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ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *Crocus sativus* (SAFFRON) AGAINST MULTI- DRUG RESISTANT PATHOGENS

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ABSTRACT

The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains. This present study was designed to investigate the preliminary phytochemical and antibacterial activity of saffron extracts against multi- drug resistant pathogens. The antibacterial activity of these extracts was checked against methicillin resistant *Staphylococcus aureus*, vancomycin resistant *enterococcus and Escherichia coli* by agar well diffusion method. The phytochemical screening of different solvent extracts of saffron revealed the presence of various secondary metabolites glycosides, saponin, steroids, flavonoids, terpanoids and tannins.

KEYWORDS: Saffron, vancomycin resistant *enterococcus*, methicillin resistant *Staphylococcus aureus*, phytochemical, glycosides, *Crocus sativus*.

INTRODUCTION

The wide use of Antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains. The emergence of multiple drug resistant bacteria (MDR) has become a major cause of failure of the treatment of infectious disease.[1] Antibiotic toxicity and multi- drug resistant pathogens are the two greatest challenges being faced by today's medical world. India has been recognized as home of spices since long gap of times. Spices are used as substances that increase the taste and variation of food.[2] According to world health organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs.[3] Spices have a unique aroma and flavor which are derived from compounds known as Phytochemicals or secondary metabolites.[4] The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents.[5] The inhibitory effects of spices are mostly due to the volatile oils present in their composition.[6]

Saffron (*Crocus sativus*) is an important spice belongs to Family *lridaceae*, known for its aroma, colour and medicinal properties and is regarded as the most costly spice in the world.[7] It is widely used plant, especially as a food additive and coloring agent. Medicinal plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Clinical microbiologists have great interest in screening of Medicinal plants for new therapeutics. Phytochemicals such as glycosides, saponin, flavonoids and tannins etc. are not essential nutrients that are required by human body for sustaining life. There are many Phytochemicals and each works differently.[8] Most Phytochemicals have anti-oxidant activity and protect cells against oxidative damage and reduce the risk of developing certain types of cancer Example flavonoids.[9] In the present study we have evaluated the antibacterial activity of different solvent extracts of saffron against three multi- drug resistant pathogens and also report various phytochemicals responsible for this antimicrobial activity.

MATERIALS AND METHODS

Collection and preparation of saffron extract

The fresh saffron was collected from Kashmir. The saffron was shade dried at room temperature and powdered by electric blender. 10 gram of powder were weighed and mixed with 100 ml of five different solvents (methanol, ethanol, acetone, chloroform and distilled water) in conical flasks and kept in rotatory shaker at 150 rpm for 24 hours. After 24 hours the filtrates were evaporated in a hot air oven at 40^{o}C until dry. One gram dried extracts were resuspended in 10ml of Dimethyl Sulphoxide (DMSO). The extracts were stored in sterile bottles at 4^{o}C prior to use.

Test microorganisms
The test microorganisms are used in this study are multi-drug resistant pathogens. They are *Escherichia coli*, methicillin resistant *Staphylococcus aureus and* vancomycin resistant *enterococcus*. The blood, urine and sputum samples were procured from Perundurai Medical College, cultured and identified by conventional methods.

Antibiotic sensitivity testing
The antibiotic sensitivity profile of the *Escherichia coli*, *Staphylococcus aureus and enterococcus* were determined according the method of Kirby- Bauer (Bauer et al., 1966) using 17 antibiotics placed on the surface of MHA medium seeded with the test organism. Antibiotic susceptibility was determined from the size of the inhibition zone.

Antibacterial activity testing using agar well method
The selected strains of bacteria were inoculated into 10 ml of sterile nutrient broth, and incubated at 37^{o}C for 16-18 hours. Using a sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile Mueller-Hinton Agar plates and left to dry for few minutes at room temperature. Agar wells were prepared with the help of sterilized cork borer. Different volume of saffron extracts (50µl, 100µl, 150µl, 200µl) were added to different wells in the plate. The plate was incubated in upright position at 37^{o}C for 24 hours. The diameter of inhibition zone was measured in mm and the results were recorded.

