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Antimicrobial activity of Yemeni myrrh mouthwash

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ABSTRACT

This study aimed to prepare pharmaceutical formulations of mouthwashes, containing extracted Yemeni myrrh as a single active constituent, and testing their quality criteria and antimicrobial activity on common pathogens of the oral cavity. In order to determine the best extracting solvent for myrrh antimicrobial constituents, different solvents were used to prepare myrrh extract. The antimicrobial activity of those extracts against Staphylococcus aureus was then investigated. The extract that showed the best activity was also investigated against Streptococcus mutans and Candida albicans. It was found that the hydroalcohol extract extracted by ethanol: phosphate buffer pH 7 (85:15) showed the best antimicrobial activity. Thus, this extract was used, thereafter, to prepare 10 pharmaceutical formulations of myrrh tinctures. Each 100-ml tincture formulation was prepared from 65 ml of myrrh hydroalcohol extract (equivalent to 2.6 g extracted constituents of myrrh). All formulations contained a solubilizing agent, antioxidant, sweetener, flavor and colorant. In order to prepare corresponding aqueous myrrh mouthwash formulation form each tincture, 1ml of the tincture was diluted up to 50 ml with water. The viscosity, pH and palatability of those mouthwash formulations were tested. It was found that two formulations (M9, M10) prepared from the myrrh tinctures (F9 and F10, which contained 9.5 and 10.5 % w/v of sodium lauryl sulphate, respectively, showed accepted results. However, formulation M9 showed better antimicrobial activity than the other formulation. The antimicrobial activity of formulation M9 was also superior to those of two commercial mouthwashes and one oral antifungal suspension. Moreover, the formulation exhibited good isothermal short-term stability when stored at three different conditions for 9 weeks.

Keywords: Yemeni, Myrrh, mouthwash, antimicrobial, sodium lauryl sulphate.

INTRODUCTION

Myrrh is an oleo-gum-resin obtained from the stem of different species of *Commiphora* tree [1]. The schizogenous cavities of the stem and branches of the tree produce myrrh [2]. The genus *Commiphora* genus (family: Burseraceae) has over 150 species distributed around the red Sea in east Africa, and with few species also growing in Arabia and India; twelve of which are wild-growing in Yemen. *Commiphora* species are used widely in the Yemeni traditional medicine [3]. Investigations have revealed that myrrh contains about 2 to 8% essential oil (myrrhol), 23 to 40% resin (myrrhin), 40 to 60% gum, and 10 to 25% bitter principles. Regarding the essential oil of myrrh, it was reported that furanosesquiterpenoids were rich in the exudates, and around 20 different compounds of this type have been isolated and identified [4, 5]. Several pharmacological effects

of *Commiphora* genus such as anti-inflammatory, antibacterial, antioxidant, hepatoprotective, antimalarial, anticandidal, antimycobacterial and antischistosomal activities were reported [3]**.** The antimicrobial effects in myrrh is due a mixture of furanosesquiterpenoids mainly furanodiene-6-one & methoxyfuranoguaia-9-ene-8-one [1]. Myrrh is used in perfumery and is an ingredient of toothpastes, mouthwashes and dentifrices [2].

Extracts are concentrated preparations of vegetable or animal drugs obtained by removal of the active constituents of the respective drugs with suitable menstrua, evaporation of all or nearly all of the solvent, and adjustment of the residual masses or powders to the prescribed standards. On the other hand, tinctures are alcoholic or hydroalcoholic solutions prepared from vegetable materials or from chemical substances. Depending on the preparation, tinctures contain alcohol in amounts ranging from approximately 15% to 80%. The alcohol content protects against microbial growth and keeps the alcohol-soluble extractives in solution [6].

