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RESEARCH ARTICLE

EVALUATION OF ANTIMICROBIAL SCREENING AND ANTIMUTAGENIC ACTIVITY OF ETHANOLIC EXTRACT OF SOME SELECTED DYE YIELDING PLANTS OF SOUTH WEST BENGAL, INDIA

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ABSTRACT

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. Due to its less pollution and less side effect, natural dyes are used more often in food products as well as other important regular uses. Methanolic extract of different seven dye yielding plant parts like *Acacia catechu* (L.f) Willd., *Basella alba* Linn. var. *rubra* Linn., *Beta vulgaris* Linn., *Bixa orellana* Linn., *Carthamus tinctorius* L., *Terminalia arjuna* (Roxb.) Wight. & Arn., *Terminalia chebula* Retz. Were used to investigate the antimicrobial properties and antimutagenic activity. The methanolic extract of the plant parts are tested by agar well diffusion method (AWDM). The investigate showing the positive response against one gram +ve bacteria and three gram -ve bacteria. The result showing the positive response against the all microorganism. The tested plant parts showing the varying level of antimutagenic activity. The concentration dose 25, 50 and 100 µg/plate of dyeing parts of methanolic extract are widely used for this investigation. It was found that the dyeing parts of methanolic extract of the plant a dose 100 µg/plate exhibit inhibition of His+ revertants against acting mutagens, sodium azide (NaN₃) induced mutagenicity in *Salmonella typhemuium* tester strains TA98 and TA100. The over all result showed that the tested methanolic plant extract both have antimicrobial properties and antimutagenic activity. Enumeration includes details about the plant with prospecting proper medicinal value.

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INTRODUCTION

Colour has influenced human psyche since ancient times. It was provide a basic to his varied imagination as reflected in various literary and artistic creativities. Colour has been a highly effective tool in breaking the monotony of life (Chandramouli 1993, Heerk 1994). Archaeological evidences show that dyeing was a widespread industrial enterprises in Egypt, India and Mesopotamia around third millennium BC (Roy, 1978). It was practice during the Indus river valley civilization at Mohenzadaro and Harappa (3500 BC) former Egyptian and China period. In Egypt mummies have been found wrapped in coloured cloth, chemical tests of red febric found in tomb of king Tutankhamen in Ezypt showed the presence of alizarin a red pigment extracted from madder (*Rubia cordifolia*) (Siva, 2007). Use of natural colour in food known from Japan in the Shosoin text of the Nara period (8th century) which contain references regarding colouring soybean and adzuki bean cakes (Rymbai *et al.*, 2011). Research has shown that the synthetic dyes are suspected to release harmful chemical that are allergic, carcinogenic and detrimental to the human health. On the other hand natural dyes are fugitive and need a mordant for enhancement of their fastness of their fastness properties, which are hazardous. Yet

there are nearly 450 taxa known to yield dye in India (Chandramouli, 1995), and over 50 taxa have been most exploited commercially and 150 pigments (Das *et al.*, 2011). The dyes are mostly produce from different parts of the plants like leaf, bark, root, flower, fruits and seeds etc. It has been noticed in the development of new natural dye colourants for use in food industry, which is apparently use to strong consumer demand for more natural products, at least in some countries (Siva *et al.*, 2011). For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. The antimicrobial efficiency attribute to some plants in treating diseases has been beyond belief.

It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientist (Khan *et al.*, 2009). Many of the plant used for dye extraction and some of there have been found posses antimicrobial properties (Gerson, 1975; Hussain *et al.* 1997; Schuerch and Wehrli, 1978; Singh *et al.*, 1989). The nutagen are involved in the initiation and promotion of several human diseases including cancer, the significance of novel bioactive phytochemicals in counteracting the promutagenic and carcinogenic effect are gaining credence. Such chemicals that reduce the mutagenicity of physical and chemical mutagens are called as antimutagen. The antimutagen have been first

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reported almost four decades ago, and since numerous studies have been carried out in order to identify compounds which might protect human against DNA –damage and its consequence. Natural antimutagens from edible and medicinal plants are of particular importance because they may be useful for human cancer prevention and have no undesirable xenobiotic effects on living organism (Aqil *et al.*, 2006; Stavric, 1994 and Flora; 1998). The present study is to investigate the seven dye yielding plant in the South West Bengal region and study their local name, habit, dye yielding parts, produce dye, colouring compound, , uses of dye and medicinal uses have been enumerated in Table -1. Also investigate their antimicrobial properties and antimutagenic activity.

MATERIAL AND METHODS

Extensive field survey (Fig. 1) and plant collection will be undertaken from three district of South West Bengal since 2009-2011. In South West Bengal there are three district viz. Bankura (23°14'N Latitude and 87°07'E Longitude), Purulia (23°2'N Latitude and 88°28'E Longitude) and Paschim Medinipur (21°47'N-23°00'N Latitude and 86°40'E-87°52'E Longitude) district having rich in biodiversity. The ethnic communities of this region used the natural dye in their daily day life. They used of this plants not only for dyeing the house holds, cloths or cultural decoration but also treatment of some common human ailments (Chatterjee and Pakrashi, 1991-2001; Das and Mondal, 2009). The collected plant specimens are deposited in Botany department herbarium, Vidyasagar University.

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 powdered materials were then used for extraction of dyes.

