ANTIMICROBIAL SUSCEPTIBILITY OF SOME NATURAL DYES

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خلاصہ

اس موجودہ تحقیقی کام میں پانچ مختلف پودوں کے بیکٹیریائی مخالف طاقت کا مطالعہ کیا گیا جس میں غیر نا میاتی محلل (پانی) اور نا میاتی محلل (ایسی ٹون اور ہیگزین) شامل ہیں۔ان تمام پودوں کے مخالف بیکٹیریائی کارکردگی کی جانچ کے لئے اگر وال نفوذی طریقہ استعمال کیا گیا۔جس سے بہترین نتیجہ حاصل ہوا۔جبکہ گرام مثبت اور گرام منفی بیکٹیریا میں 'پرپل کوبھی' کا عرق بیکٹیریا کے اگاؤ کے لئے ناموافق ثابت ہوا۔اس کے علاوہ نارنگی کے چھلکے،مہندی کے پتوں اور دارچینی کے عرق میں مخالف بیکٹیریائی کارکردگی بیماری والے زہریلے بیکٹیریا کے خلاف بہتر کارکردگی ظاہر ہوئی۔مختلف پودوں میں ضروری تیل جیسا کہ لوکٹس پی سوپی ٹیلس اور ایس اوریس میں فلیو ینوائیڈ کی موجودگی لوسونیا انرمس میں بیٹا ایسوروزن کی میکھرا کیٹلز کی موجودگی جبکہ سٹرس میں ایلبیڈو اور فلیو یڈو آف پیری کارپس جبکہ دارچینی میں سینامل ایلڈی ہائیڈ اور ایم اینڈ یکا میں میگنی فیرن کی موجودگی بیماری والے بیکٹیریا کی گروتھ کی شدید مخالفت کرتی ہے۔

Abstract

Antibacterial potency of aqueous and non aqueous extract of five plants were studied in vitro. Methanol, Acetone and Hexane were used as a non aqueous solvents for the extraction of dyes. The agar well diffusion method were used during the entire study for the evaluation of antibacterial activities of different plants. The remarkable results were obtained from aqueous as well as non aqueous extracts against gram positive and against gram negative bacteria respectively. Furthermore, among them the aqueous and non aqueous purple cabbage extract exhibit most inhibitory effect on bacteria. It suppressed the bacterial growth by effecting the metabolite of certain bacteria. Orange peel, Henna leaf, Mango Bark and Cinnamon extract showed antibacterial activities against 1 to 4 pathogen as well as food poising bacteria. The growth inhibition effect of methanolic cinnamon extract against *M. luteus, B. subtilis*and *S. aureus*almost equal about 12mm. The presence of flavonoids in *Brassicaoleracea var. capitata f. rubra*, β-asarones in *Lawsoniainermis*,, nutraceuticals in albedo and flevedo of pericarp of Citrus fruits, Cinnmaldehyde in *CinnamomumVerum*, essential oils in different plant and mangiferin in *M.indica*, make them to survived against pathogenic bacteria.
Keywords: Gram Positive bacteria, Gram Negative bacteria, Essential oils, albedo, flevedo,

Introduction

Now a days, finding the methods that free the textile industries from pathogenic micro-organisms is a challenge. In modern world the upsurge in advanced technology demanding functionality in products like water repelling, fade resistance and resistance to microbial invasion (Kamel *et al*., 2015). Consumer worldwide looking for the food remain fresh and odour free able to be use. This is all beholden to protect the materials from microorganisms. The plant is one major source of food. Majority of plants contain aromatic compounds. Aromatic plants constitute large part of natural flora and different part of plant materials used in various field such as pharmaceutical, flavour, fragrance, perfumery and cosmetic industries (Swany *et al*., 2016). Moreover these plant are also the source of natural dyes. Dyes obtained from nature are safe and eco-friendly but mostly unstable and oxidize easily, resulting discoloration (Chao *et al.,* 2017) and susceptible to micro organisms. Many natural dyes have medicinal properties (Gupta *et al*., 2006). Natural dyes are used as a textile material which are sensitize to microbial attack (Singh *et al*., 2004). Due to physical properties of natural dyes such as Large surface area and absorption of moisture make natural dyes hypersensitive for the microbial onslaught.
Literature review revealed that the massive work was done on antimicrobial properties of natural as well as synthetic dyes. Similitude between antibacterial activities of some natural dyes and silver nano particles were studied by (Mirjalili and Abbasipour, 2013). Synthesis, fastness properties, colour assessment and antimicrobial activity of some azo reactive dyes heaving pyrazole moiety was researched by (Rizk *et al*., 2015). Dyeing phenomenon was carried out on acrylic fiber through antimicrobial cationic dyes (Ma *et al*., 2005). The antimicrobial properties of photosensitizer cyanine dyes were examined by (Shindy *et al*., 2006). Antimicrobial activities of synthesized monomethinecyanin dyes were researched out by (Abd-El-Aal, 2004). Antimicrobial

