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Research

CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SOME NATURAL DYE YIELDING PLANT SPECIES OF KASHMIR VALLEY

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ABSTRACT

The present study was taken up as an exploratory study to test some natural dyes of Kashmir valley for their inherent antimicrobial activity with a view to develop a natural food dye. The plant species including *Bidens tripatita* L., *Celosia argentia* L. var plumose, *Rubus fructicosus* L., *Indigofera heterantha* Wall., *Rubia cordifolia* L., *Bistorta amplexicaule* D. Don., *Calendula officinalis* L., *Amaranthus hybridus* L., *Prunella vulgaris* L., *Fragaria nubicola* Lindl. ex Lacaita collected from different locations of Kashmir valley were characterized and identified in terms of morphological attributes, inflorescence/floral features and fruit size at maturity. Further, their extracts were analyzed for their anti-microbial activity against selected microbes (*Escherichia coli*, *Staphylococcus aureus*, *Aspergillus* spp. and *Pencillium* spp.). Most of the plant extracts showed antibacterial activity against *Escherichia coli* and *Staphyloccocus aureus*; and antifungal activity against A*spergillus* and *Pencillium* species with zones of inhibition ranging between 7.25 mm to 16 mm. Among the tested samples, the highest antimicrobial activity was recorded in *Rubia cordifolia* L. root extract showing maximum zone of inhibition (16 mm).

INTRODUCTION

Mankind has always used edible colour for desirability of food. Colour provides visual information about food's quality, condition and also influences the perception of its flavour. It enhances the aesthetic value, appetite and makes a less desirable food more desirable. The colour of a food substance indicates freshness and safety that are indices of good aesthetic and sensorial values (Pritam, *et al*., 2008). Further, food materials are known to be susceptible to microbial attack, as these provide food and moisture required for microbial growth. Food provides basic requirements such as moisture, oxygen, nutrients and temperature for bacterial growth and multiplication. This often leads to food spoilage, product deterioration and other related diseases. This necessitates the development of a product that could provide a desired antimicrobial effect and colour to the food.

Synthetic colours are widely used in the food, pharmaceutical and chemical industries. Artificial food dyes impair and disrupt the behaviour of the children (Goyette, *et al*., 1978). Industrialization of the food system, including a rise in food processing, has increased the use of food additives including food dyes as synthetic food dyes are less expensive and have intense hue than natural edible dyes but have general toxicity (Carvalho, 1992). Several studies on the application of natural dyes have been reported (Hebeish *et al*., 2012) to protect the environment

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for indiscriminate exploitation and pollution by industries. Recently, the potentiality of using natural dyes in textile coloration as UV-protection and antimicrobial has been investigated (Ali, *et al*., 2011). Synthetic dyes are complex compounds with a big complicated molecular structure and toxic properties. It can affect aquatics life, human health and ecological system.

Although known for a long time for dyeing as well as medicinal properties, the structures and protective properties of natural dyes have been recognized only in the recent past. Many of the plants used for dye extraction are classified as medicinal, and some of these have recently been shown to possess remarkable antimicrobial activity (Hussein, *et al*., 1997). Turmeric natural dye is reported to show antibacterial activity against all the bacterial isolates (Selvam, *et al*., 2012). Carthamin, a red quinochalcone isolated from safflower, has been used extensively as a natural color additive for foods and cosmetics and as a nutraceutical in food industry (Wu, *et al*., 2013). *Carthamus tinctorius* flowers at fructification stage and is a suitable source of natural dyes as an alternative to food synthetic colorants (Nidhal, *et al*., 2014).

Studies have been conducted earlier to report the antimicrobial activity of natural dyes from various regions of the world against several microbes. No study has been conducted so far to check the antimicrobial activity of the natural dyes of Kashmir valley. The present study was thus undertaken to determine the bactericidal and fungicidal properties of ten dye extracts against some common human pathogens. The common human pathogenic bacteria (*Escherichia coli* and *Staphylococcus aureus*) and fungus (*Aspergillus* spp. and *Pencillium* spp.) were used in the study as human beings are commonly exposed to these pathogens in day to day life. The usage of plant based antimicrobials can be alternatives for chemicals used in food preservation.

MATERIAL AND METHODS

Experimental material

The experimental material comprised of 10 plant species namely *Bidens tripatita* L., *Celosia argentia* L. var plumose, *Rubus fructicosus* L., *Indigofera heterantha* Wall., *Rubia cordifolia* L., *Bistorta amplexicaule* D. Don., *Calendula officinalis* L., *Amaranthus hybridus* L., *Prunella vulgaris* L., *Fragaria nubicola* Lindl. ex Lacaita collected from different regions of Kashmir valley. Plant species collected from different locations were identified after Bentham and Hooker in conformity with recent literature and were tagged for recording the following observations.