Preparation of methanolic extracts of the plant sample

10gm of powdered material of each 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 extracts were 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).

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 (BaSO₄) 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 extracts (100µl) of the different dyes were 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, 1977; Ulusoylu *et al.*, 2001). Each test was performed twice and the average of the results was taken.

Antimutagenicity assay

Bacterial strains

Salmonella typhimurium strains TA 98 (MTCC NO. 1251) and TA 100 (MTCC NO. 1252) were procured from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Sector 39 A, Chandigarh-1600036, India.

Preparation of methanolic extract of the plant sample

All the selected plant materials were collected from different zone of the South West Bengal and the specimens are deposited in the botany department herbarium, Vidyasagar University. For methanolic extraction, the seven dye yielding plant (Fig. 2-8) materials *Acacia catechu* (L.f) Willd. (Heart wood), *Basella alba* Linn. var. *rubra* Linn. (Sap from the fruits), *Beta vulgaris* Linn. (Root), *Bixa orellana* Linn. (Seed), *Carthamus tinctorius* L. (Flower), *Terminalia arjuna* (Roxb.) Wight. & Arn. (Stem bark), *Terminalia chebula* Retz. (Fruit) were collected and prepared dried powder form. Then the powdered material 100 gm soaked in 250 ml of 97 % methanol for 7-10 days and kept on a rotary shaker for 20 hours (every day). It was then filtered with the help of a Whatman No. 1 filter paper. The methanolic extraction was then collected and placed on rotary evaporator at 37° C until completely dry and then added in minimum amount of DMSO. The extracts were kept in sterile air tight bottles at 4°C until further use (Ahmad and Aqil, 2007).

Test method

In the antimutagenicity test the *salmonella* histidine point mutation with some modification (Kaur *et al.*, 2002) as used to test the antimutagenicity activity of the methanolic extract of the dyeing parts of the plant material. In this method the inhibition of mutagenic activity of the sodium azide by the test sample was determined. In the pre incubation experiment, the method for medium preparation for this test also followed by standard literature (Maroon and Ames, 1983). The test sample (25, 50 and 100 µg/plate) were assayed by plating with 0.1 ml of bacterial culture of *Salmonella typhimurium* tester strains TA 98 and TA 100, 0.1 ml mutagen and followed by the addition of 2.5 ml top agar at 45° C (containing 0.5 ml NaCl and 0.6% agar) supplemented with 0.5 mM histidine biotin. These

Table 1. Details about seven dye yielding plants with proper medicinal value

Sl. No.	Name of the plant	Local name	Family	Habit	Dye yielding parts	Product colour	Colouring Compound	Use of dye	Local medicinal uses
1.	<i>Acacia catechu</i> (L.f) Willd.	Khair	Mimosaceae	Tree	Heart wood	Reddish brown	Catechin, Catechin red	Cutch obtained by boiling the heartwood chips in water and cooling the liquid of certain consistency is the most important dye product, which yields reddish-brown colour. The said popular dye is used for dyeing cotton, silk and canvas for boat sails. Also used in calico printing.	Cutch obtained from heartwood is used in medicine as an astringent. Katha obtained from heartwood is commonly used for chewing with pan. Medicinally it is used as an astringent and digestive. Externally it is used as a cooling application to ulcers, boils and eruptions.
2.	<i>Basella alba</i> Linn. var. <i>rubra</i> Linn.	Poi	Basellaceae	Climbing herb	Sap from the fruit	Maroon	Betalain, Gomphrenin 1	Sap from the ripe fruits yielding the maroon colour which is used for dyeing sweets, jellies, silk, cotton cloth and also used in painting purposes like 'Patchitra'.	Leaves: used as diuretic; useful in gonorrhoea; leaf juice given in constipation to children and pregnant woman. Mucilaginous leaves are pulped and used as poultice.
3.	<i>Beta vulgaris</i> Linn	Beet	Chenopodiaceae	Herb	Root	Red	Betalain, Betacyanin, Betaxanthin.	It is mainly used in cooking purposes, also used in, icecream and sugar confectionary.	Seeds: Used as diaphoretic and cooling. Leaves also applied to burns and bruises.
4.	<i>Bixa orellana</i> Linn.	Sindur-e, Latkan	Bixaceae	Small Tree	Pulp surround-ed the seeds	Orange	Bixin, Norbixin, Orellin, Betacaroten-e	Seeds yields Annato an orange dye, which was used for colouring silk and cotton. It is mainly used for colouring foodstuffs, such as ghee, butter, chocolate, etc. May also be suitable for floor polishes, shoe polishes, colouring hairs etc.	Fruits: uses as an astringent and purgative. Seeds: used as an astringent, febrifuge, remedy for gonorrhoea. Leaves: useful in jaundice.
5.	<i>Carthamus tinctorius</i> L.	Kusu-m	Asteraceae	Herb	Flower	Red and Yellow	Ccarthamin (Saarlet red). Carthamon	Flowers heads source of a red and yellow dye, called safflower, used for colouring butter, liqueurs, candles and cloth; also employed in cosmetic industry in the production of rouge.	Whole plant: used to treat paralytic limbs, itch, rheumatism and intractable ulcer. Flower: used as laxative, sedative and stimulant; hot infusion; diaphoretic in jaundice; useful in cold infusion.
6.	<i>Terminalia arjuna</i> (Roxb.) Wight. & Arn.	Arjun	Combretaceae	Tree	Stem bark	Magenta	Arjunic acid	The stem bark sources of magenta colour which is used for dyeing silk and cotton cloth.	Bark: Powdered bark is taken to treat phthisis, spermatarrhoea, leucorrhoea and with milk taken to treat fractures with excessive ecchymosed and externally it is applied to remove spot from the face; decoction of the bark used for washing ulcers.; decoction of fresh bark is prescribed in palpitation, low blood pressure, blood dysentery.
7.	<i>Terminalia chebula</i> Retz	Harita-ki	Combretaceae	Tree	Fruit	Gray	Chebulinic acid	Fruit yield gray dye. People suffering from the graying hair used aqueous extract of Triphala (dried fruits of <i>Belleric myrobalans</i> , <i>Embelic myrobalans</i> and <i>Chebolic myrobalans</i>).	Fruits: used as alternative, astringent, laxative, stomachic and tonic. Cold infusion as a gargle in stomatitis and used in chronic ulcers, carious teeth; in cough and asthma, urinary diseases; highly efficacious in chronic diarrhoea, dysentery and flatulence. Triphala (<i>Belleric myrobalans</i> , <i>Embelic myrobalans</i> and <i>Chebolic myrobalans</i>) is an important ayurvedic formulation used in the treatment of liver and kidney dysfunctions.