A mouthwash is defined as a non-sterile aqueous solution used mostly for its deodorant, refreshing or antiseptic effect and also these rinses are designed to reduce oral bacteria, remove food particles, temporarily reduce bad breath and provide a pleasant taste. Many different mouthwashes are commercially available and patients and health professionals struggle to select the most appropriate product for a particular need [7]. The active ingredients in mouthwashes may be an antibacterial (to reduce the bacterial flora around the lesion), antihistamine (for local anesthetic effect), antifungal (to stop any fungal growth), a steroid (to reduce inflammation), a local anesthetic/pain reliever, or a combination of those ingredients [8]. Among antimicrobials, chlorhexidine gluconate is currently the most effective one for reducing plaque and gingivitis [7]. Solutes other than the medicinal agent are usually present in orally administered solutions. These additional agents are frequently included to provide color, flavor, sweetness, or stability [6].

Many myrrh-containing herbal products, for topical use on the oral cavity, are available in the global market. These products are either in the form of myrrh oil (to be diluted with alcohol) or as tinctures containing, in addition to myrrh extract, other herbal or chemical active constituents. Most of these products reveals no information on their country of origin of myrrh or data of their the antibacterial activity, palatability, physicochemical properties and stability. Therefore, this study was undertaken to prepare pharmaceutical mouthwash formulations of Yemeni myrrh, as a single active constituent, with appropriate quality criteria and tested antimicrobial activity on pathogens commonly infecting the oral cavity.

EXPERIMENTAL SECTION

2.1. Materials

2.1.1. Apparatuses

Rotary evaporator (R-210 V-700 V-850, Buchi, Switzerland), mechanical stirrer (X230D-Labtech, UK), pH-meter (3510, Jenway , UK) , incubator (D-6450 , Heraeus, Germany), electric thermostatic oven (DHG, Extra, China), autoclave (386-A, Asahi, Japan), Silica gel plates for thin layer chromatography (F_{254}, Merck, UK) , Ostwald- U tube viscometer and UV lamp (UVL-14, UVP, Canada)

2.1.2. Materials and Reagents

Yemeni myrrh *(Commiphora myrrha*) was collected from Hadramout area ,Yemen. Mueller- Hinton agar , Sabouraud dextrose agar (Remale, India), Blood agar (Conda, Spain); Sodium lauryl sulfate, saccharine sodium, sorbitol, anhydrous disodium hydrogen phosphate, sodium dihydrogen phosphate monohydrate, phosphoric acid, sodium carbonate (Himedia, India); Glycerin, ethanol, n-hexane , ether and chloroform (Scharlab, Spain) Natural dyes and mint flavor (Saudi factory for food colors, Saudi Arabia); Vitamin E (Riedel, Australia); Specimens of *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans* were isolated in the medical laboratory of Al-Aqsa Hospital, Hodiedah, Yemen.

2.2. Methods

2.2.1. Identification of myrrh

The identity of Yemeni myrrh was investigated morphologically and chemically as described in the USP-2007 [9]. Myrrh was also identified chromatographically as described in the literature [10]. The chemical identification involved reactions of dried etheric extract of myrrh with nitric acid as follows**:** 0.4 g of crushed Myrrh was triturated with 1 g of washed sand, shaken for a few minutes with 10 mL of ether, and filtered. the filtrate was evaporated to dryness in a porcelain dish. Few drops of nitric acid was added to the residue. Thin layer chromatography was carried out on silica gel using a mixture of toluene and ethyl acetate (93:7) as mobile phase. The test sample was prepared as follow: 0.5 g of finely powdered myrrh was transferred to a 10-mL centrifuge tube. 2 ml of ethanol was added and shaken for 1 minute, centrifuged then

filtered. Detection was carried out by UV 365 nm lamp. Values of R_f of bands produced were compared to those published in the literature [10].