Morphological attributes

Morphological attributes of plant species were recorded for characterization and identification of plant species.

a) Field observations

i) Habitat

Habitat of the plant species was recorded by observing the natural environment in which the plant species normally occurred.

ii) Plant habit

Plant habit was recorded by observing whether the plant is erect, sub-erect, climbing, creeping etc.

iii) Plant and flower/inflorescence colour

Plant colour was recorded throughout growth season and flower and inflorescence colour during flowering stage.

b) Vegetative features

i) Stem type

In this parameter the stem type was recorded by observing whether the plant stem is erect, sub-erect, climbing, creeping etc.

ii) Macro and micro morphological leaf characteristics

The macro morphological characteristics of leaves were recorded by observing the leaf type, leaf margins, leaf venation type, leaf arrangement, etc. The micro morphological features of the leaves were recorded by observing the leaf surface, presence or absence of trichomes, etc.

iii) Reproductive features

Inflorescence/floral features

The inflorescence/floral features of the plant species were recorded at the flowering stage by recording floral arrangement, the number of petals, sepals, stamens, pistils, petals, sepals of the flowers of the selected plant species.

Fruit type

The fruit type whether berry, drupe, pome, etc. was recorded by observing the fruit of each plant species at maturity.

Antimicrobial activity

Collection of plant material

Fresh plant samples collected from different sites of Kashmir valley were air dried and homogenized to a fine powder with the help of a

mixer grinder. The powdered material was then used for extraction of dyes.

Preparation of plant extracts

1 g of dried plant sample was ground in 10 ml of acetone. The supernatant was collected and filtered with the help of Whatman No. 1 filter paper. Filtrate was allowed to evaporate till completely dry. The extract was dissolved in DMSO and kept in sterile air tight Eppendorf tube at 4°C till further use.

Test organisms

Pure cultures of bacterial strains (*Escherichia coli* and *Staphylococcus aureus*) and fungal strains (*Aspergillus* spp. and *Pencillium* spp.) were obtained from department of microbiology, Govt. medical college, Srinagar, Kashmir.

Preparation of bacterial inoculum

Stock cultures were maintained at 4°C on slants and plates of Mueller Hinton Agar. Active cultures were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth medium and incubated without agitation for 24 h at 37°C to achieve optical densities corresponding to 2.0×10^6 colony forming units (CFU/ml) for bacteria.

Preparation of fungal inoculum

Stock cultures were maintained at 4°C on slants and plates of sabour and dextrose agar. Active cultures were prepared by mixing carefully small amount of test organisms in a sterilized petri plate with autoclaved water under UV laminar air flow and then spreaded.

Antimicrobial susceptibility test

All the plant extracts were screened against bacterial and fungal (two each) pathogenic strains. The disc diffusion method was used to test the antimicrobial activity of the plant extracts against *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus* spp. and *Pencillium* spp. 20 ml of sterilized Mueller Hinton agar for bacteria and 20 ml sabour and dextrose agar for fungi were poured into each sterile Petri plates. The plates were allowed to solidify for 5 minutes and inoculum suspension was swabbed uniformly. The entire agar surface of each plate was inoculated with this swab, in the horizontal vertical direction to ensure the even distribution of organism over the agar surface. Whatman filter paper no. 1 was used to prepare discs approximately 6.0 mm in diameter, which were placed in a Petri dish and sterilized. The sterile filter paper discs were soaked in the plant extract and placed on the surface of the bacterial and fungal seeded agar plates with sterile forceps and tapped gently to ensure the adherence to the agar. The compound was allowed to diffuse for 5 minutes. The plates were incubated in an inverted position at 37°C (24 h) for bacteria and at 25°C (72 h) for fungi. Inhibition zones formed around the discs were measured from back of petri plate including the diameter of the discs with the help of transparent ruler. These experiments were performed in triplicate. All experiments were performed under aseptic conditions.