combined solutions were poured onto minimal glucose plate (having 40% glucose solution and Vogel Bonner medium). Then His⁺ independent revertants were counted after incubation of the plates at 37 °C for 48 hour. To check the toxicity of test sample parallel controls were run with extract alone at all concentration tested with mutagen (Aqil *et al.*, 2006). Sodium azide was used as a diagnostic mutagen (1.5 µg/0.1 ml/plate) in the positive control and plates without sodium azide and plant samples were considered as negative control (Negi *et al.*, 2003). In this test the non toxic concentration were categorized as those where there was a well developed lawn which is almost similar size of colonies and where there was no statistical difference in the number of spontaneous revertants. The experiment was repeated twice in each of the triplicate plates. The calculation of inhibitory activity was expressed as percentage decreased of reverse mutation.

$$\% \text{ inhibition} = [(A-B)/(A-C)] \times 100$$

Where

A = number of histidine revertants per plate induced by positive mutagen,
B = number of histidine revertants per plate induced by positive mutagen in the presence of each plant extract and
C = number of spontaneous revertants per plate induced in negative control.

The inhibition of each bacterial extract was considered as strong, moderate and weak when the value was higher than 60, 40 to 60 or 20 to 40 respectively. The values of less than 20 % are considerable negligible (Calomme *et al.*, 1996).

RESULTS

Antimicrobial activity

In this study the methanolic extracts of the dye yielding plant sample showed the antimicrobial activity against selected four microorganism and obtained the different inhibition zone in different diameter which have been enumerated below in Table-2.

Table 2. Detection of zone of inhibition for antimicrobial activities by methanolic extract of the selected dye yielding plants.

Sl. No.	Plants	Parts used in test	Diameter of the inhibition zone (mm)			
			<i>Bacillus cereus</i> (Gram positive)	<i>Klebsiella pneumoniae</i> (Gram negative)	<i>Vibrio cholerae</i> (Gram negative)	<i>Escherichia coli</i> (Gram negative)
1	<i>Acacia catechu</i> (L.f) Willd.	Heartwood	11	8	9	9
2.	<i>Basella alba</i> Linn. var. <i>rubra</i> Linn.	Sap from the fruits	6	4	11	8
3.	<i>Beta vulgaris</i> Linn	Root	8	5	4	5
4.	<i>Bixa orellana</i> Linn.	Seed	6	4	7	6
5.	<i>Carthamus tinctorius</i> L.	Flower	5	8	4	5
6.	<i>Terminalia arjuna</i> (Roxb.) Wight. & Arn.	Stem bark	7	5	7	7
7.	<i>Terminalia chebula</i> Retz	Fruit	11	11	11	13

Table 3. Effect of the methanolic extracts of the plant sample on the induced mutagenicity in *Salmonella typhemurium* tester strains of TA 98 and TA 100.

Sl. No.	Treatment of the Plant sample (Pre incubation)	Using dye yielding plant parts for this treatment	Concentration dose (µg/plate)	% inhibition of histidine revertans (value are mean of 3 experiments)	
				TA 98	TA 100
1.	<i>Acacia catechu</i> (L.f) Willd. + mutagen	Heartwood	25	25.2	28.3
			50	38.4	39.7
			100	62.2	62.5
2.	<i>Basella alba</i> Linn. var. <i>rubra</i> Linn. + mutagen	Sap from the fruits	25	28.4	29.2
			50	39.2	40.4
			100	70.2	72.2
3.	<i>Beta vulgaris</i> Linn + mutagen	Root	25	20.2	25.1
			50	36.3	37.2
			100	56.3	57.6
4.	<i>Bixa orellana</i> Linn. + mutagen	Seeds	25	30.4	32.4
			50	50.2	57.2
			100	69.3	72.3
5.	<i>Carthamus tinctorius</i> L. + mutagen	Flowers	25	23.7	26.2
			50	35.1	36.3
			100	57.2	59.2
6.	<i>Terminalia arjuna</i> (Roxb.) Wight. & Arn. + mutagen	Stem bark	25	34.3	36.1
			50	60.3	62.8
			100	78.2	83.1
7.	<i>Terminalia chebula</i> Retz + mutagen	Fruits	25	35.5	40.4
			50	67.6	69.2
			100	73.4	81.2

Negative control (Plant extracts) showed no sign of mutagenicity at the above tested concentration.
Sodium azide (NaN₃) used as (1.5 µg/plate) for TA 98 and TA 100 tester strain.

