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Antibacterial Activity and Phytochemical Analysis of a mixture of sweet green pepper and molasses dates

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Abstract Streptococci strains of the anginosus group isolated from various oral and maxillofacial infections (OMF) were screened for their susceptibility to the following antibiotic: Amoxicillin, Ampiclox and Tetracycline. In conclusion is the drug of choice in infections caused by streptococci of the anginosus group.

Keywords Antibacterial Activity, Phytochemical Analysis, sweet green pepper, molasses dates

Introduction

The world is rich with natural and unique medicinal plants. Medicinal plants are now getting more attention than ever because they have potential of myriad benefits to society or indeed to all mankind, especially in the line of medicine and pharmacological field. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body [1]. The medicinal plants are those that provide people with medicines to treat illness, maintain and promote health [2-3]. The phytochemicals are the wide variety of compounds produced by plants manipulated wisely in the pharmacognostic drug development and treatment of the major ailments [3]. Chemicals present universally in all the plants can be classified as primary and secondary metabolites. Primary metabolites include proteins, amino acids, sugars, purines and pyrimidines of nucleic acids, chlorophylls etc., while secondary phytochemicals as alkaloids to terpenoids and acetogenins to different phenols [2, 4]. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [5]. Three species compose the *Streptococcus milleri* group (SMG): *Streptococcus intermedius*, *Streptococcus constellatus*, and *Streptococcus anginosus*. Investigators who have used phenotypically differentiated strains within the *Streptococcus milleri* group have suggested that these three species have similar antibiotic susceptibilities [6]. *Streptococcus constellatus* has two subspecies, *Streptococcus constellatus* subspecies constellatus and Streptococcus constellatus subspecies pharyngis [7]. Identification of the anginosus group is complicated by wide phenotypic and antigenic diversity, even within 1 species. Although most anginosus group isolates belong to the non-β-hemolytic oral streptococci, β-hemolytic strains are found in all 3 species. Some anginosus group strains carry a typeable Lancefield group antigen, which belongs to group F, C, G, or A [8].

The SMG are an important part of normal microbial flora in humans and animals. They are indigenous to the upper respiratory tract, the female genital tract and all regions of the gastrointestinal tract. They are most prevalent in the oral cavity. This is especially true of *S. anginosus*, which is present in subgingival plaque [4, 9]. SMG organisms

occasionally produce abdominal infections and are frequently found in abscesses in these and other sites. Pathogenicity in these cases has been demonstrated by the recovery of the species in pure culture, or in association with other anaerobic genera. Concurrent bacteraemia is a frequent feature [7].

In the literature, the designation *Streptococcus milleri* has often been used for streptococci of this group, although it has never been an officially approved name. Streptococci of the anginosus group can reside commensally in the human oral cavity but have a certain propensity to cause pharyngitis, bacteremia, and serious purulent infections in the deep neck and soft tissue and in internal organs such as the brain, lung, and liver [10–12]. The bacteria cause severe infections after surgical treatments and infect implanted material, thereby posing a problem of substantial clinical relevance [13-14]. The species diversity within the GCGS highlights the limits of Lancefield grouping by agglutination assays, the typically applied method in the diagnosis of streptococcal infections. The genetically distinct GCGS species differ in pathogenesis, virulence mechanisms, and antimicrobial drug susceptibility. Thus, finding the optimal treatment regimen can be facilitated by species determination. Diagnosis of anginosus group infections is particularly difficult [8].

Pepper belongs to the genus *Capsicum*, which is comprised of more than 200 varieties, with *Capsicum annuum*, *Capsicum baccatum*, *Capsicum chinense*, *Capsicum frutescens*, and *Capsicum pubescens* being the main five species [15-16]. Peppers are consumed worldwide and their importance has increased gradually to place them among the most consumed spice crops in the world not only because of its economic value, but also for the combination of color, taste and nutritional values of its fruit. They are usually consumed as food and used as additives in the food industry. They also have a significant role in traditional medicine [17]. In fact, in Indian, Native American, and Chinese traditional medicine, Capsicum species have been used for the treatment of arthritis, rheumatism, stomach aches, skin rashes, dog/snake bites, and flesh wounds. These therapeutic applications are related to the capsaicinoid, phenolic compound, and carotenoid content of peppers [18].