RESULTS AND DISCUSSION

Characterization of selected plant species

Characters of the ten (10) selected plant species from different regions of Kashmir valley are presented in Table 1 and Fig. 1. The results revealed that ripe berries of European blackberry (*Rubus fructicosus* L.) were blackish red in colour. The stem and leaves of Mountain fleece flower (*Bistorta amplexicaule* D. Don) were pink in colour while as that of Red amaranth (*Amaranthus hybridus* L.) were magenta in colour. The inflorescence of Self heal (*Prunella vulgaris* L.) was purple in colour reflecting the presence of anthocyanins while as in Cockscomb (*Celosia argentia* L. var plumose), it was yellow in colour. The petals of both the varieties of Pot marigold (*Calendula officinalis* L.) were bright yellow and orange in colour reflecting the presence of carotenoids.

Antimicrobial activity of natural dye extracts

The results showed that most of the above tested plant extracts showed antibacterial activity against *Escherichia coli* and *Staphyloccocus aureus*, and antifungal activity against *Aspergillus* and *Pencillium* spp. with zones of inhibition ranging between 7.25 mm to 16 mm (Table 2 and Fig. 2). The highest antimicrobial activity was recorded in Indian madder (*Rubia cordifolia* L.) root extract with 16 mm zone of inhibition followed by European blackberry 15.75 mm in *Rubus fructicosus* L. and Pot marigold (*Calendula officinalis* L. var. Gitana orange) against *Aspergillus* spp., Red amaranth (*Amaranthus hybridus* L.) demonstrated good inhibition activity (14 mm) against *Aspergillus* spp and Indian madder (*Rubia cordifolia* L.) root extract against *Staphylococcus aureus* (13.75 mm). A clear zone of inhibition by Indian madder (*Rubia cordifolia* L.) for *Aspergillus* spp. could be seen as a typical example in Fig. 1. Lowest antibacterial activity was demonstrated by Red amaranth and Self heal (*Prunella vulgaris* L.) against *Escherichia coli*; Burr marigold (*Bidens tripatita* L.), Himalayan indigo (*Indigofera heterantha* Wall.), Pot marigold (var. Gitana orange) and Red amaranth (*Amaranthus hybridus* L.) against *Staphylococcus aureus*; Self heal and Himalayan/Wild strawberry (*Fragaria nubicola* Lindl. ex. Lacaita) against

Table 1. Collection, characterization and identification of the selected plant species

Plant species	Characters
1) ***Bidens tripartita* L.**	
Common name	Bur Marigold
Habitat	Fallow fields, wastelands, roadsides
Habit	Erect herb.
Flower/inflorescence colour	Yellow
i) Morphological attributes	
a) Vegetative features	
Stem	Stout, annual, erect, glabrous.
Leaves	Opposite, 3-lobed, 3-partite or pinnatifid; segments lanceolate, toothed
b) Reproductive features	
Floral attributes	Flowers in lax or dense spikes, unisexual, small; bracts acicular, longer than sepals.
Fruit	Achenes, glabrous, with 2-3 short awns
2) *Celosia argentia* L. var *plumose*	
Common name	Cockscomb
Habitat	Cultivated in lawns as ornamental
Habit	Annual, erect herb
Flower/inflorescence colour	Yellow
i) Morphological attributes	
a) Vegetative features	
Stem	Stout, slender, glabrous, branched or unbranched
Leaves	Alternate, linear or lanceolate
b) Reproductive features	
Floral attributes	Flowers in long peduncled, cynlendric or oblong or ovoid spikes, top of the spike branched out in a cock's comb form; sepals scarious
Fruit	Urticle with glabrous black seeds
3) *Rubus fructicosus* L.	
Common name	European Blackberry
Habitat	Found in a variety of habitats including woodlands, hedgerows, gardens, roadsides, wastelands
Habit	Prickly, sarmentose shrub
Flower/inflorescence colour	White or pinkish white
i) Morphological attributes	
a) Vegetative features	
Stem	Arched, obtusely angled, channeled; prickles stout, scattered hooked; branches robust
Leaves	Alternate, leaflets 3 or 5, orbicular obovate or elliptic obtuse or acute, hoary beneath with white stellate tomentum, prickles short; stipules setaceous, subulate, velvety
b) Reproductive features	
Floral attributes	Flowers in dense thiyrsoid terminal panicles; petals obovate, white or pinkish white
Fruit	Drupes many, fleshy, black, crowded on receptacle
4) *Indigofera heterantha* Wall.	
Common name	Himalayan Indigo
Habitat	Forests, dry slopes
Habit	Small copiously branched shrub often forming dense scrubs
Flower/inflorescence colour	Pinkish purple
i) Morphological attributes	
a) Vegetative features	
Stem	Branclets, argente-canisent