potency and absorption of some novel hetrocyclicdisazo dyes were studied by researcher team of (Karchi *et al*., 2009).

The main interest of this work was to analyse the antimicrobial characteristics of natural dyes. The study on susceptibility of natural dyes towards microorganisms give broad spectrum information about the sustainability toward microbial attack as well as stability of natural dyes. The information from antimicrobial surveillance studies can be use to establish a trend in pathogen antimicrobial resistance and emerging pathogens at the national and global level (Masterton, 2008). The information about development of targeted approaches of microorganisms enable us to control the microorganisms. By controlling microorganisms in dyes the shelf life of dye in foods, dyeing and cosmetics increase because dyes are in several ways.

Materials and Methods

Plant extraction preparation

Plant material of five plants species included in this study presented in (**Table 1**) were collected from different local market of Karachi, Pakistan. These plants were watery washed, disinfected and rinsed by distilled water. Plant materials were dried and grounded in fine powder. After washing and converting into powder form the dyes were extracted in different solvents (**Table 2**) by using soaking method of extraction.

Table 1: Plant Species with their Specific parts were used in study

S.No	Plant	Family	Local Name	Plant part used
1	*M.indica*	Anacardiaceae	Mango	Fruit
2	*CinnamomumVerum*	Lauraceae	Cinnamon	Bark
3	*Lawsoniainermis*	Lythraceae	Henna	Leaf
4	*Brassica oleracea var. capitata f. rubra*	Brassicaceae	Purple Cabbage	Vegetable
5	*Citrus X sinensis*	Rutaceae	Orange	Fruit peel

Table 2:- Non aqueous solvent for extraction of dyes

S.No	Samples	Solvents
1	Mango Bark	Water
		Methanol
		Acetone
2	Cinnamon Bark	Water
		Methanol
		Hexane
3	Orange Peel	Water
		Methanol
4	Henna	Water
5	Purple Cabbage	Water
		Acid
		Methanol

Antimicrobial activity of plant extract

Bacterial Strain: The antibacterial potency of dyes examined by using seven bacterial species which have ability to food poising and life threatening diseases in human beings. Three species of Gram Positive bacteria (Micrococcus luteus, Staphylococcus aureus and Bacillus subtilis) and five species of Gram negative bacteria (Escherichia coli,Pseudomonas aeruginosa, Klebsiella pneumoniae, *Proteus mirabilis* and Salmonella typhi) were used to study the antibacterial activity of natural dyes in presented work.

Inoculum Method : The 24h fresh Cultures were used. Fresh culture transferred aseptically into sterile saline. Then vortex the saline tube and measured with McFarland standard no. **0.5**, turbidity which adjusted to give 1.5×10^{8}CFU/mL count.

Antibacterial activity of plant extract: The agar well diffusion method was used for evaluation of antimicrobial activity of each plant extract. The inoculum of culture was spread into surface of nutrient agar plates and then made a 100µL hole by using cork borer. Sample of interest transferred with the help of 100µL juster pipette aseptically. Then nutrient plates were incubated at **37ºC** for 24h**.**

