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Research Article

Antimicrobial activity of ethanolic dye extract of onion scale leaves

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Abstract

The phytochemical composition and pharmacological value of natural dye extracted in ethanol from scale leaves of *Allium cepa* was investigated. The dye showed the presence of alkaloids (7.10%), flavonoids (0.127%), tannins (0.013%) phenols and (71.6736 mg gallic acid eq. /g). Extract of dye was evaluated against 3 strains of bacterial (*Klebsiella pneumonia, Staphylococcus aureus* & *Acinetobacter baumannii*) and 3 strains of fungi (*Aspergillus .fumigatus, Aspergillus niger* and *Aspergillus flavous,*). The zone of inhibition against *S. aureus* was 21 mm, *K. pneumoniae* was 19 mm and *A. baumannii* was 18.5 mm. The Maximum anti-fungal activity of dye was reported against *A. flavous.* The antioxidant activity was done as percent inhibition of DPPH (1, 1-diphenyl-2-picryl-hydrazyl), free radicals. The DPPH radical scavenging activity was observed for dye and was higher at 200 µg/ml. It is concluded that *Allium cepa* dye could be a potential source of pharmacologically important compounds.

Keywords: Antimicrobial activity; Ethanolic dye; Leaves; Onion

Introduction

Natural dyes were used by human beings since time immemorial for dying of wool, fibers, food and as sources of inks. These dyes were obtained from natural resources such as animals, plants, lichens and minerals **[1]**. Natural dyes are environment friendly, less toxic and exhibit medicinal properties. Natural dyes are exhibit various kinds of plant chemicals such as phenols and saponins etc. as they are responsible for their color and other organoleptic properties **[2]**. Majority of natural dyes are vegetables dyes taken from sources of plants i.e. berries, toots, leaves, and wood as well as from lichens and fungi. Sometime whole plant is used for making die. Prominent dyes are made from lichens which grow on rocks and in pollution free zones. Natural dyes exhibit antimicrobial and antioxidant activities. **[3]** Explained that natural dye obtained from turmeric showed antimicrobial activity against different bacterial strains. **[4]** Interrogated the anti-microbial effect of natural dyes on various pathogenic bacteria. **[5]** Found on anti-bacterial activity for dye extracted from Pomegranate. While considering the value of natural dyes in the treatment of different ailments, textile, food, paper industries, the present investigation was taken to find the

anti-oxidant activity and anti-microbial of the dye extracted in ethanol from scale leaves of onion. Bulb or onion is widely cultivated vegetable from genus *Allium*. The onion has a green, hollow leaves and bulb swells when it gain a length. Seasonally the leaves fall down in autumn and the bulb of onion become brittle and dry. After this process the species is put to dry for storage and use. The species is sensitive to pathogens as onion eelworm, onion fly and many fungal species, few species in bulb are having the capability to produce multiple bulb **[6]**. The species is used in spicy and savory foods. It is also use for the storage purposes of many food items.

Keeping in sight the value of natural dyes in the curing of different ailments, textile, food & paper industries, present studies were designed to get the following two objectives;

To evaluate antimicrobial and antioxidant activity of onion dye.

To determine phytochemical composition of the onion dye.

Materials and methods

For drying, onion scale leaves were kept at room temperature (25^0C).when the leaves were completely dried, they were grinded and put in duly labeled sealed bottles of plastic.

Preparation of dye extract

Simple maceration technique was used for dye preparation. Total of 25g of onion leaves were soaked in 500 ml ethanol for 2 days at 25^0C. The mixture obtained was mixed thoroughly on daily basis with mechanical shaker. After 2 days by whatman filter paper the extract was filtered. The filtrated extract was rotary evaporated in order to separate the gummy extract from solvent. The solvent recovered was put in residue for further extraction and the procedure was recycled thrice. The ethanolic extract was allowed to evaporate till complete dryness. (Fig. 1).

Determination of pH of dye extract

The pH determination was carried out by immersing pH electrodes in the dye solution at room temperature and the pH reading was noted as 4.76 (Fig. 1).