Leaves	Pinnnate, short petioled, leaflets small, opposite, 17-25, lanceolate-oblong, sub coraceous, covered with white bristles above, glaucous and thinly argenteo, canisent below
b) Reproductive features	
Floral attributes	Flowers in racemes; racemes pedicillate, 12-20-flowered; calyx companulate; corolla purpurlish red, canisent externally
Fruit	Pod sub cylindrical, glabrous
5) *Rubia cordifolia* L.	
Common name	Indian Madder
Habitat	Near shady and moist places
Habit	Perennial herbaceous climber or creeper
Flower/inflorescence colour	Greenish white
i) Morphological attributes	
a) Vegetative features	
Root	Perennial, long, cylendric, flexuose; bark thin, red
Stem	4-angled, branches scabrid
Leaves	Cordate-ovate, 4-8 in a whorl, petiolate, lower petioles long
b) Reproductive features	
Floral attributes	Flowers in panicled cymes, cymes terminal; branches trichotomous, spreading with opposite sessile leafy cordate bracts; flowers minute 5-(4) merous
Fruit	Globose, blackish
6) *Bistorta amplexicaule* D. Don	
Common name	Mountain Fleece flower
Habitat	Moist areas, near irrigation channels and rice fields
Habit	Perennial, glabrous herb
Flower/inflorescence colour	Pink.
i) Morphological attributes	
a) Vegetative features	
Stem	Erect, cylinder, tufted
Leaves	Leaves alternate, ovate-cordate, caudate-accuminate, crenulate, minutely hairy on nerves beneath, lower long petioled, upper leaves amplexicuale
b) Reproductive features	
Floral attributes	Flowers in racemes, bracteate, bracteolate, pedicellate; perianth pink.
Fruit	Nut black, shining, 3-gonous
7) *Calendula officinalis* L.	
Common name	Pot Marigold
Habitat	Cultivated in lawns
Habit	Annual erect herb
Flower/inflorescence colour	Yellow and Orange
i) Morphological attributes	
a) Vegetative features	
Stem	Hispidly pubescent, corymbosely branched above
Leaves	Lower spathulate, entire; upper lanceolate, toothed or subentire, base cordate, amplexicaul, hispid
b) Reproductive features	
Floral attributes	Flowers in terminal peduncled heads, heterogamous; ray florets ligulate, ligules bright orange yellow, entire or 3 toothed; disc florets tubular limb dilated, shortly 5 fid.
Fruit	Achenes glabrous, longer than the involucres
8) *Amaranthus hybridus* L.	
Common name	Red Amaranth
Habitat	Cultivated vegetable fields, wastelands, roadsides
Habit	A tall, annual herb
Flower/inflorescence colour	Reddish green
i) Morphological attributes	

a) Vegetative features	
Stem	Robust, striate, reddish
Leaves	Alternate, long petioled, elliptic- or ovate-lanceolate, tip obtuse; petiole and veins reddish.
b) Reproductive features	
Floral attributes	Flowers in lax or dense spikes, unisexual, small; bracts acicular, longer than sepals.
Fruit	Urticle reddish green, compressed
9) *Prunella vulgaris* L.	
Common name	Self-Heal
Habitat	Near irrigation channels, rice fields and moist areas
Habit	Erect herb
Flower/inflorescence colour	Purple
i) Morphological attributes	
a) Vegetative features	
Root	Rootstock creeping
Stem	Erect or ascending
Leaves	Ovate or oblong, entire toothed or pinnatifid, upper sessile
b) Reproductive features	
Floral attributes	Flowers dimorphic, larger bisexual, smaller female; calyx-teeth very variable, awned or not
Fruit	Nutlets oblong, smooth
10) *Fragaria nubicola* Lindl. ex. Lacatia)	
Common name	Himalayan/Wild strawberry
Habitat	Meadows, mountain slopes, forests
Habit	Perennial, scapigerous herbs, with creeping stolons
Flower/inflorescence colour	White
i) Morphological attributes	
a) Vegetative features	
Stem	Runner, filiform, rooting at nodes
Leaves	Trifoliate, long petioled; leaflets ovate, deeply and coarsely toothed, sessile.
b) Reproductive features	
Floral attributes	Calyx persistent, 5-lobedwith 5 bracteolates at its base
Fruit	Of many small achenes sunken in the surface of fleshy red receptacle

