 00.650; cv = 12.195	L = 06.500 ± 00.712*;cv = 10.954 W = 03.000 ± 00.450*; cv = 15.000
5.	Flowers per plant	SD = 10.230 ± 00.490; cv = 04.790	SD = 06.230 ± 00.620*; cv = 09.952

* = Significant at 0.1%

Microscopical Studies:

Stem: The transverse section of non-polluted stems showed single layered epidermis covered with thin cuticle and both type of trichomes non-glandular and glandular; hypodermis 3-4 layered chlorenchymatous, 4-5 layered collenchyma. Cortex have regular shaped parenchymatous cells; endodermis composed of barrel-shaped cells and pericycle sclerenchymatous. Vascular bundles were conjoint, collateral and arranged in a ring. Cambium was 3-4 layered, xylem vessels radially arranged. Intraxylary phloem lies just inside the ring of the xylem tissue. Micro and aggregate crystals of calcium oxalate and starch grains were present in pith cells, whereas polluted stem contained thick cuticle; chlorenchyma 2-3 layered; collenchyma 4-5 layered; endodermis a discontinuous layers, pericycle absent, phloem in patches, xylem vessels more in number but smaller in size, crystals more in number and bigger in size, number of starch grains is decreased (**Plate -2**).

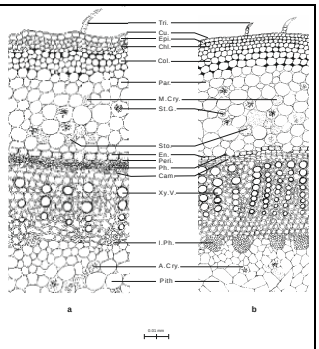

Plate-2: Anatomical differences in stem of ***S. xanthocarpum*** **Schrad. & Wendl.** growing in non polluted (a) and polluted (b)areas .

Leaf:

Midrib: Transverse section of a non-polluted leaf showed single layered epidermis covered by cuticle having glandular and non-glandular trichomes; stomata anisocytic present on both the surfaces; meristele composed of cresent-shaped, bicollateral, vascular bundle. Micro and aggregate crystals were present in parenchymatous cells. While in case of polluted leaf trichoms were more in number; epidermis followed by 6-7 collenchymatous cells on upper side and 5-6 layered on the lower side; arch shaped vascular bundle in the centre and crystals were more in number with bigger size. **Lamina** is differentiated into 4-5 layer palisade and 3-4 layers spongy parenchyma but in case of polluted leaf, stomata less in number, palisade 3-4 layered and spongy parenchyma. The stomatal index was 23.87 ± 2.59 on upper epidermis and 24.52 ± 2.58 on lower epidermis in non-polluted leaves samples whereas in polluted leaves it was 21.17 ± 2.39 on upper epidermis and 22.16 ± 2.12 on lower epidermis. The vein islet number was found to be higher in leaves collected from polluted area. The values of vein termination were higher in the leaves collected from polluted area. Results were presented in (**Plate-3).**

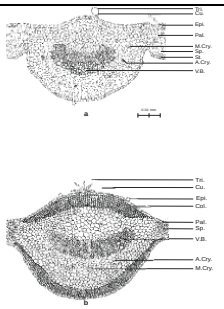

Plate-3: Anatomical differences in the leaves of ***S. xanthocarpum*** **Schrad. & Wendl.** growing in non polluted (a) and polluted areas (b)

Abbreviations of plates

A. Cry. – Aggregate crystal;Cam.-Cambium;Chl. - Chlorenchyma; Col.-Collenchyma; Cu.-Cuticle; Epi.-Epidermis; En.-Endodermis; M. Cry.-Micro Crystal; Par.-Parenchyma; Pal.-Palisade Layer; Peri.-Pericycle; Ph.-Phloem; R. Cry.-Rosette Crystal; Ra.-Raphides; Sto.-Stomata; S.P.-Spongy parenchyma; Tri.-Trichome; V.B.-Vascular Bundle; Xy.V.-Xylem Vessels

Plant Powder Studies: The colour of plant powder was green in non-polluted samples and brown green in polluted samples.Powder of the whole plant, was studied and its salient features under microscope were observed such as fragments of trichomes;parenchymatous cells with micro and aggregate crystals, rounded to oval shape starch grains.Xylem vessles showed spiral and annular thickenings with bordered

pits.Xylem tracheids elongated with pits and irregular walls. Numerous elongated fibres, thick walled, lignified with tapering and pointed ends.

b. Chemical Analysis:

Preliminary Colour Reaction Test: The preliminary colour reaction tests showed the presence of alkaloids, lignin, tannin, carbohydrates, proteins, sugars, subernin, glycosides, saponin, steroid and oil and absence of flavins in both the cases. Differences were tabulated in table–3.

