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STUDIES ON TRADITIONAL 'MEHENDI' USED AS HERBAL COLOUR WITH SPECIAL REFERENCES TO ITS ANTIMICROBIAL ACTIVITY AND PIGMENT PROFILES BY TLC

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ABSTRACT
'Mehendi' the common name of *Lawsonia inermis* Linn. belonging to the family Lythraceae is a good herbal natural dye of all times of traditional art. The principle colouring matter 'lawsone' present in leaves. In anicient times it used largely as a medicinally and also traditional art of weaving and design. The leaves have positive responses an antimicrobial test. We have studied with microorganism like gram +ve and gram –ve bacteria. Methanolic and aqueous extract of leaves are tested by agar well diffusion method (AWDM). The experiment showed the significant results. The cromatograpgy test has also been done for actual pigments are present.

KEY WORDS: Traditional, natural dye, gram +ve and two gram –ve microorganisms, antimicrobial, pigments profiles, TLC.

INTRODUCTION

During the last few years it is noticed that everybody is getting concern about natural dyes. Mainly the sources of natural dyes have three categories viz. plants, minerals and animals, but plants take great role for obtaining the natural dyes. The art of dyeing has a long past and many of the dyes go back into prehistory. It was practiced during Indus river valley civilization at Mohenjadaro & Harappa (3500BC), China and Egyptian period. Although the plants exhibit a wide range of colours, not all these pigments can be used as dyes (Siva, 2007). Even some natural dyes are fugitive and need a mordant for enhasment of fastness properties, which few are hazardous. In India there are more than 450 plants have been recorded that can produce dyes and over 2000 pigments synthesized by various parts of plants (Chandramouli, 1995). Among these plants have potential medicinal values. Out of these only about 50 taxa have been most exploited commercially and about 150 pigments. But our indigenous knowledge system has now diminished due to easy availability of synthetic dyes. Research has been shown that vast uses of synthetic dyes affect the body system; it causes the skin cancer, temporary or permanent blindness and also the respiratory system etc. (Dubey, 2007). Many of the plants used for dye extraction are classified as medicinal, and some of these have been found to possess antimicrobial activity (Gerson, 1975; Hussein *et al.*, 1997; Schuerch and Wehrli, 1978; Singh *et al.*, 2005; Wagner *et al.* , 1989; Das *et al.,* 2011). The present paper designed to focus about a very common especially in traditional art, also commercial point of view that is 'Mehendi' (*Lawsonia inermis* Linn.) (Fig. 1) growing in Paschim medinipur district and its habit, description of the plant, dye yielding parts, properties of the dye, nature of the pigment, local uses of this dye and potential antimicrobial activity against gram+ ve and gram-ve bacteria of ethanolic and aqueous extracts from plant parts. Also focus on pigment profile of leaves with the help of Thin Layer Chromatography (TLC). As well as ethnomedicinal value of various parts of the plant in the local ethnic communities of Paschim Medinipur district and gathered information about its various traditional knowledge.

MATERIALS AND METHODS

A preliminary survey was done in different parts of the district of Paschim Medinipur to collect the plant and gathering knowledge about traditional art and various ethnobotanical information with the help attaching person of making this natural dye during March 2009 to February 2011. The surveying zone of this district are (1) Jhargram: - 22º26'59" N latitude and 87º00'4" E longitude (2) Belpahari: - 22º41'10" N latitude and 86º36'56" E longitude 87º02'33" E longitude (3) Pingla:- 22˚16'1" N latitude and 87˚37'36" E longitude (4) Sabang:- 22˚8'15" N Latitude and 87˚38'5" E Longitude.

1) ANTIMICROBIAL ACTIVITY DETERMINATION

a) Collection and preparation of plant material for extraction

Plant parts were washed with 70% alcohol and then rinsed with sterilized distilled water and air dried. Clean dry plant sample were stored in cotton bags. The material were homogenized to a fine powder with the help of a mixer grinder. These powered material were then used for extraction of dyes.

b) Preparation of methanolic extracts

10gm of powdered material of sample was soaked in 30ml of 70% methanol and kept at 37°C for 24hrs on a rotary shaker. After 24hrs the previous portion of added methanol was evaporated and the same volume of methanol was again added and placed on a rotary shaker for another 24hrs at 37°C. It was then filtered with the help of a Whatman No. 1 filter paper. The filtrate was

centrifuged at 2000 rpm for 10 min. The supernatant was then collected and allowed to evaporate until it was completely dry. The extract was kept in sterile air tight bottles at 4°C until further use. Before use 30mg of dry extract was re-suspended in 1ml of 70% methanol so that the final concentration of the extract was 30mg/ml (Ushimaru *et al.*, 2007).