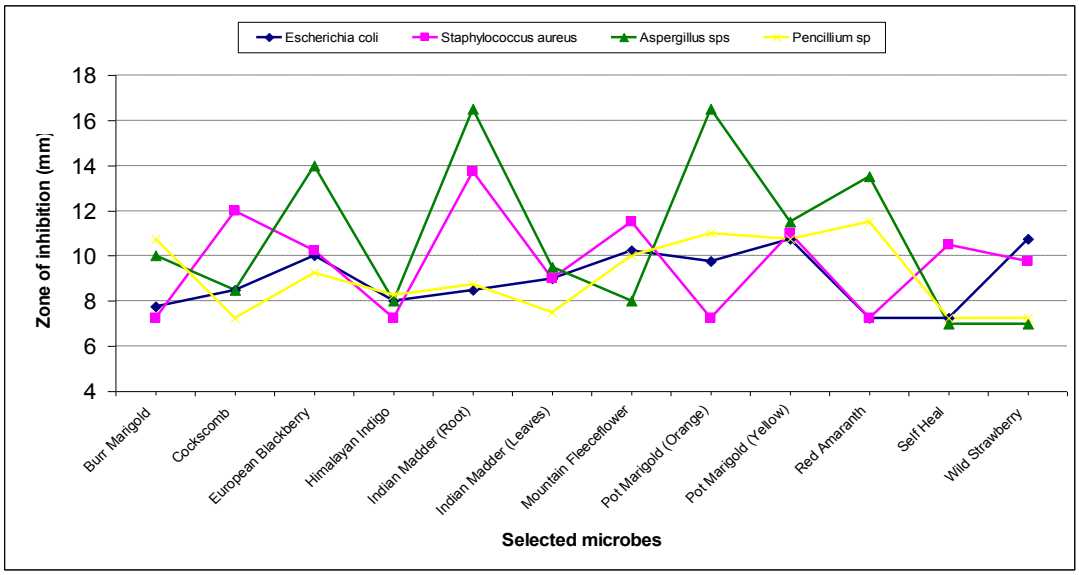
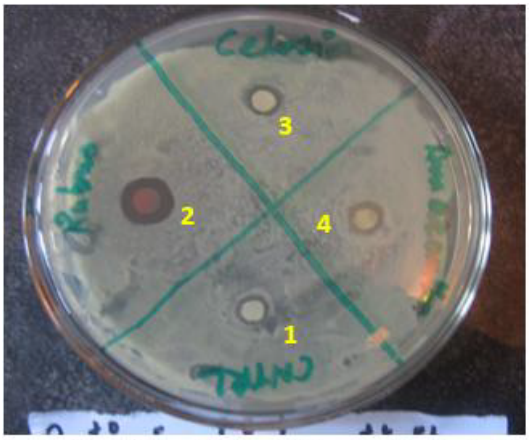


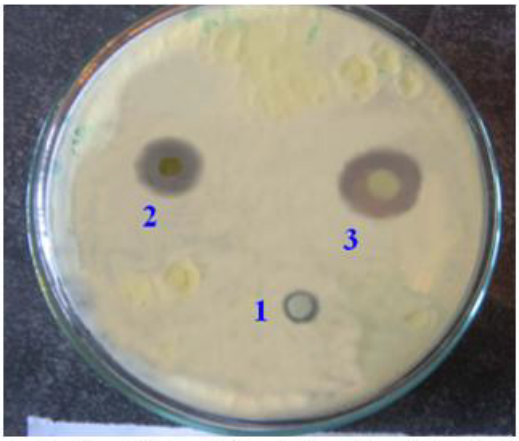
Fig. 1 Antimicrobial activity of natural dyes against common human pathogenic bacteria and fungi.

Table 2. Antimicrobial activity of natural dyes against common human pathogenic bacteria and fungi