2.2.2 Preparation and evaluation of Myrrh Extracts

(i) Preparation of extracts

Yemeni myrrh was first grounded and sieved to yield a coarse powder of particle size of 300 µg. Extraction of the antimicrobial constituents from the powder was carried out by maceration method as described in the USP 2007 for preparation of myrrh topical solution [9]. In order to obtain myrrh extract with high proportion of antimicrobial constituents, the process was carried out several times using a different solvent at each time. Solvents tried separately to extract myrrh constituents were water, n-hexane, ethyl acetate, ether, ethanol and a mixture of ethanol: phosphate buffer pH 7 (85:15) . The hydroalcoholic cosolvent is recommended by the USP 2007 [6]. However, in our study water was substituted with Phosphate buffer pH 7.0 (0.063M) for the purpose of pH compatibility with that of saliva pH 6.35-6.85 [11]. The maceration process was conducted as follows: two hundred grams of myrrh powder was macerated with 900 ml of the solvent in a close container fitted with a lid of mechanical stirrer (200 rpm) for 48 hours. After maceration, the mixture was filtered and the volume was completed to 1000 ml with the same solvent.

(ii) Determination of yield %

In order to determine the yield % (the percentage of myrrh constituents extracted by the solvent), 20-ml sample of each extract was tested as follows: the sample was concentrated by a rotary evaporator at 30^oC under reduced pressure and the mass obtained was left overnight on air to completely dry. The weight of dried residue (extracted constituents of myrrh) was then determined.

Yield (Y %) was determined as follows:

$$Y\% = (A_E/V_E) \times (V_I/A_I) \times 100$$

Where (A_I) is the amount of myrrh (g) introduced into maceration tank, (V_I) is the of volume of the prepared extract, (A_E) is the amount of dried residue (extracted constituents of myrrh) in a sample volume (V_E) of the extract.

(iii) Antimicrobial activity of Myrrh extract

Although the yield % was an important property, the antimicrobial activity of the extracts was the decisive property to evaluate those extracts. The activity was tested, using the disk diffusion method, on *Staphylococcus aureus*, *Staphylococcus aureus* and *Candida albicans* which are common pathogens in the oral cavity [12,13]. At first the activity was investigated against *Staph. aureus*. Then, the extract that showed the best activity was also investigated against *Strep. mutans* and *C. albicans.* Mueller-Hinton agar, blood agar and Sabouraud dextrose agar were used as culture media for the three pathogens, respectively. Circular pieces of sterile Whatman filter papers No.1 with diameter of 7 mm were prepared and used as disks. A volume of the tested extract (equivalent to 0.2 g of extracted constituents of myrrh) was diluted up to 100 ml of same solvent to produce a solution of a concentration of 2000 µg/ml of extracted myrrh. Then, 1 ml of the resultant solution was further diluted to 10 ml with the same solvent to produce a solution of a concentration equals to the MIC of extracted constituents of myrrh (200 µg/ml) [14]. 100 µl of that solution was used to saturate the disk The discs were applied into the culture medium and incubated for 24 hours at 25^oC. Pure solvents were used as blanks and were subjected to the same procedures applied to the tested extracts. The inhibition zones, produced after incubation, were observed and measured in mm . For the purpose of verification, five myrrh extracts of concentrations of lower than (200 µg/ml) of extracted myrrh were prepared as described earlier. The concentrations ranged from 25- 150 µg/ml. The antimicrobial activity of these extracts against *Staph. aureus* were tested and the lower concentration that produce appropriate activity was determined as the MIC of extracted myrrh.

2.2.3. Preparation of myrrh mouthwashes

The hydroalcoholic myrrh extract, which exhibited the best antimicrobial activity as shown later in results, was used to prepare 10 pharmaceutical myrrh tincture formulations (Table 1). Each 100 ml of the tincture was prepared from 65 ml of myrrh extract. Sodium lauryl sulphate was included in the formulation as a solubilizing agent at concentrations ranging from 1.5 – 10.5 % w/v. Sodium carbonate was used to enhance the effect of sodium lauryl sulphate. Other excipients included were vitamin E (α-tocopherol) as antioxidant, flavor (mint), natural green dye as colorant, saccharine sodium as sweetener and sorbitol to overcome the metallic taste produced by saccharine sodium.