Results and discussion

Antibacterial Activities of Aqueous extract

Potency of aqueous dyes against the gram positive as well as gram negative bacteria species were depicted in Table 3 and Table 4 respectively. It was observed that henna leaf dye was susceptible to *Staphylococcus aureus.Staphylococcus aureus*cause severe disease in human such as bacteremia, infective endocarditis, skin and soft tissue infections (Tracey *et al*., 2018). It was notice that Henna leaf showed resistance against two species of bacteria that were *Micrococcus luteus*and *Bacillus subtilis.* The potency of henna leaf extract due to presence of β-asarones active ingredient present in leaf, root and rizhomes tissues active against bacteria (Kannah and Vinotha, 2013). While extract of orange peel showed resistance against *Bacillus subtilis.* This was due to presence of magnesium, Calcium, Copper, Vitamin A, folate and phytochemicals in orange pericarp which make it defy towards gram positive bacteria. There was no zone of growth inhibition observed by orange peel extract against *Micrococcus luteus*and *Staphylococcus aureus.* The antimicrobial activity of samples against mentioned pathogens were due to presence of tennins, Saponins, phenolic compounds, essential oil and flavonoids (Yerou *et al.*, 2017). Flavonoids, phenolic compounds, phytochemicals and essential oils are bio-active ingredient of any fruit, leaf and vegetable which have antimicrobial properties. Antibacterial potency of henna and orange peel dyes were negative against three gram negative bacteria that were *Proteus mirabilis, Salmonella typhi*and *Pseudomonas aeruginosa*.Pseudomonas aeruginosaishuman pathogen which cause life threatening acute infection and chronic airway colonization during cystic fibrosis. *P.aeruginosa* form a bio-film characterized by secretion of extra-cellular matrix of various poly-saccharides and grow in number of genes involved in surface adherence (Eckert *et al*., 2016). Cell related to bio-film less active metabolically than their planktonics counterparts and especially resist to small molecule antibiotics as a result of this, potency of extracts against *P.aeruginosa* was negative.

It was also ascertained the mango bark dye was showed zone of growth inhibition against *Bacillus subtilis*and *Staphylococcus aureus.* It was due to mangiferin. Mangiferin(1,3,6,7-tetrahydroxyxnthone-2-C-β-D-glucopyranoside), a C-glucosylxanthone was isolated from mango bark (Castellanos *et al*., 2014) which is antibacterial agent (Hannan *et al*., 2013) and showed their potency against gram positive bacteria's. Cinnamon bark dye appeared their antibacterial activity against only Micrococcus luteus. there was no zone of growth of inhibition observed for gram negative bacteria's. In the studied of antibacterial activity of cinnamon aqueous extract studied by researchers showed antibacterial activity against *E.coli* (Puangpronpitag and Sittiwet, 2009).Cinnamaldehyde and eugnol bio-active compounds showed antibacterial activity (Nabavi *et al*., 2015) against Micrococcus luteus. The purple cabbage showed zone of growth inhibition against three gram positive and one gram negative bacteria which where depicted in table 3 and 4 respectively. The presence of phytochemical in brassica oleracea var. capitata f. rubra were responsible for their antimicrobial potency.

Table 3: Antibacterial activity of aqueous extracts against Gram Positive bacteria's

S.NO	*Samples*	*Zone of Growth Inhibition (mm)*		
		M. luteus	*B. subtilis*	*S. aureus*
1	*Henna leaf*	*20mm*	*10mm*	*-tive*
2	*Orange Peel*	*-tive*	*20mm*	*-tive*
3	*Mango Bark*	*-tive*	*11mm*	*10mm*
4	*Cinnamon*	*10mm*	*-tive*	*-tive*
5	*Purple Cabbage*	*12mm*	*11mm*	*10mm*