Phytochemical screening
Phytochemical screening was carried out on methanol, ethanol, acetone, chloroform and distilled water extracts of saffron for its chemical composition.[10]

Screening for alkaloids (Mayer's test)
To 2ml of the extract was boiled with dilute hydrochloric acid and the mixture was filtered and to the filtrate a few drops of Mayer's reagent was added. A cream or white colour precipitate produced immediately indicates the presence of alkaloids.

Screening for carbohydrates
To 1ml of extract, 1ml of Benedict's reagent was added. The mixture is heated on a boiling water bath for 2 minutes solution appeared green showing the presence of reducing sugar.

Screening of phenol
To 1ml of the extract 3ml 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.

Screening for glycosides (Keller Kilianin test)
5ml of each extract was added with 2 ml of glacial acetic acid which was followed by the addition of few drops of ferric chloride solution and 1ml of concentrated Sulphuric acid. Formation of brown ring at interface confirms the presence of glycosides.

Screening for terpanoids (Salkowski test)
5ml of extract was taken in a test tube and 2ml of chloroform was added to it followed by the addition of 3ml of concentrated sulphuric acid. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpanoids.

Screening for flavonoids (Alkaline reagent test)
2ml of extracts was treated with few drops of 20% sodium hydroxide solution formation of intense yellow colour, which becomes colorless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Screening for saponins (Foam test)
2ml of extract was taken in a test tube and 6ml of distilled water was added to it. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Screening for proteins
5ml of extract was mixed with 10% NaOH solution and added few drops of copper sulphate to it. The formation of reddish violet colour indicates the presence of proteins.

Screening for steroids
1ml of extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids.

Screening for tannins
2ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicates the presence of tannins.

RESULTS AND DISCUSSION
The present study aimed at testing the antibacterial activity of saffron against Methicillin Resistant *Staphylococcus aureus*, *E.coli*, and Vancomycin Resistant *Enterococcus*. Antimicrobial activity of saffron was studied using different solvents like ethanol, methanol, acetone, chloroform and distilled water. The antimicrobial test results of the saffron extracts shown in Table1 and 2. At the end of the analysis, the saffron extracts were found to have an inhibitory effect against *E.coli*, and Vancomycin Resistant *Enterococcus*. The saffron extracts did not have any significant antibacterial activity against Methicillin Resistant *Staphylococcus aureus*. Ethanol and chloroform saffron extract showed highest (16 mm) antibacterial activity against Vancomycin Resistant *Enterococcus*. Methanol, acetone and distilled water showed moderate (13 mm) antibacterial activity against VRE. Ethanol saffron extract showed highest (19 mm) antibacterial activity against E.coli. Acetone and distilled water showed

moderate (17 mm) antibacterial activity against E.coli. Methanol and chloroform showed least activity against E.coli

The saffron extract were subjected to phytochemical screening for the presence of flavonoids, alkaloids, glycosides, saponin, phenol, steroids, terpanoids, protein, tannins and carbohydrates according to standard procedure as described above. The phytochemical analysis of saffron is presented in Tables 3. The result reveals that some of the phytochemicals analyzed were present in the extracts.On the ten phytochemicals screened glycosides, saponins, steroids, terpanoids and tannins were present in all the saffron extracts.

Spices have been added to foods since ancient times as flavoring agent, also as food preservatives and folk medicines. Basically when spices are used for medicinal purpose, their value is depend on the phytochemicals they possess.[11] The spices, herbs, plant extract and their phytoconstituents have been reported for anti-inflammatory, antidiarrheal, antimicrobial, antioxidant and insecticidal activities.[12]

In the present investigation glycosides are present in all the saffron extracts. The glycosides are useful in lowering blood pressure.[13] They are also used in the treatment of congestive heart failure and cardiac arrhythmia. Steroids are present in all the extracts of saffron. Steroids and Sterols are great importance in pharmacy as they possess compounds like sex hormones and can be used for drug production.[14] Terpanoids are also present in the studied spice. Terpanoids are used in the treatment of cough, asthma and hay fever. Saponins are present in saffron extracts. Traditionally saponins have been extensively used as detergents and pesticides, in addition to their industrial applications as foaming and surface active agents and also beneficial health effects.[15] Saponins protect against hypercholesterolemia and antibiotics properties.[16] Tannins are present in all saffron extracts. The growth of many fungi, yeast, bacteria and viruses was inhibited by tannins.[17] Phenols and tannins acts as antioxidants.[18]