To boiling the heartwood chips in water the plant *Acacia catechu* (L.f) Willd. yielded the reddish brown colour. The dye is largely composed of xanthophyll and the dye pigments is soluble in water as well as in other solvents like methanol, actone, petroleum ether etc. The crude methanolic extract antimicrobial activity ranging 8 to 11 mm against the selected microorganism. In *Basella alba* Linn. var. *rubra* Linn., sap from the ripe fruit yielded the maroon colour. In the test result of antibacterial activity the crude extract of this dye shown that the inhibition zone ranging from 4 to 11 mm against all the tester strains. In *Beta vulgaris* Linn Root yield a red dye which is used for colouring cotton cloth and showing the inhibition zone ranging from 4 to 8 mm respectively which produce equal inhibition zone of flowers methanolic extract of *Carthamus tinctorius* L. In *Bixa orellana* Linn, seeds yielded an orange dye. The crude methanolic extract of this dye shows antimicrobial activity and grteast zone of inhibition was observed 7 mm against *Vibrio cholerae* and 4 mm in case of the remaining three strains. On the other hand the two species *Terminalia arjuna* (Roxb.) Wight. and *Terminalia chebula* Retz. & Arn belonging to the family combretaceae provide protent positive response against all the selected microorganism. Stem bark of *Terminalia arjuna* (Roxb.) Wight. Yielded the magenta colour and showing the inhibition zone 5 to 7 mm. The fruits methanolic extract of *Terminalia chebula* Retz. Shows the highest inhibition zone 11 to 13 mm remaining all the seven dye yielding plant.

Antimutagenicity activity

The antimutagenic activity of all the seven dye yielding plants against sodium azide (NaN_3) was evaluated by means of the Ames test using two tester strains TA 98 and TA 100. The methanolic extract of the dye yielding parts of the selected plants exhibit non toxic in nature in both *Salmonella typhemuium* tester strains at all concentration dose 25, 50 and 100 $\mu\text{g}/\text{plate}$. Effect of the methanolic extracts of the plant sample on the induced mutagenisyy in *Salmonella typhemuium* tester strains shown in Table-3. All the methanolic extract o the selected plant showed in increase in inhibition of mutagenisyy with provide concentration dose.

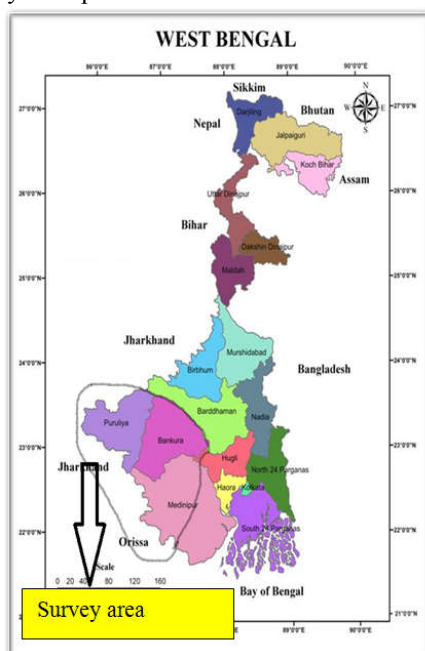


Fig. 1. The surveying zone of South West Bengal (Bankura, Purulia and Paschim Medinipur).

DISCUSSION

Acacia catechu (L.f) Willd. A moderate sized tree. Bark dark grayish-brown, nearly 1.3cm in thickness, exfoliating in long narrow strips. Sapwood yellowish white but heartwood red. To boiling the heartwood chips in water and cooling the liquid of certain consistency obtained cutch that is the most important dye product which yields reddish brown colour. Cutch obtained from the heart wood is used in medicine as an astringent. Katha obtained from heartwood is commonly used for chewing with pan (*Piper betle* L.) and it is also used as digestive. This plant showed the second anti microbial activity, inhibition ranging from 8 to 11 remaining all the seven dye yielding plant. *Basella alba* Linn. var. *rubra* Linn. Common name Poi, it is Winding or creeping. Stems & petioles usually red or green. Leaves broadly ovate to oblong, green. Spikes simple; rachies thick; flowers at first close together, gradually more spaced; elliptic. Stamens 5, epipetalous included; Ovary 1-celled; ovule 1, basal; styles 3; stigmas linear. Pseudoberry depressed-globose, lobed, showing black, containing a viole/maroot juice. Sap from the ripe fruits yielding the maroon colour which is used for dyeing sweets, jallies, silk, cotton cloth and also used in paintain purposes like 'Patchitra'. The plant containing amino acid, vitamin, organic acid and bioflavonoides. A glycoprotein present in the leaves which shown strong antiviral activity (Anonymous, 1988).



