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Full Length Research Paper

Antibacterial activity of some selected dye yielding plants in Eastern India

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***Acacia catechu* (L.) Willd. (Mimosaceae), *Pterocarpus marsupium* Roxb. (Papilionaceae), *Toddalia asiatica* (L.) Lamk. (Rutaceae) and *Ventilago denticulata* Willd. (Rhamnaceae), growing in West Midnapore district of West Bengal, an eastern state of India, yields dyes which showed strong antimicrobial activity against four bacterial strains (*Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Vibrio cholerae*). Among the dyes of the four plants tested, *Toddalia asiatica* showed the highest antimicrobial activity with the inhibition zones ranging from 14 to 16 mm. Thin layer chromatography of the dyes, which were either substantive dyes or adjective type of dyes requiring mordents for fixing to fabrics, showed that most of these pigments were composed of xanthophylls and anthocyanin. The extracts of different plant parts are also used for various medicinal purposes by the local tribal people. The over exploitation of these plants for dyeing and pharmaceutical uses has led to the depletion of these species, particularly *P. marsupium* and *V. denticulata,* from this region and immediately demands more focused conservation strategies.**

Key words: *Acacia catechu*, *Pterocarpus marsupium*, *Toddalia asiatica*, *Ventilago denticulata*, dye, antibacterial activity, Thin Layer Chromatography (TLC), pigments, IUCN red list, vunerable, biodiversity, conservation, strategies.

INTRODUCTION

India, considered to be a forerunner in the art of natural dyeing has a rich biodiversity of approximately 490,000 plant species including 17,500 angiosperms. Being one among the world's twelve mega diversity countries, it harbors a wealth of useful germplasm resources, with the plant kingdom a rich source of diverse natural products, including dye. Natural dyes are used in textiles, inks, cosmetics, drugs, food, etc. and plants exhibit a wide range of colors ranging from the ubiquitous green pigment chlorophyll helping to capture sun's energy and convert it to chemical energy, to the beautiful vibrant colors of the flowers and fruits to attract insects or animals either for pollination or for the purpose of seed dispersal. However the role of a large number of plant pigments still remains a dilemma. Scientists believe that some of these pigments have role in the protection of the plant from diseases (Siva, 2007). In spite of being detrimental to human health, having toxic, allergic and carcinogenic effects, synthetic dyes have dominated the market, sidelining the natural dyes largely because of the tedious extraction procedure from the raw material for natural dyes, low color value and longer time of extraction, which makes them expensive. Even some natural dyes are fugitive and need a mordant for enhancement of their fastness properties, which are hazardous. Yet, there are nearly 450 taxa known to yield dyes in India (Chandramouli, 1995) and over 2000 pigments synthesized by various parts of plants (Siva, 2007). Out of these only about 50 taxa have been most exploited commercially and about 150 pigments. These dyes are produced by different parts of the plant including the flower, fruit, seed, leaf, bark, etc. Unfortunately all these pigments cannot be used as dyes. While working with natural dyes, several problems are faced. First and foremost, there is lack of precise standardized technical

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knowledge of extracting and dyeing technique for all dyes. The ethnic groups inhabiting different areas have indigenous knowledge systems and have evolved methods for utilizing the vast plant resources available (Sarmah et al., 2000).