c) Preparation of aqueous extracts:

2 gm powered material were mixed with 20 ml of sterile distilled water and kept at 24 hours at 37°C for 24hrs on a rotary shaker. Therefore, it was filtered with the help of Whatman No. 1 filter paper. The filtered was then centrifuged at 2000 rpm. for 10 min. Then the supernatant was collected and stored at 4°C for further use.

d) Bacterial strains

Pure cultures of four bacterial strains *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Vibrio cholerae* were obtained from Department of Microbiology, Vidyasagar University, Midnapore and Department of Microbiology, Lady Brabourne College, Kolkata, West Bengal, India.

e) Agar well diffusion

Antimicrobial activity was determined by the agar-well diffusion method. Mueller Hinton Agar was used as media. To standardize the inoculum density for sensitivity test, a Barium Sulphate ($BaSO_4$) turbidity standard, equivalent to 0.5 Mac Farland standard was used and was cultivated on agar medium. Thereafter 6mm diameter wells were punched in the agar plates. Methanolic and aqueous extract (100µl) of the dyes was added to the wells. Streptomycin sulphate was used as positive control (30µg/ml). The plates were then incubated at 37°C for 24hrs. After incubation the antimicrobial activity was evaluated by measuring the inhibition zone diameter observed (NCCLS, 1997; Ulusoylu *et al.*, 2001). Each test was performed twice and the average of the results was taken.

2) THIN LAYER CHROMATOGRAPHY OF PIGMENTS

0.5gm of fresh plant material was combined with 0.5gm of anhydrous magnesium sulfate and 1gm of sand and transferred to a mortar. Using a pestle the mixture was grinded until a fine dry powder was obtained. The anhydrous magnesium sulfate was used to remove the water from the plant material. The powder was transferred to a small test tube and combined with 2ml of acetone. The test tube was stopped with a cork and shaken vigorously for approximately one minute so that the solid and solvent were well mixed. This mixture was allowed to stand for 10min. Then using a pipette the solvent above the solid was carefully transferred into a small micro-centrifuge tube and centrifuged gently at 2000 rpm for 5min for any remaining debris to settle down. TLC was performed on DC-Alufolien Kieselgel 60 aluminium sheets (Merck). The pigment was spotted on the sheets with a fine capillary tube and chromatographed using a mixture of petroleum ether: acetone (9:1) as solvent system. Rf values for each of the pigments was determined by using the formula.

$$R_f = \frac{\text{distance moved by solute (pigment)}}{\text{distance moved by solvent}}$$

The pigments were identified by comparing with the Rf values of standard pigments.

RESULTS & DISCUSSION

Generally local and ethnic communities of this district followed some process to preparation of dyes from 'Mehendi' plant for dying their personal adornment and colouring leathers, silk and cotton (Panigrahi and Murti, 1989-1999; Anonymous. 1994-1996). They produce the 'Heena' powder that they took from dry leaves of 'Heena' and then it mixed with water (mixed two teaspoonful of 'Mehendi' powder in some water and stir well) and produce red-brown colour (Fig. 2). The local ethnic people used this red brown dye for stain their skin (Fig. 3). The plant common in garden and mostly available in Jhargram, Belpahari and Pingla areas. The yellow pigment Lawsone from *Lawsonia inermis* Linn. having rich amount of napthoquinones. The crude methanolic and aqueous extracts of plant when subjected to antimicrobial activity showed inhibition zones of different diameters, which have been enumerated in Table 1.

TABLE 1: Detection of zone of inhibition for antimicrobial activities by methanolic and aqueous leaves extracts of selected plant taxa.

Plant	Parts used in test	Type of extraction	Diameter of the inhibition zone (mm)			
			Bacillus cereus (Gram positive)	*Klebsiella pneumoniae* (Gram negative)	*Vibrio cholerae* (Gram negative)	*Escherichia coli* (Gram negative)
Lawsonia inermis Linn.	Leaves	Methanolic extracts	10	9	10	9
		Aqueous extracts	5	5	7	5

TABLE 2: Thin layer chromatography (TLC) result of selected plant taxa *Lawsonia inermis* Linn.