Fig. 2. The plant *Acacia catechu* (L.f) Willd.

In the presence of phenolic compounds *Basella alba* Linn. var. *rubra* Linn. extract shows high antimutagenic activity (Jayaprakasha *et al.*, 2007). The herbal extract of *Basella alba* Linn. var. *rubra* Linn. which posses potent antimutagenic activity, have high potential to be further developed as functional foods for cancer prevention (Phadungkit *et al.*, 2012). Mucilagenous leaves are pulped and used as poultice. Crude methanolic extract showed moderate antimicrobial activity against all the four strain of bacteria, with the gretest

diameter of inhibition zone 11 mm in case of *Vibrio cholerae* and 4 mm, 6 mm and 8 mm in *Klebsiella pneumoniae*, *Bacillus cereus* and *Escherichia coli*. *Beta vulgaris* Linn herb with deep red fleshy root. Leaves alternate, ovate or oblong, obtuse; cantine smaller upwards and often rhomboid or lanceolate; lowermost long petiole, upper ones sessile. Root yields a red dye which is used for colouring cotton cloth. A betalain, betacyanin, betaxanthin, dropaxanthin and indixanthin present in this dye. In the respect of antimicrobial activity the dye shows the positive response against all the tester strains and the inhibition zone ranging from 4 to 6 mm respectively. *Bixa orellana* Linn. A small evergreen tree. Leaves cordate, acuminate, veins curved, longer than broad. Flowers pink or white in terminal panicles. Fruit an ovoid or subglobose capsule, softly echinate. Seeds 50, small, trigonous, with a pulpy outer covering. Occurs in two forms: One with white flowers and green capsules and the other with pink flower and red capsules. Seeds yields Annato an orange dye, which was used for colouring silk and cotton. A carotenoid, bixin is the principle colouring matter present in the seeds. It comprises 70-80% of the total pigments in each seeds. In addition to bixin, water soluble yellow dye, orellin, methyle-bixin, β -carotene, cryptoxanthin, lutein and zeaxanthin and also are also reported. Bixin on saponification gives a water soluble product nor bixin. Bixin is the main pigment of oil soluble annato preparation and norbixin is the principle colouring matter of water soluble products (Anonymous, 1988).



Fig. 3. The plant *Basella alba* Linn. var. *rubra* Linn. with dye yielding parts fruit (Berries).

The dye is extensively used in the dairy industry for colouring butter, ghee, cchees, margarins, icecream, chocolate, meat, cereals, confectionery, spices etc. The dye also used as an ingredient in hair oils, shoe polishes, nail gloss, furniture, soap, cosmetics, pharmaceuticals and ointments. The crude methanolic extract of this dye shows antimicrobial activity and grteast zone of inhibition was observed 7 mm against *Vibrio cholerae* and 4 mm in case of the remaining three strains. Medicinally the Fruits used as an astringent and purgative. Seeds used as an astringent, febrifuge, remedy for gonorrhoea. *Carthamus tinctorius* L. common name Safflower of the family asteraceae, a slender, much-branched, annual herb. Leaves alternate, oblong or oblong-lanceolate, lower shortly spinulose-serrate, upper with semi-amplexicaul base. Heads 2-3cm across, orange yellow, surrounded by a cluster of leafy bracts. Green, spinous or not; inner ones ovate-oblong, acute,



Fig. 4. The plant *Beta vulgaris* Linn with dye yielding parts root.

spine-tipped. Flowers orange- red, yellow, some times white. Corolla segments linear. Anthers with sagittate base. Fruits 4- angled, obovoid achenes. Pappus absent. Safflower occupied a central position so far as development of various other derivatives from it is concerned and it is provided a major basis to the trade in natural colours in Bihar in the later of the 19 th century (Grierson, 1885). Safflower florets contain principally two colouring matters, carthamin which is scarlet-red and safflower yellow which is soluble in water. The dye used for colouring butter, liqueurs, candles and cloth; also employed in cosmetic industry in the production of rouge. The crude methanolic extract of this dye showing the antimicrobial properties and measured the inhibition range from 4 to 6 mm. Medicinally the flower used as laxative, sedative, stimulant, hot infusion also useful in cold infusion. *Terminalia arjuna* (Roxb.)



Fig. 5. The plant *Bixa orellana* Linn. with dye yielding parts fruit seeds.

Wight. & Arn, a medium to large size tree; bark smooth, flaky; branches spreading or inclined. Leaves alternate or sub-opposite, oblong or elliptic-oblong, flowers whitish-greenish, in terminal and axillary paniced spikes; calyx glabrous, 3.5-4 mm long, lobes triangular; petals absent; stamens much exserted; ovary covered with crisped hairs. Fruits 5-angled, 5-winged, marked with ascending striations, glabrous, ovoid or obovoid-oblong. Stem bark of this plant present arjunolic acids, tomentosic acid, ellagic acid, arjungenin tannins containing catechin, galocatechin, epicatechin (Rastogi and Mehrotra 1993a,b,c). The stem bark yield the magenta colour which is used for colouring silk and cotton cloth. The plant also have medicinal properties. Decoction of the bark used for washing ulcers.; decoction of fresh bark is prescribed in palpitation, low blood pressure, blood dysentery; bark pounded in water over night and the water is given to treat haematemesis, , menorrhagia leucorrhoea (Padmaa, 2010). In the study of antimicrobial properties, it shows the positive response and inhibition ranging from 5 to 7 mm against the entire supplied microorganism. Strong antibacterial activity shown by the methanol extracts of this plant against multi-drug resistant *Salmonella typhi* (Rani and Khullar, 2004).