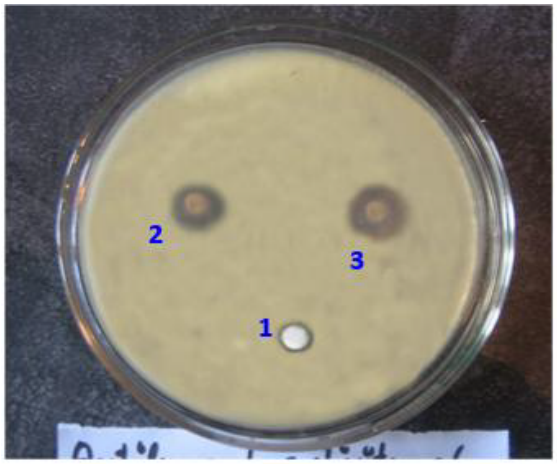
S. No	Common name	Botanical name	Part used	Zone of inhibition (mm)											
				Escherichia coli			*Staphylococcus aureus*			*Aspergillus* sps			*Pencillium* sps		
				2010-2011	2011-2012	Pooled	2010-2011	2011-2012	Pooled	2010-2011	2011-2012	Pooled	2010-2011	2011-2012	Pooled
1	Burr Marigold	*Bidens tripatita* L.	Leaves and stem	8	7.5	7.75	7.5	7	7.25	8	10	9	11.5	10	10.75
2	Cockscomb	*Celosia argentia* L. var *plumose*	Inflorescence	9.5	7.5	8.5	13	11	12	8	8.5	8.25	7	7.5	7.25
3	European Blackberry	*Rubus fructicosus* L.	Fruit	8.5	11.5	10	11	9.5	10.25	17.5	14	15.75	9	9.5	9.25
4	Himalayan Indigo	*Indigofera heterantha* Wall.	Leaves and stem	8.5	7.5	8	7	7.5	7.25	9	8	8.5	9	7.5	8.25
5	Indian Madder	*Rubia cordifolia* L.	Root	9.5	7.5	8.5	13.5	14	13.75	15.5	16.5	16	8.5	9	8.75
6	Indian Madder	*Rubia cordifolia* L.	Leaves	9.5	8.5	9	9.5	8.5	9	11.5	9.5	10.5	8	7	7.5
7	Mountain Fleece flower	*Bistorta amplexicaule* D. Don	Above ground portion	9	11.5	10.25	11	12	11.5	8	8	8	10.5	9.5	10
8	Pot Marigold	*Calendula officinalis* L. var Gitana Orange	Flower	9	10.5	9.75	7	7.5	7.25	15	16.5	15.75	10	12	11
9	Pot Marigold	*Calendula officinalis* L. var Gitana Yellow	Flower	11.5	10	10.75	10.5	11.5	11	14.5	11.5	13	12	9.5	10.75
10	Red Amaranth	*Amaranthus hybridus* L.	Above ground portion	7.5	7	7.25	7.5	7	7.25	14.5	13.5	14	9.5	13.5	11.5
11	Self-Heal	*Prunella vulgaris* L.	Inflorescence	7.5	7	7.25	11	10	10.5	7.5	7	7.25	7	7.5	7.25
12	Wild Strawberry	*Fragaria nubicola* Lindl. ex Lacaita	Root	10.5	11	10.75	10	9.5	9.75	7.5	7	7.25	7.5	7	7.25
CD ($p \leq 0.05$)				0.877	0.688	0.543	1003	1.032	0.701	1.166	1.131	0.758	1.003	1.06	0.711



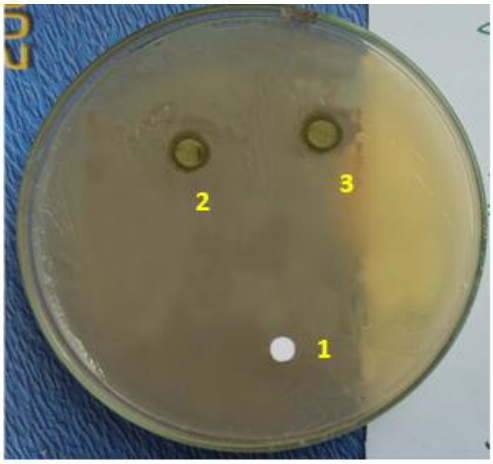
Antimicrobial activity of the (1) Control, (2) *Rubus fructicosis* L. (3) *Celosia argentea* L. var *plumose* (4) *Amaranthus hybridus* L. against *E. coli*



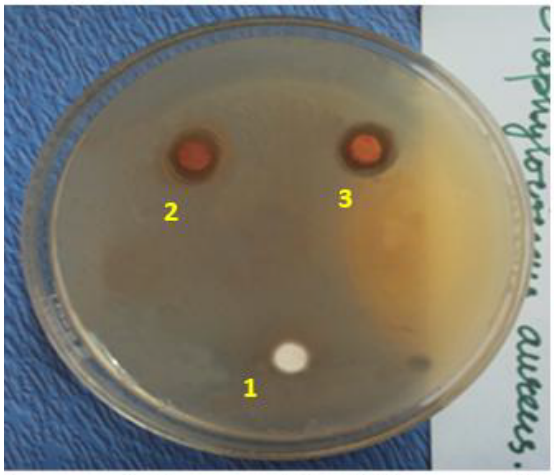
Antimicrobial activity of the (1) Control, (2) *Indigoferra heterantha* Wall., (3) *Bidens tripartita* L., against *Pencillium* species