TABLE: 1 ANTIMICROBIAL ACTIVITY OF SAFFRON AGAINST VANCOMYCIN RESISTANT *Enterococcus* [VRE]

VRE ISOLATE	ETHANOL 50 100 150 200	METHANOL 50 100 150 200	ACETONE 50 100 150 200	CHLOROFORM 50 100 150 200	D.W 50 100 150 200
VRE 1	-- -- 13 15	-- -- -- 12	-- -- -- 13	-- 12 13 15	-- 11 12 13
VRE 2	-- 11 13 16	-- -- -- 13	-- -- 11 12	-- 12 14 16	-- 11 12 13
VRE 3	10 12 14 16	-- -- 11 13	-- 11 12 13	-- 11 13 15	-- 10 12 13
VRE 4	-- 11 13 16	-- -- -- 13	-- -- 10 12	-- 12 13 16	-- 10 11 12
VRE 5	-- -- 13 16	-- -- 12 13	-- -- -- 12	-- 11 13 16	-- 10 11 13
VRE 6	10 12 14 16	-- -- 12 13	-- -- 10 12	-- 11 13 15	-- 10 11 13
VRE 7	-- 11 13 16	-- -- -- 13	-- -- 10 12	-- 12 13 16	-- 10 11 12

TABLE: 2 ANTIMICROBIAL ACTIVITY OF SAFFRON AGAINST E.coli

E.coli ISOLATE	ETHANOL 50 100 150 200	METHANOL 50 100 150 200	ACETONE 50 100 150 200	CHLOROFORM 50 100 150 200	D.W 50 100 150 200
E.coli 1	-- 14 15 16	-- -- -- 12	-- 11 13 15	-- -- -- --	-- -- 13 16
E.coli 2	-- 15 17 19	-- -- 12 14	-- 12 14 17	-- -- -- 13	-- 11 13 15
E.coli 3	-- 13 15 17	-- -- 11 12	-- 11 13 15	-- -- -- 12	-- 11 12 14
E.coli 4	-- 14 16 18	-- -- -- 13	-- 10 12 13	-- -- -- --	-- -- 12 14
E.coli 5	-- 15 16 18	-- -- 12 14	-- 11 13 15	-- -- -- 13	-- 12 13 15
E.coli 6	12 15 17 19	-- -- 12 13	-- 12 13 16	-- -- -- 12	-- 10 12 14
E.coli 7	-- 15 17 19	-- -- 12 14	-- 12 14 17	-- -- -- 13	-- 11 13 15
E.coli 8	-- 14 16 18	-- -- 11 13	-- 11 13 16	-- -- -- --	-- 11 13 15
E.coli 9	12 15 16 18	-- -- 12 13	-- 12 14 16	-- -- -- 13	-- 12 13 15
E.coli 10	-- 15 17 19	-- -- 12 14	-- 11 13 15	-- -- -- 12	-- -- 12 14

TABLE: 3 PHYTOCHEMICAL ANALYSIS OF SAFFRON EXTRACTS

NO	PHYTOCHEMICAL TEST	METHANOL	ETHANOL	ACETONE	CHLOROFORM	DISTILLED WATER
1	ALKALOIDS	_	_	_	_	_
2	GLYCOSIDES	+	+	+	+	+
3	SAPONNIN	+	+	+	+	+
4	PHENOL	_	_	_	_	_
5	STEROIDS	+	+	+	+	+
6	FLAVANOIDS	+	+	+	+	+
7	TERPANOIDS	+	+	+	+	+

8	PROTEIN	_	_	_	_	_
9	TANNINS	+	+	+	+	+
10	CARBOHYDRATES	_	_	_	_	_

'+' = present '-' = absent

CONCLUSION

In conclusion saffron was found to have important antimicrobial activity against to the test strains. This study opens up the possibility for the search of new antimicrobials as an alternative to the antibiotics. The result of this study would lead to find out some compounds which are very useful for the manufacturing of new drugs. The previous phytochemical analysis and present studies show nearly the similar results due to the presence of phytochemical constituents.

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