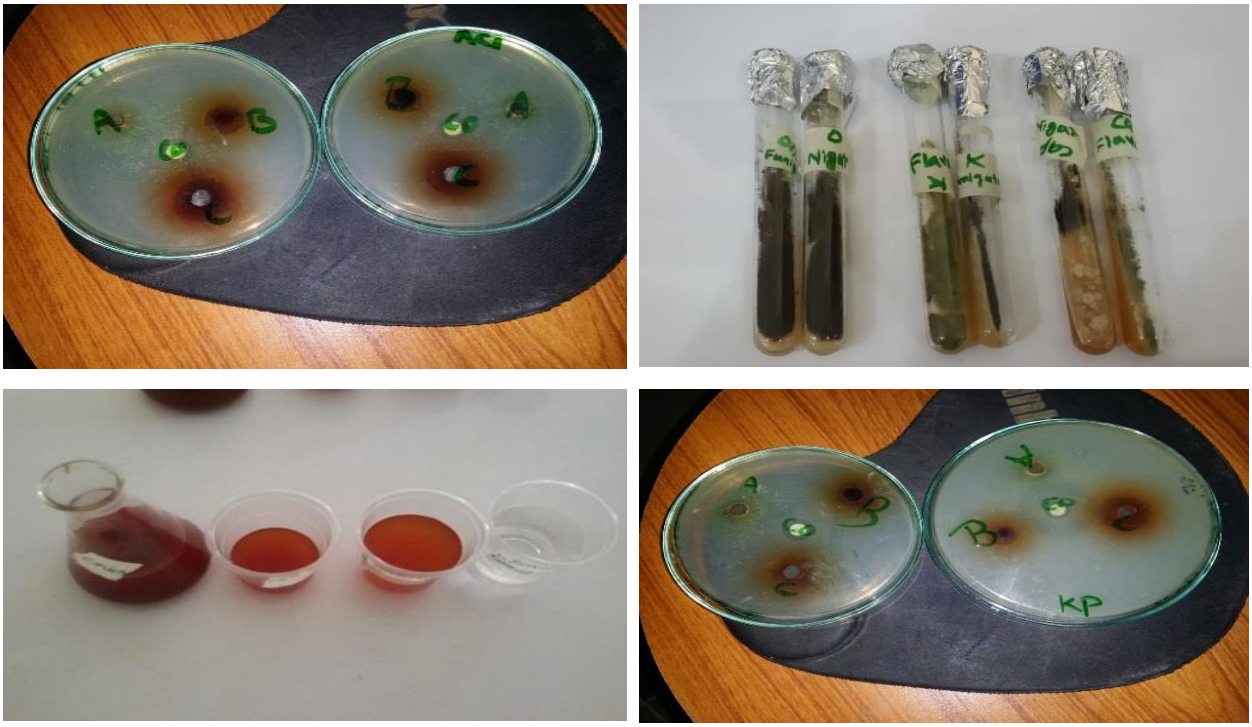

Figure 1. Natural dye extracted in ethanol from scale leaves of *Allium cepa*

Quantitative analysis

Alkaloids

Firstly the sample was mixed with 200 milliliter of 10% acetic acid. Filled beaker was protected for about 240 minutes. The sample was put for filtration process that was followed by concentration in a water bath to $1/4^{th}$ parts of its basic volume. Up to the process of precipitation conc. NH_4OH was mixed drop by drop in the extract. After precipitation, the precipitate obtained and rinsed with dil. NH_4OH followed by filtration. The extra material (alkaloid) was obtained and weighted after drying process. After that the percentage was determined **[7]**.

Tannins

In each step, the sample was taken for about 500mg in a bottle, to dilute its amount 50 ml distilled H_2o was mixed to it. The solution was jerk properly which was followed by filtration process, in a volumetric flask. The flask was labelled. About 5ml of the filtrate was taken out and was combined with two milliliter of 0.1M $Fecl_3$ in 0.1 M Hydrogen Cloride & 0.008ml $K_4[Fe(CN)_6]$. After that, within 10 minutes the immersion was determined at 120nm **[8]**.

Total phenols

Total of 0.001gram of the dye was mixed in 10 ml CH_3OH in a test tube. When the dye was fully dissolved, 125 µl of the solution was dissolved in 500 µl distilled H_2O & 125 µl folin reagent. The mixture was left for 5 minutes. The volume was raised up to 3 ml with the help of distilled water, to make the passage of rays easy across the spectrophotometer. Then 1.25 ml of 7% $NaHCo_3$ solution in distilled H_2O was added to the sample. After that, sample was placed for about half an hour, and its optical density was measured by passing 760 nm wavelength through spectrophotometer. Methanol was used as blank. **[9]**.

Flavonoids

At a temperature of 25^0C 10 gm of the sample was mixed to 100 ml of 80% dilute CH_3OH. Whattman filter paper of pore size 125nm was used to filter the solution. The remaining portion was then shifted into a crucible followed by evaporation process for dryness on a water bath. The substance was measured & percent amount was determined **[10]**.