Fig. 6. The plant *Carthamus tinctorius* L. with dye yielding parts flower.

The extract from the *Terminalia arjuna* (Roxb.) Wight. & Arn exhibit potent antibacterial activity against, *Escherichia coli*, *Klebsiella aerogenes*, *proteus vulgaris* and *Pseudomonas aerogenes* (gram negative bacteria) at 1000-5000 ppm by the disc diffusion method (Perumal Samy *et al.*, 1998). It was established that our work also posses this similarity. *Terminalia chebula* Retz. of the family Combretaceae, it is a small to medium sized tree, to 25m tall; young branchlets, leaf-buds, and leaves with long, soft, shining, rust-coloured, sometimes silvery hairs. Leaves mostly subopposite, distant, ovate or oblong-ovate, sub acute at apex, deciduous in the cold season. Spikes usually branched, 5-7cm long. Flowers dull-white or yellowish. Fruits obovoid or ellipsoidal from a broad base, glabrous, glossy, horned, yellowish-green, 2-3.5cm across, more or less 5- ribbed when dry. Fruit yield the gray dye, which is used for colouring rope and decoration of house etc. Medicinally the fruits used as alterative, astringent,

laxative, stomachic and tonic. Cold infusion as a garglein stomatitis and used in chronic ulcers, carious teeth; in cough and asthma, urinary diseases; highly efficacious in chronic diarrhoea, dysentery and flatulence. Triphala (*Belleric myrobalans*, *Embelic myrobalans* and *Chebulic myrobalans*) is an important ayurvedic formulation used in the treatment of liver and kidney dysfunctions. The fruits contain chebulinic acid, tannic acid, terchebin and vitamin- c. In the antimicrobial activity testing it shows the highest inhibition zone 13 mm against *Escherichia coli* and remaining 11 mm inhibition in all the seven dye yielding plants extract. In antimutagenicity test *Terminalia arjuna* (Roxb.) Wight. & Arn and *Terminalia chebula* Retz. showed decrease in the number of revertants colonies against sodium azide iduced mutagenisity in between two tester strains (TA98 and TA100) of *Salmonella typhemuium*. The percent of inhibition in 100 µg/plate against sodium azide (NaN₃) is 78.2 and 83.1% in between two tester strains of *Terminalia arjuna* (Roxb.) Wight. & Arn . Where percent of inhibition in 25 µg/plate it was ranged from 34.3% and 36.1% and in 50 µg/plate it was ranged from 60.3% and 62.8%. The above result, it was suggest that whenever concentration dose is increase the percent of inhibition also increase and it was shows strong antimutagenicity activity. Another great percent of inhibition 73.4% and 81.2% in *Terminalia chebula* Retz. was recorded. The fruit of this plant having rich in tannic acid. Both of this plant (*Terminalia arjuna* (Roxb.) Wight. & Arn . and *Terminalia chebula* Retz.) yielded the tannin based dye and their antimutagenicity activity differs greatly.

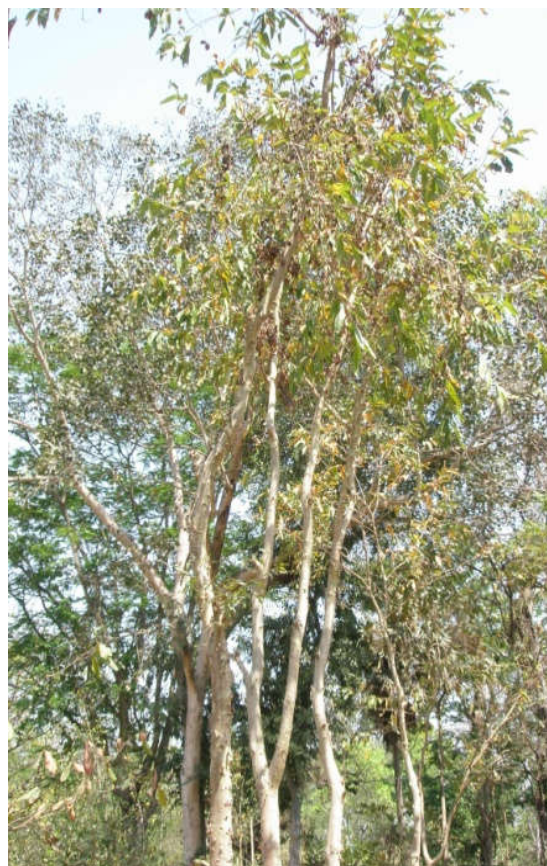


Fig. 7. The plant *Terminalia arjuna* (Roxb.) Wight. & Arn.