Methods and Materials

Plant Extraction

Collection and preparation of plant sample

Bell peppers or green sweet pepper (*Capsicum annuum*) was obtained from market in Erbil city, then was washed with tap water, then with distill water, then left for air drying until become completely drying, after drying was converted into powder form and stored in polyethylene sack in refrigerator at 4 °C for further process. While the Molasses dates was obtained from market in Erbil city.

Extracts preparation

150 ml of sterilized distilled water was added to 15 g of ground dried plant, heated below the boiling point and stirred for 2½ - 3 h. The extract was filtered by muslin cloth, then by filter paper (Whatman No. 1) then stored in the refrigerator at 5 ◦C for using [2, 8].

Qualitative analysis

Alkaloids

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent [19].

Preparation of Maeyer's reagent: 0.355 g of mercuric chloride was dissolved in 60 ml of distilled water. 5.0 g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water [2].

To 2 ml test solution, 8 ml of 1 % HCl was added, warmed then treated with 2ml of Mayer's reagent. Formation of white turbidity or precipitate indicates the presence of alkaloids [2].

Steroids

2 ml of test solution was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids [2].

Flavonoids

2 ml of the test solution was mixed with 4 ml of 1 % aluminium chloride in methanol in a test tube and the colour was observed. Formation of yellow colour indicated the presence of flavonols, flavones and chalcones [2, 20].

Tannins and phenols

Crude extract was mixed with 2 ml of 2 % solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins [21].

Phlobatannins

The extract (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2 % HCl solution. Red precipitate shows the presence of phlobatanins [22].

Coumerin

To the test sample 10% of sodium hydroxide and chloroform were added. Formation of yellow colour indicates the presence of coumerin [23].

Triterpenoids

Noller's test: The substance was warmed with Tin and Thionyl chloride. Purple coloration indicates the presence of Triterpeniods [23, 24]

Quinones

To the test substance, sodium hydroxide was added. Blue green or red colour indicates the presence of quinine [23].

Anthraquinones

About 0.5 g of the extracts was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heat. Formation of rose-pink colour, indicates the presence of authraquinones [19].

The bacteria employed in the study

Streptococcus anginosus was isolated from patient in Rezgary Teaching hospital in Erbil city-Iraq. The isolate was cultured on nutrient agar to obtain single colonies, and then identified according to the cultural, morphological and some biochemical tests (Vitek system 2). The isolate was maintained and preserved on nutrient agar slants. For every experiment, freshly prepared sterile nutrient broth (10ml) was inoculated from the slants and incubated at 37 ^oC for 24 hours.

Preparation of inoculums

Two to three colonies from pure growth of tested organism were transferred to (5) ml of nutrient broth, and then incubated overnight at 37 ^oC. The suspension was diluted with sterile distilled water to obtain approximately $1*10^6$ CFU/ ml (9).

Well diffusion technique

Screening of antibacterial activity of plant extracts (Pepper, Molasses dates and Molasses dates/Pepper 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10) V/V at 50℃, and Molasses dates/Pepper (at concentration was made the biggest zone of inhibition), at 60℃, 70℃, 80℃ and 90℃, in addition to control represented with antibiotics (Amoxicillin,

Ampiclox and Tetracycline) then was performed by well diffusion technique (10). The Nutrient agar (NA) plates were seeded with (0.1) ml of the inoculums of tested organism. The inoculums were spread evenly over plate with loop. A standard cork borer of (8) mm diameter was used to cut uniform wells on the surface of the NA and (100) µl of each concentration of plant extracts was introduced in the well, the plates were incubated for 24 hours at 37 ºC, and the zones of inhibition was measured to the nearest millimeter (mm).

Determination of minimal inhibitory concentration (MIC)

Agar dilution method with slight modifications was used to determine the MIC of plant extract. Equal volumes of bacterial strain culture containing approximately 10^6CFU/ml, were applied onto nutrient broth tubes supplemented with correlated plant extract at concentration 1:5 V/V (Molasses dates/Pepper) with concentrations ranged from 1% (v/v) to 15% (v/v) in tubes. Cultures were then incubated at 37 °C for 24 h, subsequently, 100 ul of each culture was inoculated on nutrient agar and further incubated at 37 °C for 24 h. MIC was defined as the lowest concentration of plant extract that inhibiting the visible growth of organism on the agar plate. The presence of one or two colonies was disregarded. All experiments were applied in triplicates (Ahmed A. Tayel*, Wael F. El-Tras, 2009).
All experiments were applied in triplicates.

Phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out in extracts as well as powder specimens.

Table 1: Qualitative analysis of different types of Pepper.

Class of compounds	S.G	S.R	S.O	S.Y	H.G	H.R
Alkaloids	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Flavonoids	-	-	-	-	-	-
Phlobatannins	-	+	+	+	+	+
Tannins	-	-	-	-	+	-
Phenols	+	+	+	-	-	-
Coumerin	-	-	-	-	-	-
Triterpenoids	-	-	-	-	-	-
Quinones	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-

+: Present
-:Apsent

Table 2: Biochemical Details of *Streptococcus anginosus* by Vitek 2

Biochemical Details by Vitek 2																		
2	AMY	(-)	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	+	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	+	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	-	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	-
47	NOVO	+	50	NC6.5	-	52	dMAN	-	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

Table 3: Antibacterial activity of plant extracts against *S. anginosus*.

Concentration V/V	**Zone of inhibition/ mm**					
(Molasses dates/ Pepper)	**S.G**	**S.R**	**S.O**	**S.Y**	**H.G**	**H.R**
1:1	8.5	11	-	-	23	27
1:2	18	13	-	9	23	27
1:3	25	16	-	23	23	27
1:4	27	21	-	21	19	32
1:5	27.5	25	-	24	25	27
1:6	19	-	-	-	25	9
1:7	20	13	16	-	25	11
1:8	22	15	18	9	26	21
1:9	20	9	18	9	22	10
1:10	20	-	-	12	27	22
Pepper	27	12	-	15	27	11
Molasses dates	10					
Tetracycline	-					
Amoxicillin	29					
Ampiclox	29					

⁎:Values calculated as mean of triplicates. -: No inhibition zone or less than 8 mm.
S.G: sweet green pepper, S.R.: sweet red pepper, S.O.: sweet orange pepper, S.Y.: sweet yellow pepper, H.G.: hot green pepper, H.R.: hot red pepper.

Table 4: MIC of plant extracts mixture V/V (Molasses dates/ Pepper (1:5)) against *S. anginosus*.

Concentration V/V	**S.G**	**S.R**	**S.O**	**S.Y**	**H.G**	**H.R**
1%	R	R	R	R	R	R
2%	R	R	R	R	R	S
3%	S	S	S	S	S	S
4%	S	S	S	S	S	S
5%	S	S	S	S	S	S
6%	S	S	S	S	S	S
7%	S	S	S	S	S	S
8%	S	S	S	S	S	S
9%	S	S	S	S	S	S
10%	S	S	S	S	S	S
11%	S	S	S	S	S	S
12%	S	S	S	S	S	S
13%	S	S	S	S	S	S
14%	S	S	S	S	S	S
15%	S	S	S	S	S	S

R: Resistant
S: Sensitive

Table 5: Antibacterial activity of plant extracts mixture V/V (Molasses dates/ Sweet Pepper (1:5)) against *S. anginosus* under different degrees of temperature

Temperature (°C)	Zone of inhibition/ mm
50	27.5
60	15
70	15
80	19
90	19

⁎: Values calculated as mean of triplicates.