Secondly, all dyes do not dissolve in water and some require complicated solvent systems and methods for extraction. Thirdly, some dyes do not directly absorb on to fibers and require a mordant such as metal salts, tannins or oil, which in turn can be toxic. Fourthly, natural dyes may fade away when washed or exposed to air or sunlight. Yet, the non-toxic, non-polluting, non-carcinogenic, non-poisonous and environment friendly nature of natural dyes cannot be overlooked. Further, natural products still remain as one of the best reservoir of new therapeutically drugs. 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). The aim of the present study was to investigate into four dye-yielding plants [*Acacia catechu* (L.) Willd. (Mimosaceae), *Pterocarpus marsupium* Roxb. (Papilionaceae), *Toddalia asiatica* (L.) Lamk. (Rutaceae) and *Ventilago denticulata* Willd. (Rhamnaceae)] growing in West Midnapore district of West Bengal, an eastern state of India, their availability, habit, habitat, parts yielding the dyes, properties of the dyes, nature of the pigments, local uses of these dyes by the regional tribal people, antibacterial activity of the extracts of these dyes, as well as the ethno-medicinal uses of the various parts of the plants yielding these dyes. Five rural areas (Amlachati, Jhargram, Kankrajhore, Pingla, Sabang) of the lateritic zone of West Midnapore were selected for the present study as this area of the state is largely covered by light to very dense stretches of deciduous forest, dominated by the tribal communities and endowed with a wealth of natural flora and fauna, thus a potential area for the availability of plant resources, including dyes. The tribal communities inhabiting these areas are the Santals, Lodhas, Mundas and other local communities like Chitrakar, Mahatos, etc.

MATERIALS AND METHODS

The preliminary survey was made in the different rural areas of the lateritic zone of West Midnapore during the period of January 2006 to February 2009. The places under the zone of survey included Amlachati (22° 22' 36" N latitude and 87° 02' 33" E longitude), Jhargram (22° 26' 59" N latitude and 87° 00' 4" E longitude), Kankrajhore (22° 42' 13" N latitude and 86° 36' 24" E longitude), Pingla (22° 16' 1" N latitude and 87° 37' 36" E longitude) and Sabang (22° 8' 15" N latitude and 87° 38' 5" E longitude). Plants or plant parts were collected from the different areas. Each specimen was labeled, numbered and annotated with date of collection. Collected plant specimens were identified with the help of available literature and a voucher specimen of each has been deposited in the herbarium of the Department of Botany and Forestry of Vidyasagar University, Midnapore, West Bengal, India for further reference. Field information regarding their habit, habitat, dominance, local uses and their ethno-medicinal uses are based on personal observation and detailed discussions with the tribes inhabiting these areas, particularly the aged people and the ojhas (quack doctors) during the regular field surveys.

Antimicrobial activity determination

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 samples were stored in cotton bags. The materials were homogenized to a fine powder with the help of a mixer grinder. These powered materials were then used for extraction of dyes.

Preparation of methanolic extracts

10 g of powdered material of each sample was soaked in 30 ml of 70% methanol and kept at 37°C for 24 h on a rotary shaker. After 24 h 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 24 h 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 extracts were kept in sterile air tight bottles at 4°C until further use. Before use 30 mg of dry extract was re-suspended in 1 ml of 70% methanol so that the final concentration of the extract was 30 mg/ml (Ushimaru et al., 2007).

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.

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 6 mm diameter wells were punched in the agar plates. Methanolic extracts (100 µl) of the different dyes were added to the wells. Streptomycin sulphate was used as positive control (30 µg/ml). The plates were then incubated at 37°C for 24 h. After incubation the antimicrobial activity was evaluated by measuring the inhibition zone diameter observed (NCCLS, 1977; Ulusoylu et al., 2001). Each test was performed twice and the average of the results was taken.

Thin layer chromatography of pigments

0.5 g of fresh plant material was combined with 0.5 g of anhydrous magnesium sulfate and 1 g 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 2 ml of acetone. The test tube

Table 1. Antibacterial effect of the methanolic extracts of the four plants.