Key: -tive means no zone of growth of inhibition

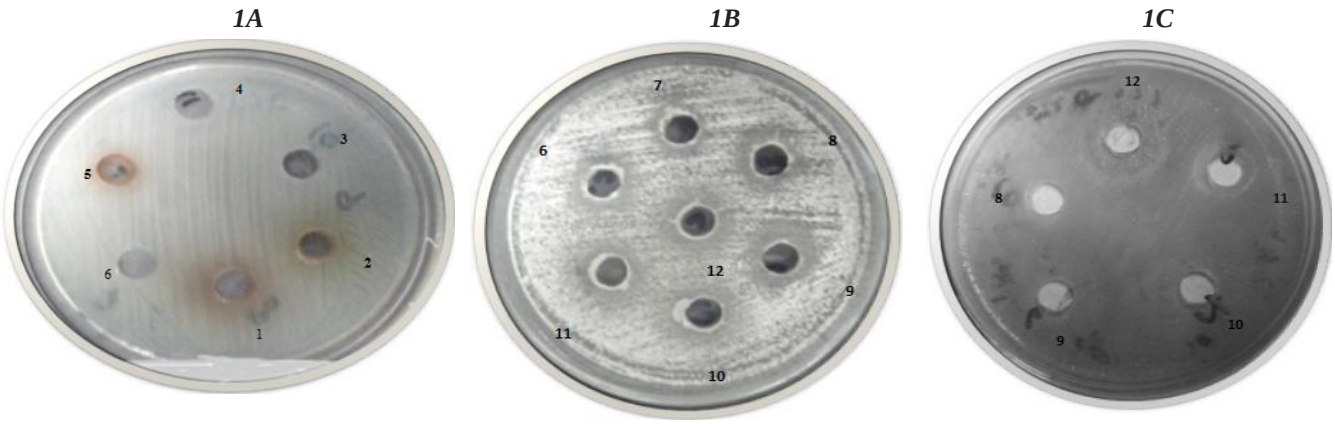

Fig (1A): Antibacterial activity of different compounds against *Micrococcus luteus* , *(1B)* Antibacterial activity of different compounds against *Bacillus subtilis, (1C)* Antibacterial activity of different compounds against *Staphylococcus aureus*

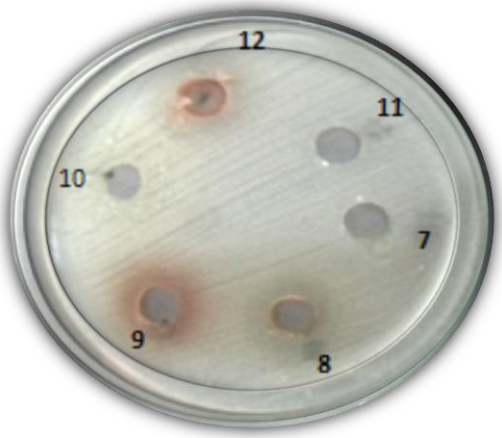

Fig 02: Antibacterial activity of different compounds against*Proteus mirabilis*

Table 4: Antibacterial activities of aqueous extracts against Gram negative bacteria's

S.NO	*Sample*	*Zone of Growth Inhibition (mm)*				
		P.mirabilis	*S. typhi*	*P. aeruginosa*	*E.coli*	*K.pneumoniae*
1	*Henna*	*-tive*	*-tive*	*-tive*	-	-
2	*Orange Peel*	*-tive*	*-tive*	*-tive*	-	-
3	*Mango Bark*	*-tive*	*-tive*	*-tive*	-	-
4	*Cinnamon*	*-tive*	*-tive*	*-tive*	-	-
5	*Purple Cabbage*	-	*-tive*	*-tive*	*8mm*	*-tive*

Key: -tive means no zone of growth inhibition
Key: - means sample not studied against these bacterial strains

Antibacterial activity of non aqueous extract: By using the non aqueous extraction the antibacterial potency of extracts were also studied presented in (Table 5). It was ascertained that the methanolic extract of orange peel exhibit antibacterial activity against *M. luteus*and *B. subtilis.* The zone of growth inhibition in *M. luteus*was 10mm than *B. subtilis* that was 9mm. The orange is citrus fruit. The pericarp of orange consist bio-active compounds, nutraceuticals and functional compounds in albedo and flavedo of peel (Yashaswini and Arvind, 2018). In orange the flavonoids are present as flavanones (neohesperidosides, rutinosoides), flvanol glycoside, flavones (polymethoxyflavones, hydroxylated polymethoxyflavones) with frequent bio active compounds like

naringin and hesperidin. Phenolic compound have antibacterial as well as antiviral activities in orange. The citrus fruit also contain the essential oils (EO_s) that were also responsible for their antibacterial activities. It was also observed three strains of gram negative bacteria survived against methanolic extract of orange peel was depicted in (Table 6). The other authors also studied the antibacterial activities of orange EO_s (Espina *et al*., 2011) which ratified our results.