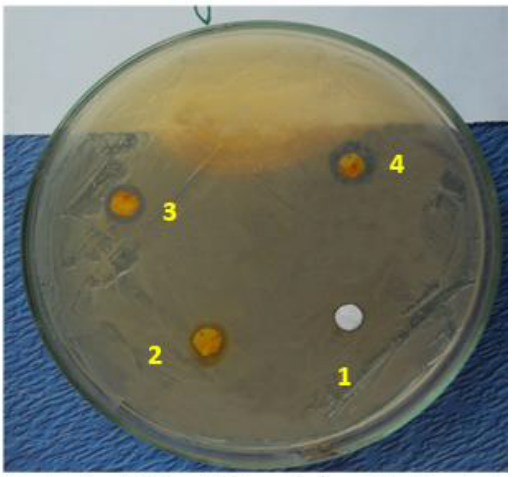
Antimicrobial activity of the (1) Control, (2) *Bistorta amplexicaule* D. Don (3) *Amaranthus hybridus* L. against *Pencillium* sp.



Antimicrobial activity of the (1) Control and *Rubia cordifolia* L. (Leaf extract) (2, 3) against *Staphyloccocus aureus.*



Antimicrobial activity of the (1) Control and *Rubia cordifolia* L. (Root extract) (2, 3) against *Staphyloccocus aureus.*



Antimicrobial activity of the (1) Control and *Calendula officinalis* L. var. Gitana Orange (2, 3, 4) against *E. coli.*

Fig. 2 Antimicrobial activity of natural dyes.

Aspergillus spp. and Cockscomb (*Celosia argentia* L. var. plumose), Self heal and Himalayan/Wild strawberry against *Pencillium* spp. All produced 7.25 mm zone of inhibition, followed by Indian madder root extract against *Pencillium* spp. (7.5 mm) and Burr marigold against *Escherichia coli* (7.75 mm). Burr marigold produced the largest zone of inhibition against *Pencillium* spp. (10.75 mm) and lowest zone of inhibition against *Staphyloccocus aureus* (7.25 mm).

Tomczykowa, *et al*., also reported that the oil from roots of *Bidens tripartite* L. possess strong antimicrobial activity against 8 Gram-positive, 11 Gram-negative species and 10 fungal strains. B. *pilosa* contains compounds like flavonoids, phenylacetylenes, alkaloids, sterols, triterpenoids and tannins which are responsible for the antimicrobial activity of this species (Brandao, *et al*., 1997; Khan, *et al*., 2001). Further, (Oladipupo, *et al.,* 2015) while investigating the *in vitro* antibacterial activity of crude extracts from the different organs of *Bidens pilosa* reported the leaf extract exhibited significant inhibition on the growth of bacteria tested than the seed and root extracts. This activity of the leaf extract have been attributed to the presence of phenylpropanoids, terpenoids, many flavonoids and glycoside derivatives, all of which have well known pharmaceutical and biological activities (Silva, *et al.,* 2011; Bartolome, *et al.,* 2013), thus supporting the results of the present study.

Further, Cockscomb produced the largest zone of inhibition against S. *aureus* (12 mm) and lowest zone of inhibition against *Pencillium* spp. (7.25 mm). Our results are in agreement with the results of (Okpako, *et al.,* 2015) who observed that the leaf extract gave both antibacterial and antifungal activities while the stem and root extracts possessed only antibacterial effects. Interestingly, its extract was more active against Gram-positive S. *aureus* than Gram-negative E. *coli* microorganisms. This is in agreement with the previous reports of several workers (viz. Smith-Palmer, *et al*., 1998; Ceylan and Fung, 2004; Lopez, *et al.,* 2005; Shan, *et al*., 2005; Buwa and Staden, 2006). The reason lies in the fact that there exists a significant difference in the outer layers of Gram-negative and Gram-positive bacteria. Gram-negative bacteria possess an outer membrane and a unique periplasmic space which is absent in Gram-positive bacteria (Duffy and Power, 2001). The resistance of Gram-negative bacteria toward antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules, and is also associated with the enzymes in the periplasmic space, which are capable of breaking down the molecules introduced from outside (Gao, *et al*., 1999). Gram-positive bacteria do not have such an outer membrane and cell wall structure. Consequently, antibacterial substances can penetrate the bacterial cells and easily destroy the bacterial cell wall and cytoplasmic membrane and result in a leakage of the cytoplasm and its coagulation (Shan, *et al*., 2007). Eventually, these disruptions may cause the loss of cell integrity and death. Further, the modes of action of bacterial agents are related to their cell wall structure and to the outer membrane arrangement.