Antimicrobial assay

Antibacterial activity

For the determination of antibacterial potential of ethanolic dye extract of onion scale leaves, process described by **[11]** was used. Strains of *S. aureus, K. pneumoniae* and *A. baumannii* were used. For the +ive control streptomycine and for the -ive control (DMSO) was used.

Antifungal assay

The **[12]** Duraipandiyan and Ignacimuthu (2009) protocol was used to find out the antifungal activity from the plant. The fungal strains used were *A. niger*, *A. flavous* and *A. fumigatus.* The samples were developed from crude of 12 mg/ml to obtain final amount of 200 µg/ml. For positive control, Solutions of terbinafine in DMSO was made. For negative control pure DMSO was used. The growth of linear fungus was measured in millimeter & inhabitation of fungal growth and was find out by formula as;

$$\text{Percent inhibition of fungal growth} = \frac{100 - \text{linear growth in test (mm)}}{\text{Linear growth in control (mm)}} \times 100$$

DPPH radical scavenging activity

The **[13]** protocol was adopted for the DPPH activity of the dye extract. To prepare stock solution 2.4 mg of (DPPH) was mixed in 100 ml ethanol and this solution was stored at 20c°.To obtain a working solution having an decoction of about 0.980(±0.02) at 517 nm by using the spectrophotometer, (DPPH) was

diluted by adding ethanol. A 3ml 0f this solution was dissolved in 100µl of fractions at different concentration. After shaking the test tubes and incubated at 25 c° for about thirty minutes. At 517 nm the absorbance was taken. The DPPH radical scavenged as:

$$\text{Scavenging effect (\%)} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100.$$

Results and discussion

Phytochemical investigation of *Allium cepa* scale leaves dye is shown in (Table 1). Ethanolic dye extract of *Allium cepa* scale leaves was determined quantitatively for the presence of different chemical constituents. tannins (0.013 %), Alkaloids (7.10%), flavonoids (0.127%) and phenols (71.674 mg gallic acid eq./gm dry weight) were present. **[14]** Determined total soluble phenolic concentration of several Jordanian plant species. They found close relationship of antioxidant activity with phenolics composition of the studied plant species. The anti-bacterial activity was findout by using AWDM (agar well diffusion method) (Table 2). The crude dye extract exhibited antibacterial activity against the tested bacterial strains. Dye extract showed antibacterial activity (zone of inhibition), against *K. pneumonia (19 mm), S. aureus* (21 mm) and *Acinetobacter baumannii* (18.5 mm). The antibiotic Streptomycin showed anti-bacterial activity trialed against the bacterial strains viz *K. pneumonia* (30 mm), *S. aureus* (13 mm) and *Acinetobacter baumannii* (29mm). Anti-fungal activity was performed according to agar tube dilution method while percent inhibition was used for linear growth of fungal strains (Fig. 2). The anti-fungal activity of crude dye extract against *A. niger (*27 % inhibition) whereas *A. flavous* (67% inhibition) *and that of A. fumigatus* (16.5% inhibition). The antifungal agent terbinafene showed 100 % growth inhibition of tested fungal strains. The DPPH free radical scavenging activity was determined and the value was recorded in terms of % inhibition of DPPH radicals. Higher antioxidant activity was shown by dye at 200 µg/ml (22.183%). The dye at 100 µg/ml showed 8.80 % inhibition of DPPH free radicals. The dye at 50 µg/ml showed 13.20% inhibition of DPPH free radicals (Fig. 3). **[15]** studied the anti-bacterial activity of green leaves medicinal plants *S. trilobatum*. According to their findings reported, there was linear relation with the phytochemical content and anti-microbial activity. The dye extracted has good antioxidant activity. This means the dye can be used as an alternative to synthetic anti-oxidants. The synthetic antioxidants have many reactions when taken under *in vivo* conditions **[16]**.

Table 1. Quantitative Analysis of *Allium cepa* scale leaves dye

Sample Code	Alkaloids (%)	Flavonoids (%)	Tannins (%)	Phenols (mg gallic acid eq./gm dry weight)
Allium cepa scale leaves dye	7.10	0.127	0.013	71.6736

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Table 2. Anti-bacterial activity of A. cepa dye

Sample	inhibition Zone (mm)		
	Klebsiella pneumoniae	***Staphylococcus aureus***	***Acinetobacter baumannii***
Dye extract	19	21	18.5
Antibiotic(streptomycin)	30	13	29