Plant sample (Species)	Colour of the dye	Chromatogram pigment		
		Colour of the spot from the top	Pigment name	Rf values
Lawsonia inermis Linn.	Brown red	Yellowish-orange	Lawsone	0.9117
		Yellow green	Chlorophyll-a	0.3352
		Grey	a breakdown product	0.2411
		Yellow	Xanthophylls	0.1352
		Red	Anthocyanin	0.0647
		Blue green	Chlorophyll-b	0.0294

Fig. 1- The Plant *Lawsonia inermis* Linn.

Fig. 2- Dye yielding plant part- leaves and produces the red brown colour.

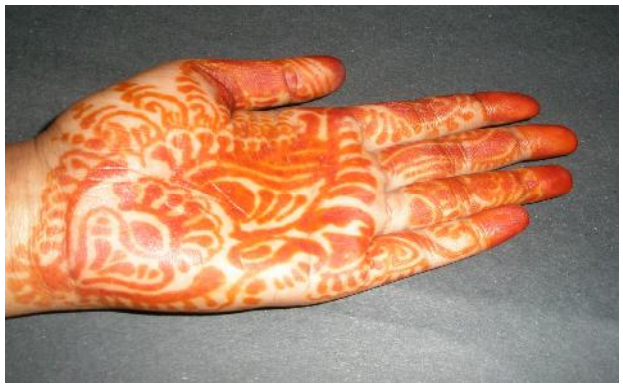

Fig. 3- Lady's staining palms of hand

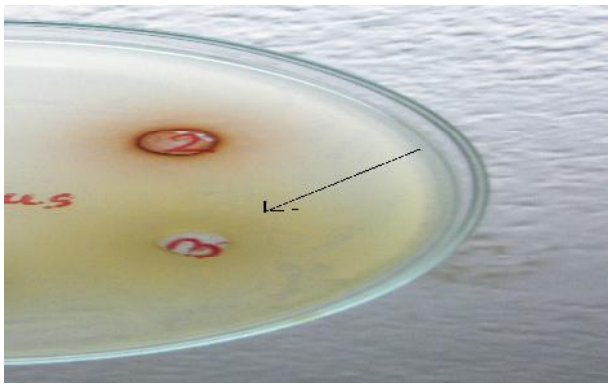

Fig. 4a- The arrow showing the antimicrobial activity of the methanolic extract of *Lawsonia inermis* Linn. against the pathogen *Bacillus cereus*.

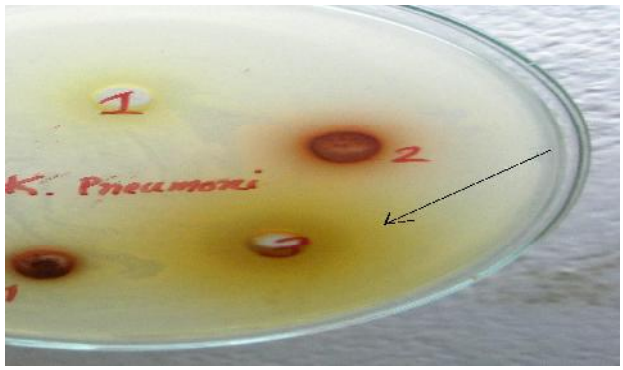

Fig. 4b- The arrow showing the antimicrobial activity of the methanolic extract of *Lawsonia inermis* Linn. against the pathogen *Klebsiella pneumoniae*.

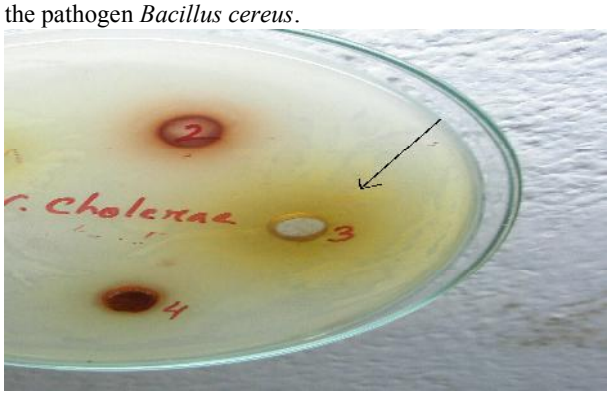

Fig. 4c- The arrow showing the antimicrobial activity of the methanolic extract of *Lawsonia inermis* Linn. against the pathogen *Vibrio cholerae*.