Sodium lauryl sulphate was dissolved in a part of the myrrh extract and the other ingredients were added gradually with the aid of a mechanical stirrer 500 rpm for 30 minutes. The mixture was filtered and the filtrate volume was made up to 100 ml by ethanol: phosphate buffer pH 7. No preservative was necessary to be added due to the high content of ethanol in the formulations (> 15 %) [6]. To prepare the corresponding myrrh mouthwash, 1 ml of the tincture was diluted up to 50 ml with water. This dilution ratio was based on the dose of myrrh mouthwash reported by ESCOP as 5 ml of the tincture is to be diluted with a glass (240 ml) of water and used several times a day [15].

Table 1. Amount of Ingredients used to prepare 100 ml of different myrrh tincture formulations

Ingredient	Formulation									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Sodium lauryl sulphate (g)	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5
Saccharine sodium(g)	3.2	3.4	3.6	3.8	4	4.2	4.4	4.6	4.8	5
Sorbitol(g)	3.2	3.4	3.6	3.8	4	4.2	4.4	4.6	4.8	5
Flavor Mint(ml)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Natural dye (green)(g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium carbonate(g)	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Vitamin E (α-tocopherol)(ml)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Myrrh extract*(ml)	65	65	65	65	65	65	65	65	65	65

** Prepared by maceration using ethanol: Phosphate buffer pH 7 (85:15)*

2.2.4. Evaluation of myrrh mouthwashes

(i) pH and viscosity

pH of mouthwashes was measured using pH mater. Viscosity of mouthwashes was measured at 25 ^{o} C using Ostwald- U tube viscometer and the results was compared to that of an equal volume of distilled water as a reference [16].

(ii) Palatability

Palatability is the property of being acceptable to the mouth. The mouthwashes were tested separately for that criteria by three research members in a blind-style. The test was done on scale of 5 levels : 5 = really good; 4 = good; 3 = not sure; 2 = bad; and 1 = really bad [17].

(iii) Antimicrobial activity of myrrh mouthwash

The antimicrobial activity of the myrrh mouthwashes, prepared form the selected myrrh tinctures (F9 and F10), was investigated against against *Staph. aureus, Strep. mutans* and *C. albicans*, using the same procedure applied to myrrh extract. Thereby, the tested myrrh mouthwash solution represented 200 µg of extracted constituents of myrrh per ml of water. The results were compared to those of blank solutions (aqueous solutions prepared by dilution of 1 ml of blank tincture containing all ingredients except the active one, the myrrh extract, up to 50 with water) and also to those of two commercial mouthwash brands, including a brand of 0.1 % chlorhexidine gluconate and a brand of 0.15% benzydamine HCl, and to that of a brand of oral antifungal suspension of 1000000 U/30ml (1g/30 ml) of nystatin. The two mouthwash brands were tested against the two previously mentioned bacteria while the antifungal suspension was tested against *C. albicans*. Test samples of each brand were prepared by dilution of a quantity of the product with water so as to contain the MIC of the active ingredients in the three brands of 20 µg/ml [18], 50 µg/ml [19] and 0.25 µg/ ml [20], respectively.

(iv) Isothermal stress stability

The selected myrrh tincture (F 9 and F10) were stored in 3 storage condition 8 °C , 35 °C and 70 °C . 3 (60-ml) samples of each tincture were stored in tightly closed amber glass bottles at each of those conditions. Periodical evaluation of those tinctures were carried out at 1 , 2 , 4 and 9 weeks intervals. 1 ml of the stored tincture was diluted up to 50 ml with water to prepare a corresponding mouthwash. The prepared mouthwash was then evaluated in terms of its antimicrobial activity against *Streptococcus mutans* , physical change such as turbidity, sedimentation and color change as well as in terms of its pH and palatability.