Plant	Parts used in test	Diameter of the inhibition zone (mm)			
		Bacillus cereus	***Klebsiella pneumoniae***	***Vibrio cholerae***	***Escherichia coli***
Acacia catechu	Heart wood	11	8	9	9
Pterocarpus marsupium	Stem bark	7	9	7	7
Toddalia asiatica	Root bark	15	16	15	14
Ventilago denticulata	Stem bark	9	9	9	12

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 10 min. 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 5 min for any remaining debris to settle down. TLC was performed on DC-Alufolien Kieselgel 60 aluminium sheets (Merck). The pigments were 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 AND DISCUSSION

The crude methanolic extracts of all the four plants when subjected to antimicrobial activity showed inhibition zones of different diameters, which have been tabulated in Table 1 and shown in [Figures 1 (c-f), 2 (c-f), 3(c-f) and 4(c-f)]. Thin layer chromatography of the freshly extracted dyes showed several spots on the chromatograms [Figures 1 (g), 2(g), 3(g) and 4(g)], the Rf values of which helped to know the composition of these dyes (Table 2). The availability of these plants in this region, their habit, habitat, common names as well as local names, parts yielding the dyes, local uses of these dyes and other plant parts for various medicinal purposes by the regional tribal people have been documented in Table 3.

A. catechu (L.) Willd. is a moderate sized deciduous, thorny tree which grows up to a height of 9 to 12 m or less. Common names for it include Catechu, Cachou and Black cutch. The heart wood of this plant yields a reddish brown colored adjective dye, which does not bind to the fabrics directly and require a mordant like alum to fix to the fabrics. Since this tree can be propagated easily by planting its seeds, *A. catechu* (L.) Willd. has been found to be frequent in all the five surveyed subdivisions of West Midnapore. The dye is largely composed of xanthophyll and the pigment is soluble in water as well as in other solvents like methanol, acetone, petroleum ether etc. Crude methanolic extracts showed moderate antimicrobial activity against all the four strain of bacteria, with the greatest diameter of inhibition zone of 11 mm (including the 6 mm diameter of the wells) in case of *Bacillus cereus* and 9 mm inhibition zones in case of the remaining three strains.

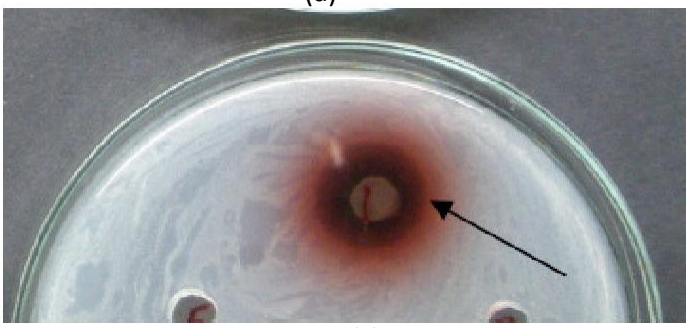
An economically important plant as well as a plant of great medicinal importance, the various parts of this plant is widely used by the tribes in this region for different purposes. This plant has been reported to contain catechins, phlobatinin, gummy matter, quercitrin, and quercitin. Gum and flowering tops of this plant are used for medicinal purpose. It is used in the diseases like worms, wounds, fever, edema, pruritis, diabetes, obesity, blood disorders, cough, asthma and anaemia etc. The powder by itself arrests the bleeding in gums. The decoction is an effective gargle in sore throat, cough and hoarseness of voice. The paste is beneficial externally in skin diseases and wounds. The decoction is effective in curing diarrhoea, dysentery and colitis, especially associated with bleeding, vaginal diseases, leucorrhea, menorrhagia etc. When given along with honey, it effectively curbs the haemoptysis (bleeding through sputum) and alleviates cough. It is also beneficial in chronic fever with enlarged spleen. It alleviates the excessive body fats in obesity and works well as an adjunct in diabetes. Its heartwood extract is used not only in dyeing but also in leather tanning, as a preservative for fishing nets and as a viscosity regulator for oil drilling.