In this study it was found that European Blackberry produce the largest zone of inhibition against *Aspergillus* spp. (15.75 mm) and lowest zone of inhibition against *Pencillium* spp. (9.25 mm). Fruit extract of the European Blackberry exhibited 10.25 mm zone of inhibition against S. *aureus* which is higher than the zone of inhibition (9 mm) reported in the leaf extract of *Rubus* spp. against the same pathogen (Joy, *et al.,* 2008). Himalayan Indigo produced the largest zone of inhibition against *Aspergillus* spp. (8.5 mm) and lowest zone of inhibition against S. *aureus* (7.25 mm). Esimone, *et al*., reported significant antimicrobial activity from aqueous extract of Indigofera dendroides against Gram-positive (S. *aureus*, *Bacillus subtilis*), Gram-negative (E. *coli* and K. *pneumoniae*) and fungal species (*Aspergillus niger* and *Candida albicans*). The current study demonstrated that Indian madder leaf extract produced the largest zone of inhibition against *Aspergillus* spp. (10.5 mm) and lowest zone of inhibition against *Pencillium* spp. (7.5 mm) while as the root extract produced the largest zone of inhibition against *Aspergillus* spp. (16 mm) and lowest zone of inhibition against E. *coli* (8.5 mm). Our results are in conformity with Qiao, *et al*., who reported antimicrobial activities in the roots of *Rubia cordifolia* L. Further, the root extract of Indian madder inhibits S. *aureus* (13.75 mm) in much better way than E. *coli* (8.5 mm), and these results are in conformity with those reported by Anwar, in case of *Rubia tinctorium* who reported zone of inhibition in between the range of 20-40 and 6-13 against S. *aureus* and E. *coli* respectively. Moreover, *Bistorta amplexicaule* D. Don (Mountain fleece flower) produced the largest zone of inhibition against S. *aureus* (11.5 mm) and lowest zone of inhibition against *Aspergillus* spp. (7.5 mm). Similar findings were reported by Mackeen, *et al*., Penna, *et al*., they reported that the extracts of the many species of *Polygonum* possess antimicrobial activity. Interestingly, Pot marigold (*Calendula officinalis* L. var. Gitana Orange) produced the largest zone of inhibition against *Aspergillus* spp. (15.75 mm) and lowest zone of inhibition against S. *aureus* (7.25 mm) while as Pot marigold (*Calendula officinalis* L. var. Gitana yellow) produced the largest zone of inhibition against *Aspergillus* spp. (13 mm)

and lowest zone of inhibition against E. *coli* and *Pencillium* spp. (10.75 mm). These results were in consistence with the findings of Zilda, *et al*., who reported that essential oil from *Calendula officinalis* L. is having good potential antifungal activity. Likewise, Red amaranth (*Amaranthus hybridus* L.) produced the largest zone of inhibition against *Aspergillus* spp. (14 mm) and lowest zone of inhibition against E. *coli* and S. *aureus* (7.25 mm). In this context (Maiyo, *et al*., 2010) also reported that A. *hybridus*, A. *spinosus* and A. *caudatus* leaf extract contained varied types of pharmacologically active compounds with antimicrobial activity. Self heal (*Prunella vulgaris* L.) produced the largest zone of inhibition against S. *aureus* (10.5 mm) and lowest zone of inhibition against E. *coli*, *Aspergillus* spp. and *Pencillium* spp. (7.25 mm), thus confirming the findings of Kirbag, *et al*., who also reported the values close to the present findings as 8 mm and 11 mm zones of inhibition in E. *coli* and S. *aureus* by *Prunella vulgaris* L., Nurdan and Ayseh also reported 11 mm zone of inhibition by *Prunella vulgaris* L. against S. *aureus*.

In conclusion, most of the studied plant extracts possessed antimicrobial activity against tested microbes. The strongest antimicrobial activity was demonstrated by *Rubia cordifolia* L. root extract, thus indicating its usage as alternative to the toxic chemicals used in food preservation. It is suggested that, further research is needed to determine the effect of dye structure on inhibition and their relevant use in food applications. New studies combining the use of antimicrobials with other methodologies of food preservation are necessary to reduce the impact of these compounds on sensory properties.

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