RESULTS AND DISCUSSION

3.1. Identification of Myrrh

Morphology of Yemeni myrrh complied with that described in the USP 2007 [9]. Chemical identification by reaction with nitric acid was positive with purplish violet color produced instantly [9]. The TLC of myrrh at UV 365 nm was in agreement to that reported in the literature [10] with light- violet zones of furanosesquiterpenoids appeared at R_f 0.2, 0.6 and 0.7

3.2. Evaluation of Myrrh extract

As demonstrated in Table 2, the non-polar solvents (ether, ethyl acetate and n-hexane) had greater yield % of extracted constituents of myrrh than those produced by the polar solvents (water, ethanol and 85:15 mixture of ethanol to phosphate buffer pH 7). In the contrary, as shown in Fig. 1 and Table 2, the extracts of polar hydroalcoholic cosolvent showed a greater antimicrobial activity than those of non-polar extract . This cosolvent extract also showed greater activity than those of a single polar solvent . These findings revealed that the antimicrobial constituent extracted, by either a single non-polar or non-polar solvent, are fewer or of low proportion than those extracted by the hydroalcoholic cosolvent.

Table 2. Yield % and antimicrobial activity against *Staph. aureus* of myrrh extracts

Extracting solvent	Yield % Mean ± SD ; (CV%)	Inhibition zone (mm) Mean ± SD ; (CV%)
Ethyl acetate	65.7 ± 4.32 ; (6.575 %)	9.200 ± 0.100 ; (1.087%)
n-hexane	68.4 ± 7.25 ; (10.599 %)	15.467±0.306 ; (1.957%)
Ether	49.5 ± 2.43 ; (4.909 %)	16.333 ± 0.252 ; (1.541%)
Water	6.2 ± 0.52 ; (8.387 %)	7.433 ± 0.135 ; (1.816 %)
Ethanol	16 ± 1.4 ; (8.711 %)	19.533 ± 0.153 ; (0.782%)
Ethanol : phosphate buffer pH 7 (85: 15)	20.1 ± 1.45 ; (7.213 %)	22.367 ± 0.306 ; (1.366%)

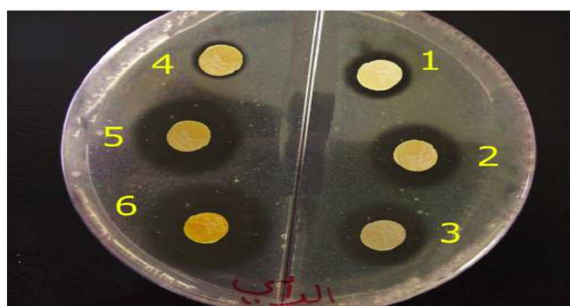

Fig. 1 Disk diffusion test of antimicrobial activity of myrrh extracted by different solvent on *Staphylococcus aureus*; (1): ethyl acetate, (2):n-hexane, (3): ether , (4): water , (5): ethanol , (6): 85:15 mixture of ethanol to phosphate buffer pH 7

The mean ± SD of amount of extracted constituents of myrrh (g) in the 20-ml tested hydroalcoholic extract samples was 0.804 g ± 0.003 . Hence, the mean concentration of extracted myrrh constituent (per 100 ml of hydroalcoholic myrrh extract) was calculated to be 4.02 %. The mean Yield % of extracted myrrh from that extract was 20.1 % (Table 2).

Since the hydroalcoholic myrrh extract showed the best activity against *Staph. aureus,* it was further investigated against Streptococcus mutans and Candida albicans. The mean ± SD ; (CV%) of inhibition zones (mm) observed in cultures of the two pathogens were 32.457 ± 0.403 ; (1.241 %) and 26.511 ± 1.23 ; (4.639 %), respectively.

Concentrations lower than 200 µg/ml of the hydroalcoholic extract showed weak antimicrobial activity against *Staph. aureus* with a higher activity exhibited by the concentration 150 µg/ml with 11.532 mm inhibition zone. This finding revealed that 200 µg/ml concentration of myrrh extract was the MIC of myrrh against that pathogen. The result, therefore, was in compliance with that reported in the literature [14].

3.3. Preparation of myrrh mouthwashes

The preparation of each- 100 ml myrrh tincture involved the use of 65 ml hydroalcoholic myrrh extract. Using the Y % equation described previously, it was found that 65 ml extract would contain 2.61 g of extracted constituents of myrrh. Hence, the concentration of extracted myrrh present per 100 ml tincture was 2.61 % w/v. With dilution of 1 ml of that tincture up to 50 ml with water, in order to prepare a corresponding mouth wash, the amount of extracted constituents of myrrh in mouthwashes was, therefore, 0.052 % w/v (520 µg/ml).