P. marsupium Roxb. or the Indian Kino Tree is a medium to large, deciduous tree that can grow up to 30m height. The stem bark of this tree yields a brick red colored dye largely composed of xanthophylls, which is water soluble, and can fix to all types of fabrics without a mordant. The colour is comparatively lasting and fades away only slightly on washing or exposure to sunlight. This can be overcome by using a mordant like alum. This tree is comparatively rare in West Midnapore and in restricted to the Pingla subdivision of this district. *P. marsupium* Roxb. has been listed as vulnerable in the IUCN red list of threatened species (World Conservation Monitoring Centre, 1998). Methanolic extracts prepared from stem bark showed inhibition zone against all the bacteria tested. The greatest zone of inhibition was observed against *K. pneumoniae* (9 mm) and 7 mm inhibition zones in case of the remaining three strains. The heart wood, leaves and flowers of the Indian Kino

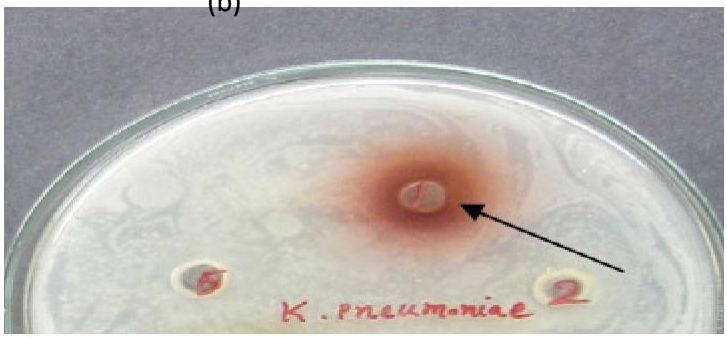
(a)

(b)

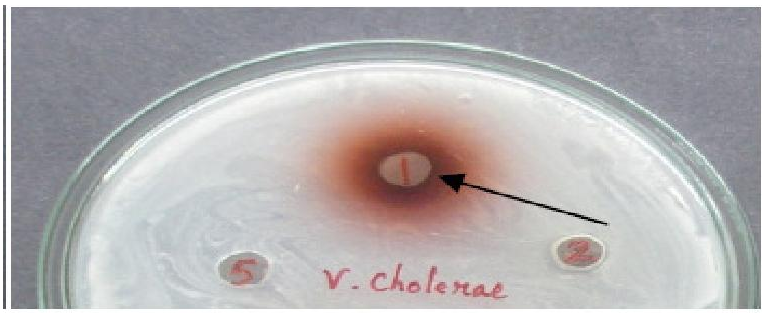
(g)



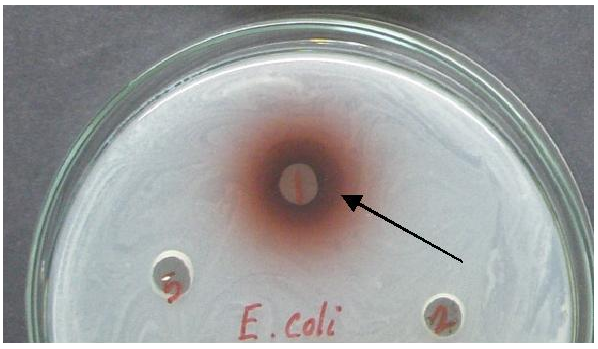
(c)



(d)



(e)



(f)

Figure 1. a) Plant *of Acacia catechu* (L.f) willd. b) Reddish brown dye yielded by the heart wood of *A. catechu*. c) Arrow showing antimicrobial activity of the methanolic extract of *A. catechu* against the pathogen *Bacillus cereus*. d) Arrow showing antimicrobial activity of the methanolic extract of *A. catechu* against the pathogen *Klebsiella pneumoniae*. e) Arrow showing antimicrobial activity of the methanolic extract of *A. catechu* against the pathogen *Vibrio cholerae*. f) Arrow showing antimicrobial activity of the methanolic extract of *A. catechu* against the pathogen *Escherichia* coli. g) Thin layer chromatography result of pigments of the plant of *A. catechu.*