TABLE 3: Details about the plant *Lawsonia inermis* Linn. with various ethnomedicinal uses

Scientific name	Family	Local name	Status	Habit	Description of the plant	Dye yielding part	Produce colour	Use of dye	Ethnomedicinal uses
Lawsonia inermis Linn.	Lythraceae	Mehedi, Mehendi, Heena	Common in garden and open dry mixed forest.	Much branched Shrub	A glabrous, much-branched shrub; lateral branches 4 gonous, often ending in thorns. Leaves 1.3-3.2 x 0.6-1.6 cm, elliptic or broadly lanceolate, acute or obtuse, base tapering; petioles very short. Flowers very small, numerous, fragrant, white or rose coloured in large terminal pyramidal cymes; pedicels short, slender; hypanthium 2- 2.5 mm long; sepals ovate, spreading; petals cream coloured, obovate-suborbicular; stamens 8, inserted in pairs on rim of hypanithum; filaments inflexed in buds; anthers oblong, connective thick. Fruits globose, slightly veined outside, supported by persistent calyx, crowned with style. Seeds trigono-pyramid, externally sub-tuberculate.	Leaves	Brown- red	A brown-red dye, which stains skin obtained from macerated, triturated or powdered leaves. The dye used by ladies for staining palms of hands, soles, nails; also used for dying hairs, beard and eye brow for personal adornment. Leaves also used for colouring skins, leathers, silk and wool.	Leaves flower and seeds are used in medicines. Root and leaf powder in milk are used for jaundice. Leaves are also used as astringent, leaver tonic, diuretic and also useful in wounds, ulcers, cough, bronchitis, diarrhoea, dysentery, leprosy, boils and anemia. The flowers are intellect promoting cardio tonic, refrigerant etc.

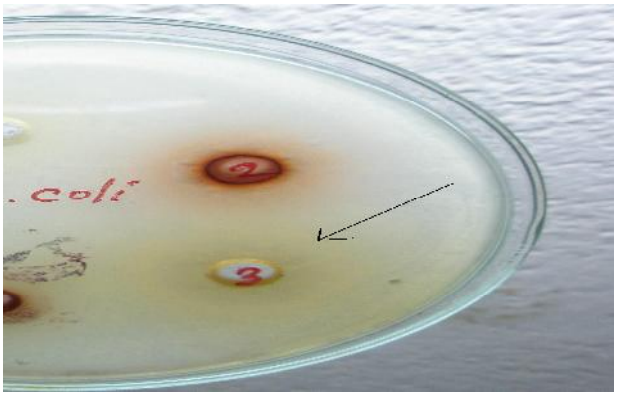

Fig. 4d- The arrow showing the antimicrobial activity of the methanolic extract of *Lawsonia inermis* Linn. against the pathogen *Escherichia coli*.

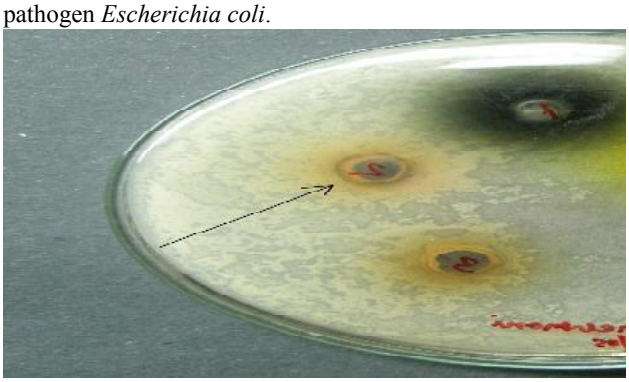

Fig. 5b- The arrow showing the antimicrobial activity of the aqueous extract of *Lawsonia inermis* Linn. against the pathogen *Klebsiella pneumoniae*.

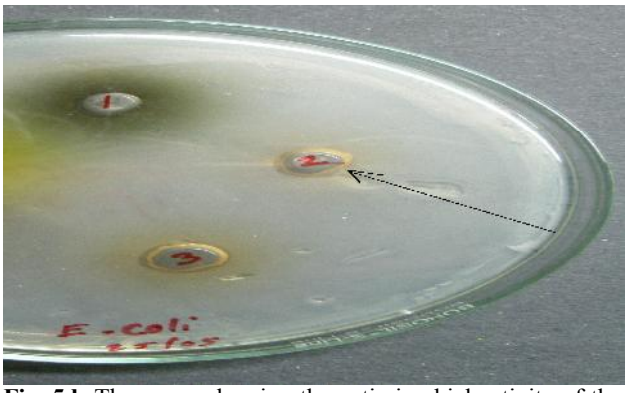

Fig. 5d- The arrow showing the antimicrobial activity of the aqueous extract of *Lawsonia inermis* Linn. against the pathogen *Escherichia coli*.