Table 3. Preliminary colour reaction tests of *Solanum xanthocarpum* Schrad. & Wendl. plant grown in non-polluted and polluted areas

S. No.	Reagents	Test for	Nature of Colour	Degree of Changes	
				NP	P
1.	Dragenorff's Reagent {Cromwell (1955)}	Alkaloid	Orange ppt	++++	++
2.	Mayer's Reagent	Alkaloid	Brown	++++	++
3.	Wagner's Reagent (Trease and Evans (1983))	Alkaloid	Brown	++++	+++
4.	Tannic Acid	Alkaloid	Turbidity	++	+
5.	Hager's Reagent	Alkaloid	Yellow	+++	++
6.	Phloroglucinol + HCl	Lignin	Dark Red	+++	++
7.	$FeCl_3$	Tannin	Black	++++	+
8.	Molisch Test	Carbohydrates	Red	++++	+++
9.	Fehling Solution	Carbohydrates	Red	+++	++
10.	Millon's Reagent	Protein	Red	+++	++
11.	Xanthoproteic Test	Protein	Yellow	++++	++
12.	Bendict's Reagent after Heating	Sugars	Yellow Red	++	+
13.	Sample+Heating with Strong KOH + H_2SO_4	Subernin	Red Black	++++	+++
14.	Molisch Test after Hydrolysis	Glycoside	Red	+++	++
15.	Plant Powder + H_2O + Shake	Saponin	Froth (W)	++	+
16.	Plant Powder + Conc. HCl	Flavin	Red	-------	------
17.	Libermann's Buchard Reagent	Steroids	Violet	+++	++
18.	Sudan IV	Oils	Red	+++	++

Thin Layer Chromatography: The spots were 4-6 in non-polluted and 4-5 in the polluted plants. The Rf values are shown in **(Plate-5) table 4**.

Table-4: TLC of *Solanum xanthocarpum* Schrad. & Wendl. plant grown in non-polluted and polluted areas.

S. No.	Wavelength	Non –polluted	Polluted
		Rf – values	Rf - values
1.	Sunlight (Visible)	0.31, 0.36, 0.51, 0.93	0.33, 0.40, 0.53, 0.93
2.	U V Light (264nm)	0.27, 0.33, 0.47, 0.53, 0.67, 0.87	0.33, 0.36, 0.47, 0.93
3.	U V Light (365nm)	0.31, 0.36, 0.51, 0.87, 0.93	0.31, 0.33, 0.47, 0.53, 0.93

Plate-5: **The Rf values of *S. xanthocarpum* growing in non-polluted and polluted areas.**

Physical Evaluation:

Fluorescence Behaviour: The fluorescence behaviour of plant powder as well as their extracts in different solvents were studied under visible and ultraviolet light. The observations were presented in **table–5**.

Table 5. Colour change by UV fluorescence of variously treated *Solanum xanthocarpum* Schrad. & Wendl. plant grown in non-polluted and polluted areas.

S. No.	Extracts	Visible (Sun Light)		UV Light (264nm)		UV Light (365nm)	
		NP	P	NP	P	NP	P
1.	Powder	G. Yellow	G. Brown	Green	Green	D. Green	Green
2.	Water	R. Brown	Brown	R. Green	Green	B. Black	Br. Blue
3.	Benzene	P. Yellow	Yellow	Yellow	Green	Orange	P. Orange
4.	P. Ether	Colour less	Colour less	Green	L. Green	Gr. Violet	Gr. Violet
5.	Methanol	D.Green	Green	Green	Green	Orange	Gr. Orange
6.	Chloroform	Lemon	L. Lemon	Green	L. Green	Gray	L. Gray
7.	Acetone	Green	Green	Green	Green	Gr. Red	Gr. Red
8.	E. Acetate	Green	Green	Green	Green	G. Red	G.Red
9.	E. Alcohol	L. Brown	D. Green	L. Green	B. Green	R. Green	R. Green

Extractive Values and Total Ash Values: The water soluble extractive values, alcohol soluble extractive and LOD were higher in non-polluted plants and comparatively lower in polluted plant sampleswhereas ash values were higher comparatively in polluted plant samples. The results were tabulated in **table 6**.

Table-9: Extractive values and Ash values of *Solanum xanthocarpum* Schrad. & Wendl. plant grown in non-polluted and polluted areas.