Figure 2. a) Plant of *Pterocarpus marsupium* Roxb. b) Brick red dye yielded by the stem bark of *Pterocarpus marsupium*. c) Arrow showing antimicrobial activity of the methanolic extract of *P. marsupium* against the pathogen *Bacillus cereus*. d) Arrow showing antimicrobial activity of the methanolic extract of *P. marsupium* against the pathogen *Klebsiella pneumoniae*. 2) Arrow showing antimicrobial activity of the methanolic extract of *P. marsupium* against the pathogen *Vibrio cholerae*. f) Arrow showing antimicrobial activity of the methanolic extract of *P. marsupium* against the pathogen *Escherichia coli*. g) Thin layer chromatography result of pigments of the plant of *P. marsupium*.

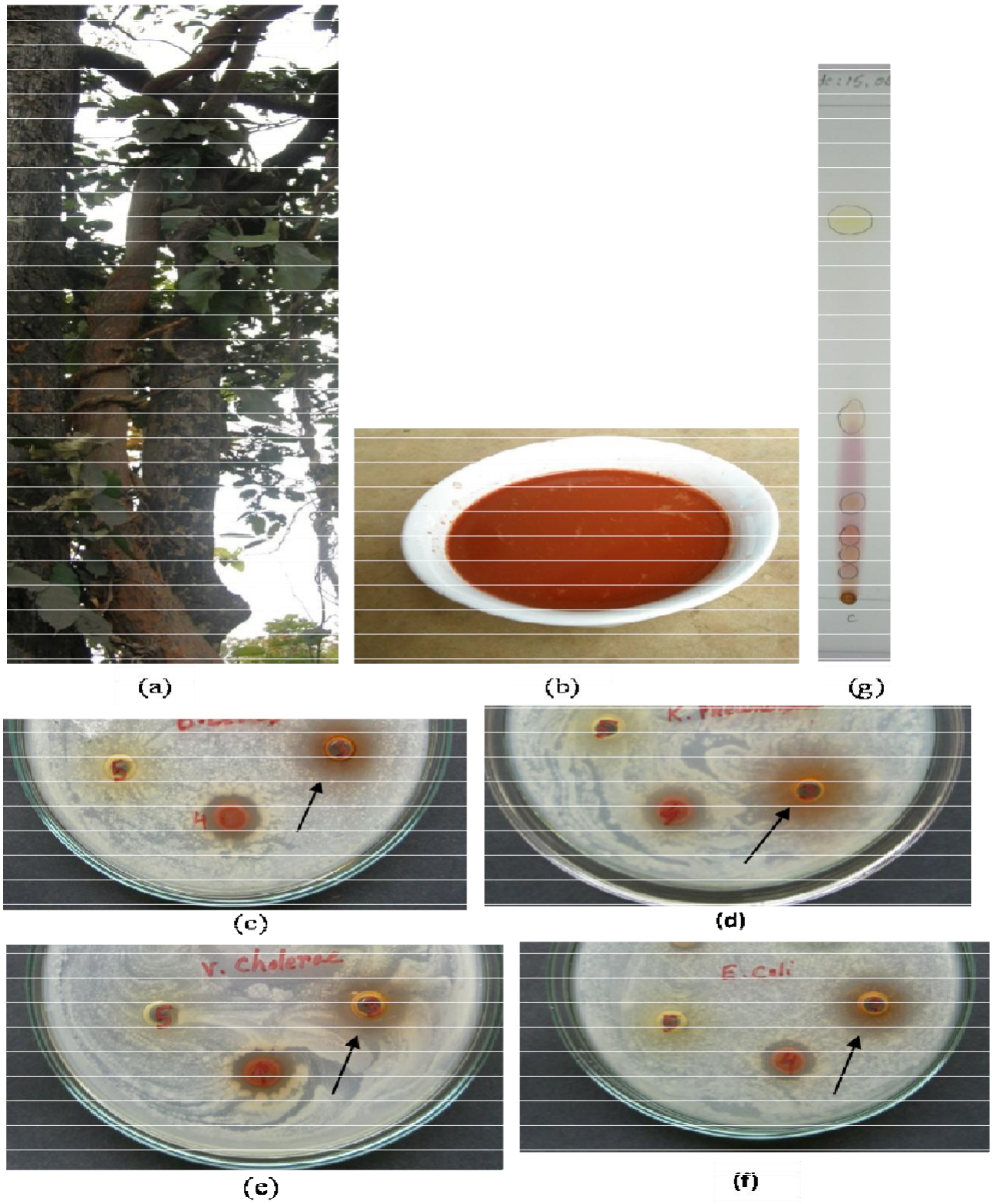


Figure 4. a) Plant of *Ventilago denticulata* Willd. b) Red dye yielded by the stem bark of the plant of *Ventilago denticulata*. c) Arrow showing antimicrobial activity of the methanolic extract of *V. denticulata* against the pathogen *Bacillus cereus*. d) Arrow showing antimicrobial activity of the methanolic extract of *Ventilago denticulata* against the pathogen *Klebsiella pneumoniae*. e) Arrow showing antimicrobial activity of the methanolic extract of *Ventilago denticulata* against the pathogen *Vibrio cholerae*. f) Arrow showing antimicrobial activity of the methanolic extract of *Ventilago denticulata* against the pathogen *Escherichia coli*. g)Thin layer chromatography result of pigments of the plant of *Ventilago denticulata*.

Table 2. Thin layer chromatography results of the pigments of the four plants.

Plant	Colour of the dye	Chromatogram pigment		
		Colour of the pigment spot	Pigment name	Rf values
Acacia catechu	Reddish brown	Yellow	Xanthophyll	0.46
Pterocarpus marsupium	Brick red	Yellow	Xanthophyll	0.48