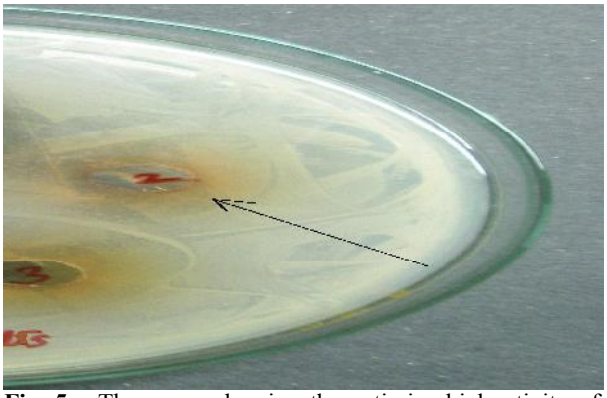

Fig. 5a- The arrow showing the antimicrobial activity of the aqueous extract of *Lawsonia inermis* Linn. against the pathogen *Bacillus cereus*.

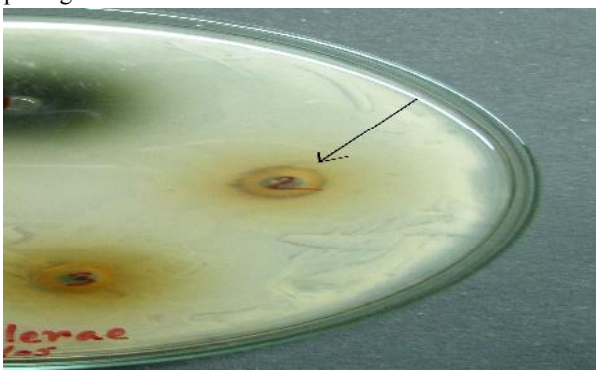

Fig. 5c- The arrow showing the antimicrobial activity of the aqueous extract of *Lawsonia inermis* Linn. against the pathogen *Vibrio cholerae*.

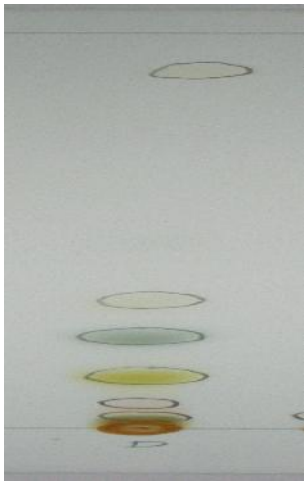

Fig. 6- Thin layer chromatography result of pigments of the plant of *Lawsonia inermis* Linn.

The methanolic extracts test showed that the inhibition zone diameter ranging from 9 to 10 mm against these four bacterial strains. The tested results results of methanolic extract showed that *Lawsonia inermis* Linn. are bactericidal in nature and not bacteriostatic (Fig. 4a, Fig. 4b, Fig. 4c and Fig. 4d). The aqueous extracts test showed that the inhibition zone diameter ranging from 5 to 7 mm. (Fig. 5a, Fig. 5b, Fig. 5c and Fig. 5d) which showed the less positive result against these bacterial strains. Thin layer chromatography of the freshly extracted dyes showed several spot on the chromatogram (Fig. 6) and Rf values of which helped to know the composition of this dye (Table 2). The documentation of this plant having their local name, family, status, habit, description of the plant, dye yielding parts, nature of the pigment, local uses

of this dye and ehnomedicinal value are enumerated in Table 3.

CONCLUSION

It is well known that the tribal population of West Bengal mainly in Paschim Medinipur district has been used the plant parts in traditional system of medicine for the treatment of various diseases and also they used various types of dyes from the sources of different plant parts for their cultural decoration and other economic purposes. From the above antimicrobial study it was found that methanolic extracts of *Lawsonia inermis* Linn. shows positive result against all the selected pathogenic bacteria than the aqueous extracts. From this study it may be conclude that *Lawsonia inermis* Linn. which is available in this district takes an important role to prevent the microorganisms which causes the various diseases. In the TLC result it was also shows that in which the pigment actual spotted on the TLC plate. So, promote proper conservation and gathering knowledge about the use of such natural dyes like 'Mehendi' and others in the local communities of this district; also should enhanced to preserve this immense treasure of traditional knowledge and documentation of this indigenous knowledge system.

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