±represent value of standard error

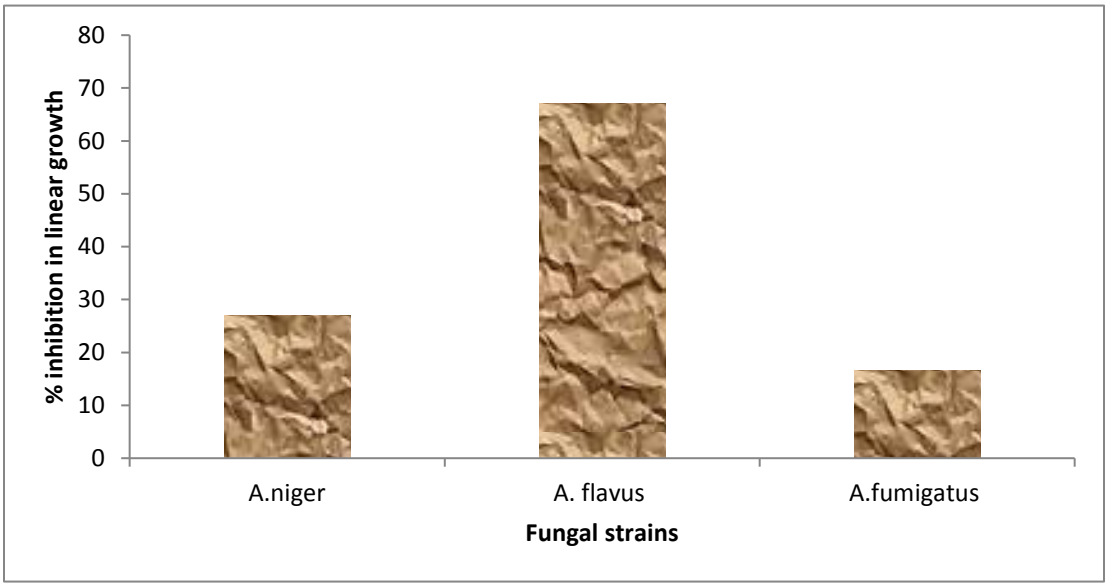

Figure 2. Antifungal activity of Allium cepa crude dye

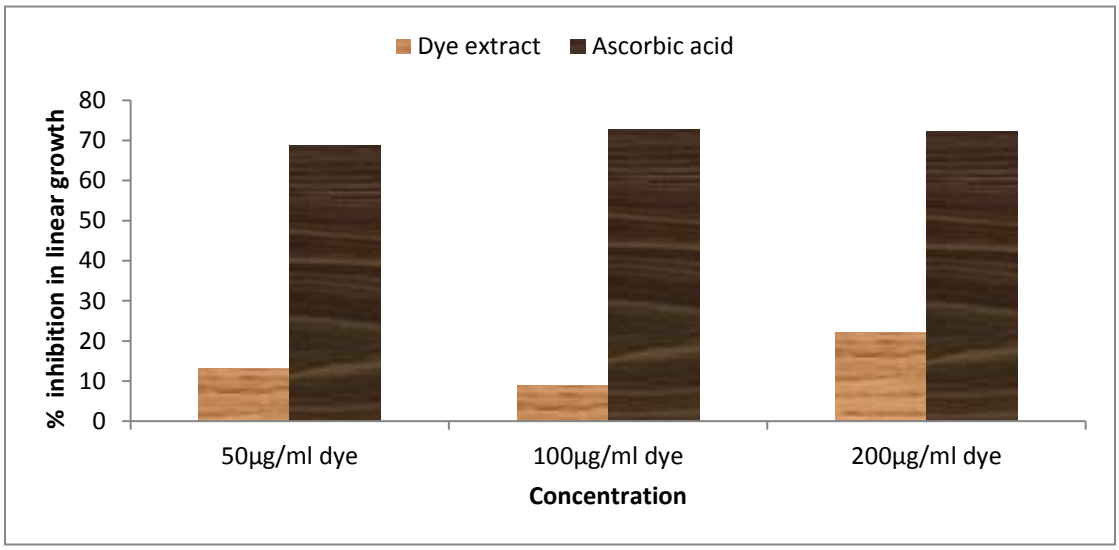

Figure 3. Antioxidant activity (DPPH free radical scavenging activity) of Onion crude dye

Conclusion

It is inferred that *Allium cepa* dye possesses some active ingredients with higher pharmacological importance. Future studies focusing on isolation, purification and identification of biologically active ingredients form *Allium cepa* is highly needed.

Authors' contributions

Conceived and design; F Ullah, Performed experiment: Z Sikandri, Data analyzed; S Mehmood, Materials and Methods; S Kamran & ZU Haq, Reviewed paper; Abdullah.

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