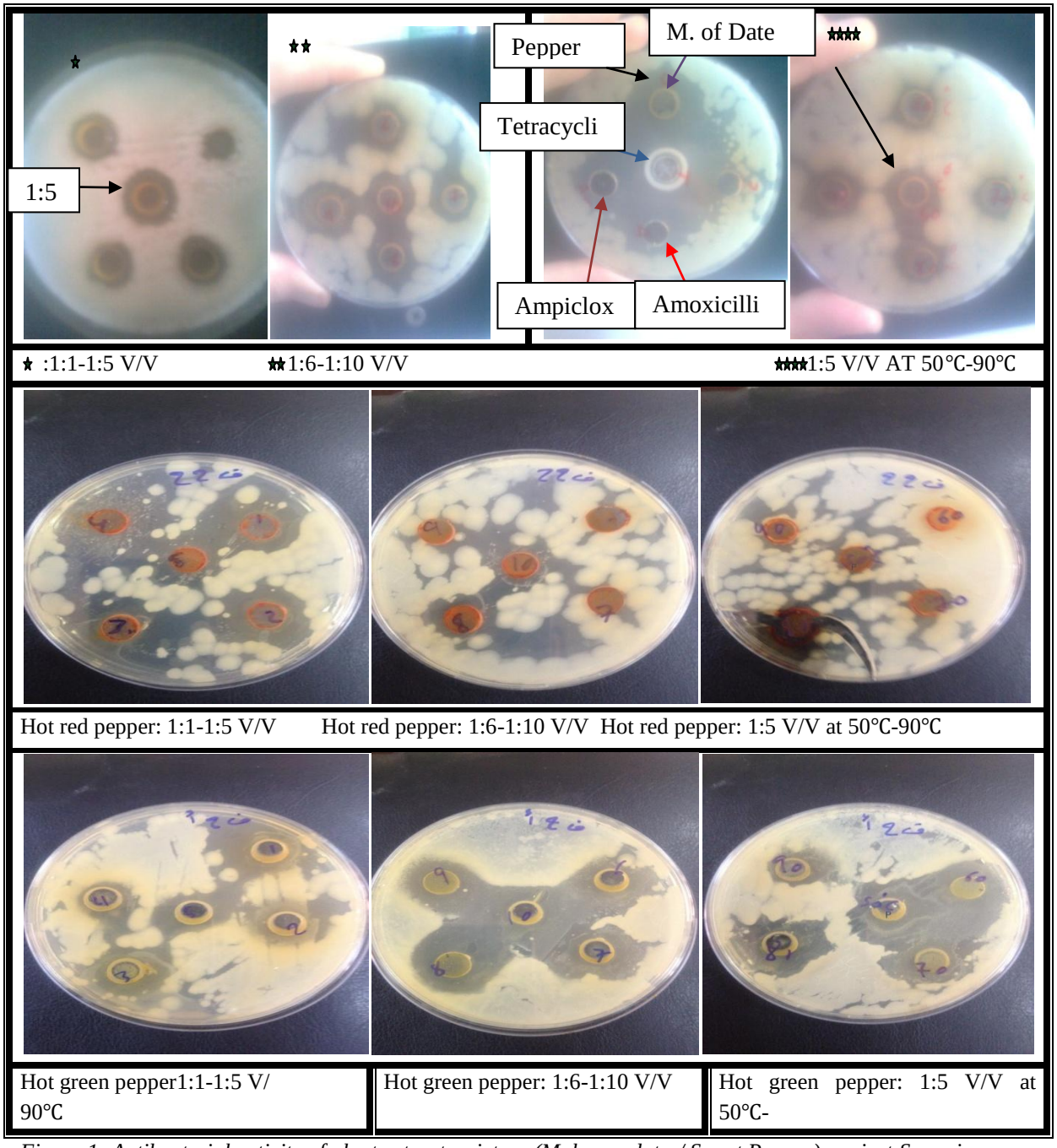
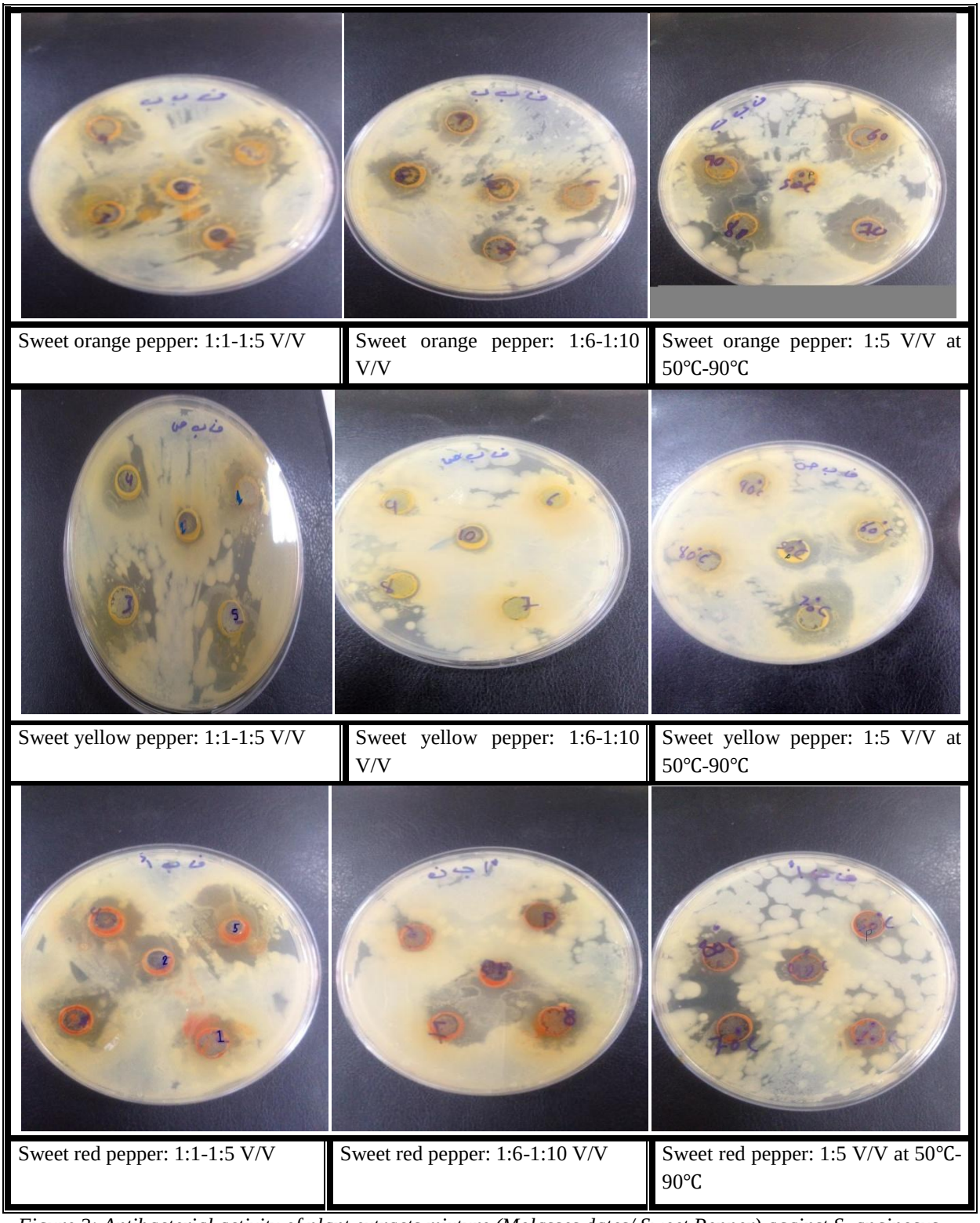