S. No.	Extractive values (%)		
	Parameters	Non-Polluted	Polluted
1.	Water Soluble	30.250 ± 1.800; cv = 05.950	27.090 ± 1.060*; cv = 03.913
2.	Alcohol Soluble	49.730 ± 3.920; cv = 07.883	30.000 ± 2.250*; cv = 07.500
3.	LOD	36.860 ± 2.450; cv = 06.647	32.712 ± 1.085*; cv = 03.317
4.	Total ash	12.750 ± 00.95; cv = 07.451	42.520 ± 04.230*; cv = 09.948
5.	Acid insoluble	01.390 ± 00.410; cv = 29.496	06.560 ± 02.250*; cv = 34.298
6.	Sulphated ash	18.650 ± 01.920; cv = 10.295	25.930 ± 03.500*; cv = 13.498

* = Significant at 0.1%

DISCUSSION

The effluent samples collected from the selected industry was analysed for different physico-chemical parameters and hashigher values than the recommended values by I.S.I. Similar results were also obtained by Subrahmanyam, 1990,Sujatha and Gupta, 1996 and Singh, *et al*., 1996.

Morphological characters were found to be decreased in the selected plant collected from polluted area. Similar observations are reported by Murray, *et al*. 1994 and Palaniswamy, *et al*. 1995.

Transverse section of stem samples collected from polluted areas showed single layered epidermis covered with thick cuticle and both types of trichomes but more frequent than the nonpolluted samples. Similar observations were found by Subrahmanyam, 1990.Collenchyma was less layered with smaller cell size in polluted samples; phloem less layered with patches; xylem vessels were smaller in size but more in number; included phloem more developed in samples from polluted area. Thick cuticle observed in the transverse sections of the stem collected from the polluted area also matched with the findings of Barnes and Brown, 1990 and Percy and Baker, 1990; Cuticle being the first point of attack of pollutants and the results indicated an increase in the thickness at the polluted sites hence by increasing the cuticle thickness, plants could have an effective barrier for the pollutants entry. Reduced length and width of xylem vessels observed in the plant samples collected from polluted areas also matched with the observationsin *Dalbergia sisoo* byGhouse, *et al.,* 1984; in *Chenopodium album* by Ghouse, *et al.,* 1985; in *Cleome viscosa* by Ahmad, *et al.,* 1987; in *Cajanas cajan by*Ghouse, *et al.,* 1989. The reduced length of vessel elements coupled with their augmented frequency appears to be the significant adaptations to the stress of pollution. In contrast to the above workers more number of parameters (Xylem vessels) observed in the plant samples collected from polluted area over to control population in *Datura inoxia* by Iqbal, *et al.,* 1986 and in *Lantana camara* by Iqbal, *et al.,* 1987.

The trichomes frequency was less where as more stomata were observed in polluted sample.The collenchymas was more layered and spongy parenchyma was less layered with smaller cell size and wide air space, ground tissue was less layered, stomatal index and palisade ratio decreased; crystals more in numbers but bigger cell size in polluted samples. Similar observations were noted by Ghouse and Khan 1984, Bhiravamurthy, *et al.* 1985 and Ramanathan &Kanabiran, 1989Low stomatal frequency in the plants grown in polluted areas, an adaptation which may be of ecotypic significance in regulating the limited and controlled entry of harmful gaseous

pollutants into the plants tissues, especially when the plant grown in polluted area.

Chemical analysis included preliminary colour reaction tests and TLC. Colour tests showed that amount of chemical constituents decreased quantitatively according to the degree of colour change in the plant samples collected from polluted areas.TLC results indicated more number of spots in the plants which were growing in non polluted areas. Observations were also matched with the findings of Tample-Smith and Koen, 1982 and Mashaly, 1988.

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 lowered collected from polluted areas. Ash values were higher comparatively in polluted plant samples.Same observations were made by Sharma and Habib, 1995. The percentage of ash content 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 observed as macronutrient and micronutrients which take up different functions

Water is one of the important prerequisites for triggering enzymatic activity at the early stages of germination. The toxic effect of pure effluent for seedling growth was also attributed due to high amount of BOD and COD in the effluent. High BOD and COD values affect the dissolved oxygen. So due to high BOD and COD values and absence of the dissolved oxygen restricting their energy supply through aerobic respiration which is necessary for growth and development of young seedlings. This damage plant cells and the plant can not grown up in the higher concentration of effluent.

On the contrary, stimulatory effects were noticed in some characters by some workers as Pandey and Soni, 1994 and Prasanna, *et al.*, 1997. The chemicals, in low concentrations, have actually enriched the soil. This indicates that the interaction between the various constituents of the effluent and native microbes might be responsible for the stimulation of seedling growth.

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