This is an interesting finding and need to depth investigation for which the dye having tannin based structure. Flavonoides and phenolics are the most likely candidate among the

methanol extract for providing the antimutagenic effect and preventing the oxidative damage (Edenharder and Grunhage, 2003). The whole antimutagenicity assay it was recorded that the lowest percent of inhibition 20.2% in TA 98 strain and 25.1% in TA 100 strain in *Beta vulgaris* Linn. of the 25 µg/plate. Antimutagenic potential of a fraction isolated from *Terminalia arjuna* has been evaluated in TA 98 and TA 100 strains of *Salmonella typhimurium* against direct and indirect acting mutagens. The fraction was quite effective against S9-dependent 2AF while it showed moderate effect against NPD. The fraction was analyzed to be ellagic acid (Kaur *et al.*, 1997).



Fig. 8. The plant *Terminalia chebula* Retz with dye yielding parts fruit.

Our experiments were performed similarity of this statement that the antimutagenicity activity factors present in *Terminalia arjuna*. In *Acacia catechu* (L.f) Willd. showed moreover similarities between two tester strain at 100 µg/plate against sodium azide (NaN₃) and percent of inhibition ranged 62.2% and 62.5%. The antimutagenic response of *Bixa orellana* Linn. and *Basella alba* Linn. var. *rubra* Linn. Showed moreover similarities between two tester strain at 100 µg/plate against sodium azide (NaN₃) and percent of inhibition ranged from 69.3% to 72.3%. and 70.2% to 72.2 respectively. *Carthamus tinctorius* L. showed similar concentration (100 µg/plate) dependent moderate antimutagenicity activity (57.2% and 59.2%) against direct mutagen (Sodium azide) in respective of two tester strain used. In the above result it was conclude that the overall antimutagenicity potential of methanolic extract of seven dye yielding plants in the order of *Terminalia arjuna* (Roxb.) Wight. & Arn. > *Terminalia chebula* Retz. > *Basella alba* Linn. var. *rubra* Linn. > *Bixa orellana* Linn. > *Acacia catechu* (L.f) Willd. > *Carthamus tinctorius* L. > *Beta vulgaris* Linn respectively.

Conclusion

The above antimicrobial test it was shows that the methanolic extract of this dye yielding plants having potential antimicrobial properties of the entire selected microorganism. It should be concluded that the addition of this natural dyes in food it not only enhanced the quality of food but also provided good antimicrobial activity. In the above mutagenicity test it was reveals that the methanolic extract of this dye yielding plants having non toxic in nature and *Terminalia arjuna* (Roxb.) Wight. & Arn. shows the highest and *Beta vulgaris* Linn lowest antimutagenic potential in the selected plant species respectively. Concentration and strain depended response are also valuable evident in this study. In recent times there has been a revival of interest in harmless, eco-friendly natural dyes. The art of dyeing with vegetable dyes is known only to some old artisans and is not common. In South West Bengal most of the dyers and villagers are practicing vegetable dyeing since generation often generation and they have followed old traditional method. To promote the production of natural dyes needs to train younger person for carrying out dyeing of the fabric with the natural dyes and encourage them for providing some job oriented scheme. Proper collection, documentation and protection should be needed of such natural dye yielding plants in this zone. It can be concluded that that there is a need for researcher to explore and uncover all the medicinal properties of the natural dye yielding plants in this zone.

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REFERENCES

- Ahmad, I. and Aqil, F. 2007. In vitro efficacy of bioactive extracts of 15 medicinal plants against ESβL producing multi drug resistant enteric bacteria. *Microbiological Res.*, 162: 264.
- Ames, B. N. 1983. Dietary carcinogens and anticarcinogens: Oxygen radicals and degenerative diseases. *Science.*, 221: 1256-1263.
- Anonymous, 1988. The wealth of India: Raw materials. Vol. 2B, 50. Publication and information directorate, CSIR, New Delhi.
- Aqil, F., Mehmood, Z. and Ahmad, I. 2006. Antioxidant and free radical scavenging properties of twelve traditional used Indian medicinal plants. *Turk J Biol.*, 30: 177.