Figure 1: Antibacterial activity of plant extracts mixture (Molasses dates/ Sweet Pepper) against S. anginosus



Sweet orange pepper: 1:1-1:5 V/V	Sweet orange pepper: 1:6-1:10 V/V	Sweet orange pepper: 1:5 V/V at 50℃-90℃
Sweet yellow pepper: 1:1-1:5 V/V	Sweet yellow pepper: 1:6-1:10 V/V	Sweet yellow pepper: 1:5 V/V at 50℃-90℃
Sweet red pepper: 1:1-1:5 V/V	Sweet red pepper: 1:6-1:10 V/V	Sweet red pepper: 1:5 V/V at 50℃-90℃

Figure 2: Antibacterial activity of plant extracts mixture (Molasses dates/ Sweet Pepper) against S. anginosus

Conclusion

Streptococci of the anginosus group cause severe infections in humans. Examination of clinical isolates from patients with purulent infections of the upper respiratory tract, the urinary tract, and invasive infections showed that SDSE is dominant in GCGS infections in Vellore, accounting for 81% of the cases. SDSE was responsible for all 7 cases of invasive infections included in this study. *S. anginosus* that possess group G antigens accounted for the remaining 19% of the suppurative infections, which indicates a considerable epidemiologic role for this species in the Vellore region.

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms *in vitro* [21].

Phytochemicals are naturally occurring and non–nutritive chemical compounds in plants having protective or disease preventive properties. They are found in fruits, vegetables, beans, grains, and other plants. Scientists have identified thousands of phytochemicals, although only a small fraction has been studied closely. Some of the more commonly known phytochemicals include beta carotene, ascorbic acid (vitamin C), folic acid and vitamin E [24].

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