Toddalia asiatica	Orange yellow	Yellow	Xanthophyll	0.46
		Yellowish orange	Toddaline	0.30
Ventilago denticulata	Red	Orange yellow	Carotene	0.98
		Yellow	Xanthophyll	0.48
		Purplish yellow	Breakdown product	0.20
		Deep red	Anthocyanin	0.12
		Red	Anthocyanin	0.09
		Pinkish red	Anthocyanin	0.05

have long been used for their medicinal properties in Ayurveda. The heart wood is used as an astringent, bitter acrid, antihelmintic, anodyne and in the treatment of inflammation, fractures, bruises, rheumatoid arthritis, diabetes and heart problems. It is good for elephantiasis, leprosy, leucoderma, diarrhoea, dysentery, constipation, depurative, ophthalmopathy, hemorrhages, rectalgia, cough and greyness of hair. The bruised leaves are considered useful as an external application for boils, sores and skin diseases. The active ingredients present in the extracts of *P. marsupium* Roxb. include pterostilbene, alkaloids and tannins. The pterostilbene helps lowers the lipid and glucose level in body and shows significant reduction of glycosylated hemoglobin and an increase in total hemoglobin level. It inhibits cell proliferation and induces apoptotic effect in tumor cell lines, shows potent antioxidant activity and inhibits electrolyte leakage caused by herbicide-induced oxidative damage. The alkaloids significantly lower the high blood sugar level in the body and raises the blood pressure. The tannins show scavenging activity against mitochondrial lipid peroxidation and causes significant decrease in cholesterol level. The extracts of *P. marsupium* Roxb. have also shown strong antihyperlipdemic activity. It causes significant reduction of serum triglyceride, total cholesterol and LDL and VLDL cholesterol levels without any effect on the level of HDL-cholesterol (Jahromi and Ray, 1993).