Methanolic extract of mango bark also resist against *B. subtilis.* While there was no zone of growth inhibition observed against gram negative bacteria. The bark of mango consist Tannins, Saponins, Phenol, Glycogen, Alkaloids and Cardiac glycoside (Abubakar, 2009). These are bio-active bio compounds which protect the plant against pathogens and predation by animals. The *Micrococcusluteus*and *Staphylococcus aureus*showed resistance against the methanolic and acetone mango bark extract. The difference in activities observed with the variation in solvents. This associated with presence of oil, fatty acids, wax , resins, and pigments which capable to blocked the active ingredient in the extract , preventing the bark extract from reaching the bacterial cell wall (Ashok *et al.*, 2014). The *Micrococcusluteus*and *Staphylococcus aureus*showed resistance against the methanolic and acetone mango bark extract.

The methanolic extract of Cinnamon showed resistance against three gram positive and one gram negative bacteria. The methanolic extract clearly indicate the antibacterial characteristic of cinnamon bark. There is a presence of tennin which has phenol group have a properties like alcohol is an antiseptic that use as antimicrobial component (Suliman *et al.*, 2017). Methanolic extract showed almost equal antibacterial activity against *M. luteus, B. subtilis*and *S. aureus.* 10mm length of growth inhibition observed against *P. mirabilis.* The cinnamaldehyde in cinnamon proven most active against fungi, virus and bacteria (Ali *et al.*, 2005). The cinnamaldehyde in cinnamon resits against *S. aureus* (Aneja *et al*., 2009). It was among the most potent chemical against gram positive bacteria. Hexane extract of Cinnamon was capability to resist against only *B. subtilis* while the antibacterial activity of hexane extract of cinnamon against gram negative bacteria was negative.

The acidic extract of purple cabbage resist againt all gram positive and gram negative bacteria's except *S.typhi*which resist against acidic extract. The acid break the cell wall of gram positive as well as gram negative bacteria's.

Table 5: Antibacterial activities of non aqueous samples against Gram positive bacteria

S.NO	*Solvents*	*Samples*	*Zone of Growth Inhibition (mm)*		
			M. luteus	*B. subtilis*	*S. aureus*
1	*Methanol*	*Orange Peel*	*10mm*	*9mm*	*-tive*
2		*Mango Bark*	*-tive*	*10mm*	*-tive*
3		*Cinnamon*	*12mm*	*12mm*	*12mm*
4	*Hexane*	*Cinnamon*	*-tive*	*10mm*	*-tive*
5	*Acetone*	*Mango Bark*	*-tive*	*12mm*	*-tive*
6	*Acid*	*Purple*	*15mm*	*18mm*	*18mm*

Key: -tive means no zone of growth of inhibition

Table 6: Antibacterial activities of non aqueous extracts against Gram negative bacteria

S.No	*Solvent*	*Sample*	*Zone of Growth Inhibition (mm)*				
			P. mirabilis	*S.typhi*	*P. aeruginosa*	*E.coli*	*K. Pneumonia*
1	*Methanol*	*Orange Peel*	*-tive*	*-type*	*-tive*	-	-
2		*Mango Bark*	*-tive*	*-tive*	*-tive*	-	-
3		*Cinnamon*	*10mm*	*-tive*	*-tive*	-	-
4	*Hexane*	*Cinnamon*	*-tive*	*-tive*	*-tive*	-	-
5	*Acetone*	*Mango Bark*	*9mm*	*-tive*	*-tive*	-	-
6	*Acid*	*Purple Cabbage*	-	*-tive*	*20mm*	*18mm*	*17mm*

Key: -tive means no zone of growth inhibition

Key: - means sample not studied against these bacterial strains

Conclusion

It was concluded Thatthe aqueous extract exhibit great potential against gram positive bacteria than non aqueous extract. The potency of non aqueous extracts against gram negative bacteria was greater than aqueous extracts. Mango bark non aqueous extract showed resistance against ***P.mirabilis.***More over the Cinnamon also exhibited antibacterial activity against ***P.mirabilis.*** Aqueous extract of purple cabbage resist against all gram positive bacteria which were under studied. While the potency of purple cabbage aqueous extract against ***E.coli***was 8mm suggest that the purple cabbage can be use in stomach relief pain drugs. The all non aqueous extracts which were under studied showed great antibacterial activity against ***B. subtilis.*** Further more the acid extract of purple cabbage possessed potency against all gram positive as well as all gram negative bacteria except ***S.typhi***. This was notable that the ***S.typhi***resist in acidic condition. The presence of flavonoids, essential oils, flavanones tennins and other compounds make fruits and vegetable to resist against wide range of microorganisms.

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