- Calomme K., Pieters L., Vietinck A. and Bergha, V. 1996. Inhibition of bacterial mutagenesis by *citrus* flavonoids. *Planta Med.*, 62: 222-226.
- Chandramouli, K. V. 1993. The colours of our lives. PPST foundation Adyar, Madras.
- Chandramouli, K. V. 1995. Sources of Natural Dyes in India – A Compendium with Regional Names. PPST Foundation, Chennai.
- Chatterjee, A. and Pakrashi, S. C. 1991-2001. The Treatise on Indian medicinal plants, 1- 6, Nat. Inst. Sci. Comm., CSIR, New Delhi, India
- Das, P. K., Mondal, A. K. and Mondal (Parui), S. 2011. Antimicrobial activity of some selected dye yielding plants in Eastern India. *Afr. J. Plant Sci.*, 5(9): 510-520.
- Das, P. K., Mondal, A. K., Singh, V. (Ed.), 2009. Ethnobotany and Medicinal Plants of India and Nepal, Scientific Publishers, India, Vol. 3, pp. 128-138.
- Edenharder, R., and Grunhage, D. 2003. Free radical scavenging ability of flavonoids as mechanism of protection against mutagenicity induced by test-butyl hydroxide or cumene hydroperoxide in *Salmonella typhimurium* TA 102. *Mut Res.*, 540: 1
- Flora, S. D. 1998. Mechanism of inhibitory of mutagenesis and carcinogenesis. *Mut Res.*, 222: 141.
- Gerson, H. 1975. Fungi toxicity of 1, 4-naphthoquinones to *Candida albicans* and *Trichophyton mentagrophytes*. *Can. J. Microbiol.*, 21: 197-205.
- Grierson, G. A. 1885. Bihar peasant life. Govt of Bengal Publication (2nd edition published in 1926, by the Bihar and Orissa Govt.).
- Heerak, K. 1994. Bharatiya Lok Sanskriti Me Per Paudhe (Plants in Indian Folk Cultur). Indian Council of Agricultural Research, New Delhi. pp. 97-100.
- Hussein, S. A. M., Barakat, H. H., Merfort, I. and Nawwar, M. A. M. 1997. Tannins from the leaves of *Punica granatum*. *Photochemistry*, 45: 819-823.
- Jayaprakasha, G. K., Negi, P. S., Jena, B S., Rao, J. M. 2007. Antioxidant and Antimutagenic Activities of *Cinnamomum zeylanicum* Fruit extracts. *J. Food Compos. Anal.*, 20: 330-336.
- Kaur, K., Arora, S., Kumar, S. and Nagpal, A., 2002. Antimutagenic activity of acetone and methanol fractions of *Terminalia arjuna*. *Food Chem Toxicol.*, 40(10): 1475.
- Kaur, S., Grover, I. S. and Kumar, S. 1997. Antimutagenic potential of ellagic acid isolated from *Terminalia arjuna*. *Indian J. Exp. Biol.*, 35: 478-482.
- Khan, R., Islam, B., Akram, M., Shakil, S., Ahmad, A., Ali, S. M., Siddiqui, M. and Khan, A. U. 2009. Antimicrobial Activity of Five Herbal Extracts Against Multi drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin. *Molecules*, 14: 586-597.
- Maroon, D. M. and Ames, B. N. 1983. Revised methods for *Salmonella* mutagenicity test. *Mut. Res.*, 113: 173-215.
- National Committee for Clinical Laboratory Standards. 1997. Methods for determining bactericidal activity of antimicrobial agents Proposed Guideline. NCCLS document M26-P Villanova.
- Negi, P. S., Jayaprakasha, G. K. and Jena, B. S. 2003. Antioxidant and antimutagenic activities of pomegranate peel extracts. *Food chem.*, 80: 393-397.
- Padmaa, M. P. 2010. *Terminalia arjuna* (Roxb.) Wt. and Arn. : A Review. *Int. J. Pharmacol.*, 6(5): 515-534.
- Perumal Samy, R., Ignacimuthu, S., Sen, H. 1998. Screening of 34 Indian medicinal Plants for antibacterial properties. *J. Ethnopharmacol.*, 62: 173-182.
- Phadungkit, M., Somdee, T., Kangsadalampai, K. 2012. Phytochemical screening, antioxidant and antimutagenic activities of selected Thai edible plant extracts. *J. Med. Plants Res.*, 6(5): 662-666.
- Rani, P. and Khullar, N. 2004. Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi*. *Phytther. Res.*, 18: 670-673.
- Rastogi, R. P. and Mehrotra, B. N. 1993a. Compendium of Indian Medicinal Plants. Vol. 1, CSIR, New Delhi.
- Rastogi, R. P. and Mehrotra, B. N. 1993b. Compendium of Indian Medicinal Plants. Vol. 2, CSIR, New Delhi.
- Rastogi, R. P. and Mehrotra, B. N. 1993c. Compendium of Indian Medicinal Plants. Vol. 3, CSIR, New Delhi.
- Roy, M. 1978. Dyes in ancient and medieval India. *IJHS*. 13(2): 83-112.
- Rymbai, H., Sharma, R. R., and Srivastav, M. 2011. Biocolorants and its implications in Health and Food Industry- A Review. *Int. J. PharmTech res.*, 3(4): 2228-2244.
- Schuerch, A. R. and Wehrli, W. 1978. β -Lapachone, an inhibitor of oncornavirus reverse transcriptase and eukaryotic DNA polymerase inhibitory effect, thiol dependence and specificity. *Eur. J. Biochem.*, 84: 197-205.
- Singh, R., Jain, A., Panwan, S., Gupta, D. and Khare, S. K. 2005. Antimicrobial activity of natural dyes. *Dyes Pigm.*, 66: 99-102.
- Siva, R. 2007. Status of natural dyes and dye yielding plants in India. *Curr. Sci. India.*, 92: 916-925.
- Siva, R. 2011. Evaluation of Antibacterial, Antifungal, and Antioxidant Properties of Some Food Dyes. *Food Sci. Biotechnol.*, 20(1): 7-13.
- Stavric, B. 1994. Antimutagens and anticarcinogens in food. *Food & Chem Toxicol.*, 32: 79.
- Ulusoylu, M., Öndersev, D. V., Soyoğul, Ü., Gürkan, E. and Tuzlaci, E. 2001. The cytotoxic and the biological (antibacterial and antifungal) activities of *Centaurea iberica* and *Ferulago confuse*. *Journal of Faculty of Pharmacy of Gazi University.*, 18(2): 75-80.
- Ushimaru, P. L., Mariama, T. N. Luiz, C., Luciano, B. D. and Ary, F. J. 2007. Antimicrobial activity of medicinal plant extract. *Braz. J. Microbiol.*, 38: 717-719.