T. asiatica Lamk., commonly called the Orange climber, is a woody liana, which can reach a height of 10 m in forests as it uses other trees for support. This plant has been also found to be rather infrequent in West Midnapore, mostly found in the Jhargram subdivision. Bees generally pollinate the flowers and birds who love the fruits of this plant and monkeys disperse the seeds. An orange yellow colored dye is extracted from the roots of this plant, which is also an adjective dye requiring a mordant like alum to fix to fabrics. The pigment has been found to be rich in xanthophylls. Among the four plants reported in this paper, *T. asiatica* Lamk. showed the highest antimicrobial activity among the dyes of the four plants tested, with the inhibition zones ranging from 14 to 16 mm with the greatest diameter of 16 mm observed in case of *K. pneumoniae.* It is also used medicinally by the local tribes. The fruit is used as a cough remedy. The leaves are used for lung diseases and rheumatism. The root and its bark are used medicinally as a tonic in the treatment of indigestion, stomach ailments, fever, influenza, malaria, cholera, diarrhoea and rheumatism.

V. denticulata Willd. commonly called the Red Creeper is an extensively branched, woody climber with hanging branches. The stem and root bark of this plant is a source of a red dye 'ventilagin', which is used for coloring cotton, wool and tasar silk. The dye was found to be largely composed of anthocyanin and xanthophyll pigments. This color is very long lasting and does not fade away on exposure to sunlight or washing. It can bind to fabrics directly without a mordant but a mordant like alum may be used for long lasting of the color of the fabrics. *V. denticulata* Willd is a very rare plant in this district being restricted to the Chilkigarh forest of the Sabang subdivision and most exploited by the tribes not only for dyeing purpose but also as a source of medicines. The bark of this plant is used medicinally in blood and heart related diseases. The paste of the root bark is applied as a cure for wounds, eye diseases, etc. Stem bark decoction is given with a paste of *Piper nigrum* Linn. (Black pepper) to treat stomach ulcer. The paste of the stem bark is also applied to relieve body pain.

An assay of the antimicrobial activities shows this plant to possess moderate antimicrobial activities showing the inhibition zones with a diameter of 9 to 12 mm. The

Table 3. Dye yielding plants with medicinal value.

Name of the plant	Synonym	Family	Common name	Local name	Status	Habitat	Habit	Flowering period	Dye yielding part	Dye colour	Use of dye	Local medicinal use
Acacia catechu (L.) Willd.	*Acacia catechu* (L.f.) Willd. *catechuoides* (Roxb) Prain. *Acacia catechuoides* (Roxb.) Benth. *Acacia sundra* (Roxb.) Bedd. *Acacia wallichiana* DC. *Mimosa catechu* L.f. *Mimosa catechuoides* Roxb.	Mimosaceae	Black catechu, Cutch tree, Catechu, Cachou	Khair	Frequent	Found in the deciduous forest in the lateritic zone	Medium sized thorny deciduous tree grows up to 13 m in height. Leaves bipinnately compound, leaflets 30 to 50 paired, main rachis pubescent, with large conspicuous gland near the middle of the rachis. Flowers found in axillary spikes, pale yellow, sessile; fruits flat brown pods bearing 3 to 10 seeds per pod, with triangular beak at the apex, shiny, narrowed at base.	Sept-Mar	Heart wood	Reddish brown	Cutch obtained by boiling the heartwo-od chips in water, which yields reddish brown colour. The said popular dye is used for dying cotton, silk and canvas.	Gum and flowering tops used in skin diseases, cough, asthma, tooth ache, diarrhea, etc.
Pterocarpus marsupium Roxb.	-	Papilionaceae	Indian Kino Tree, Malabar Kino Tree, Kino	Titsal, Piasal, Bijayasal, Munga	Rare	Found in the drier, hilly zones of the deciduous forests	A moderate-sized to large deciduous tree. The bark is grey, rough, longitudinally fissured and scaly. The older trees exude a blood red gum-resin. The leaves are compound, imparipinnate: the leaflets 5 to 7 are oblong, obtuse and partially bilobed with prominent nerves; the flowers occur in large terminal panicles, yellowish and fragrant; Fruits are pods, the pods are orbicular, flat, winged.	Aug-Apr	Stem bark	Brick red	Used for dyeing the silk cloth and many other colouring works.	In treatment of diabetes, leprosy, elephantiasis fractures, inflammation boils, skin diseases, diarrhea
Toddalia asiatica (L.) Lam.	*Toddalia aculeata* Pers.	Rutaceae	Orange climber	Kada todali, Himancha	Less frequent	Found along forest margins near rivers or streams, often climbing into the canopy of larger trees. It grows fairly well in clay soils.	Strong robust woody liana growing up to a height of 10m, branches covered with sharp, hooked knobby thorns; leaves 3-foliolate; leaflets shiny trifoliate, elliptic to obovate, shiny green, paler below, covered with translucent gland-dots, extremely aromatic with a strong citrus smell when crushed. Flowers cream to pale greenish-yellow in axillary and terminal clusters or branched inflorescences; Fruit berry-like, small, citrus-like, 5 to 7 mm in diameter, orange when ripe and taste like the skin of an orange.	Sep–Jan	Root bark	Orange yellow	Used for dyeing silk and cotton cloth.	Used in lung diseases, rheumatism, indigestion, stomach ailments, fever, malaria, cholera, diarrhoea

Table 3. Contd.

Ventilago denticulata Willd.	*Ventilago calyculata* Tul. *Ventilago macrantha* Tul. *Ventilago madraspatna* auct.non. Gaertn.	Rhamnaceae	Red creeper	Banga-sarjom, Sanga-sarjom, Raktapita	Rare	Deciduous woody climber climbing onto other trees	Large much branched woody climber; young branches and panicles pubescent; Leaves 5 to 10 x 2.5 to 3.9 cm, oblong - lanceolate or elliptic-ovate, entire or crenate; Flowers in large drooping terminal panicles; buds five angled; petals five, greenish, with an offensive odour; Fruit nut about 5 mm diameter, yellowish, globular, prolonged into a linear -oblong; one nerved wing.	Oct-June	Stem and root bark	Red	Used for colouring cotton, wool and tasar silk	Used in the treatment of heart diseases, wounds, eye diseases, stomach ulcer, body pain

highest activity was observed against *Escherichia coli* (inhibition zone of 12 mm).

Thus, *A. catechu* (L.) Willd., *P. marsupium* Roxb., *T. asiatica* Lamk. and *V. denticulata* Willd. are some rare treasures amidst the rich floral diversity of this region of West Midnapore both from the medicinal as well as dye yielding point of view. Unlike *A. catechu* and *T. asiatica*, which are still more frequently available, as they can be easily propagated by seeds, *P. marsupium* Roxb.and *V. denticulata* Willd. are fast disappearing from this region and most exploited. Because of the impervious nature of the seed coat of *P. marsupium* Roxb. (Lakshmi et al., 1992; Das and Chatterjee, 1993) the germination rate is very low and the propagation of this plant through stem cuttings poses difficulties. But at the same time this plant is being over-exploited for pharmaceutical use, which has led to the depletion of this species from its natural habitat, thereby widening the gap between its demand and supply (Tiwari et al., 2004). This has put immense pressure on this species because of which, it is on the verge of extinction and will be extinct soon if proper measures for its conservation are not taken. The same condition also prevails for *V. denticulata* Willd, in which the seed germination rate is low and largely exploited by the tribes. In view of the problems of conventional propagation and simultaneous high demand of plant material, large scale planting is required. For species with lower germination rates, large scale multiplication can only be met efficiently and economically in a short span of time by *in vitro* propagation and *ex situ* plantations. Further there is not only a need for proper documentation and assessment of these dye yielding plants and their dyes, characterization of these dyes and research to find ways to overcome the limitations of these dyes is the urgent need of the day to promote and increase awareness among people on the use of natural dyes.

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