With respect to excipients, the amount of each one in each myrrh mouthwash was 1/50 time less than that in its corresponding myrrh tincture. Therefore, the range of sodium lauryl sulphate, used as a solubilizing agent in order to reduce turbidity and enhance water solubility of hydrophobic constituents in myrrh, was 0.03- 0.21% which was within the range recommended for the use of such excipient (0.0025 – 0.5 %) [21]. Similarly, the concentrations of all other excipients after dilution were within accepted limits.

3.4. Evaluation of myrrh mouthwashes

3.4.1. Physicochemical properties and Palatability

Table 3 demonstrates the results of physicochemical and palatability tests to which the prepared myrrh mouthwashes were subjected. The mouthwashes were assigned as M1,…, M10 in correspondence to myrrh tinctures F1,…., F10, previously described. pH of all formulations ranged from 6.44-6.74 which were compatible with those of saliva [11]. Majority of the mouthwash formulations were non-palatable with exception of M9 and M10 (prepared form tincture F9 and F10, respectively). This finding could be attributed to the low extent of water insoluble myrrh matters in those formulations as a result of their higher content of solubilizing agent. The two formulations also showed accepted viscosity values that were greater than those of water (=1 mPa.s) and ethanol (1.2 mPa.s) [16]. Consequently, F9 and F 10 were decided to be the best ones and were selected for further investigations.

Table 3. Physicochemical properties and palatability of different myrrh mouthwash formulations

Property	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
pH	6.44	6.48	6.48	6.5	6.5	6.52	6.54	6.58	6.72	6.74
Viscosity(mPa.s)	2.3	2.01	1.95	1.88	1.86	1.79	1.75	1.69	1.65	1.5
Palatability▲	1	2	2	2	2	2	3	3	5	5

▲ *: 1= really bad, 2= bad , 3= not sure , 4 =good, 5= really good.*

3.4.2. Antimicrobial activity

Table 4 and Fig. 2 demonstrate the antimicrobial activity of the selected myrrh mouthwash formulations (M9 and M10) compared to brands of commercial mouthwashes and oral antifungal. The concentrations of tested product were its MIC as reported in the literature. The results revealed remarkable antimicrobial activity of myrrh mouthwash (M9) greater than all other tested products. Indeed, some of the tested commercial products showed no activity at all. In comparison of the two myrrh mouthwash formulations, formulation M10 showed inferior activity than M9, probably due to higher concentration of sodium lauryl sulphate that might interact with myrrh constituents at such concentration. Blank formulations of M9 and M10 showed minor activity against tested pathogen. Thus, the inhibition zone diameter of myrrh mouthwash was determined after subtraction the blank zone diameter from the observed diameter.

Table 4. Antimicrobial activity of myrrh mouthwash

Test	Tested concentration (µg/ml)	Inhibition zones (mm) observed on pathogenic microorganisms Mean ± SD ; (CV %)		
		Staph. aureus	*Strep. mutans*	*C. albicans*
Myrrh mouthwash (M9)	200	23.367 ± 0.379 ; (1.436 %)	32.367 ± 0.262 ; (0.809 %)	29.433 ± 0.153 ; (0.601 %)
Myrrh mouthwash (M10)	200	13.207 ± 0.679 ; (5.141 %)	22.367 ± 0.102 (0.456 %)	10.433 ± 0.202 ; (1.936 %)
A brand of chlorhexidine gluconate 0.1% mouthwash	20	11.267 ± 0.351 ; (3.115 %)	12.467 ± 0.672 ; (2.021 %)	Non tested
A brand of benzydamine HCl 0.15 % mouthwash	50	Resistant: No zone	9.433 ± 0.073 ; (0.774 %)	Non tested
A brand of nystatin (1000000 U/ 30 ml) oral suspension	0.25	Non tested	Non tested	Resistant: No zone

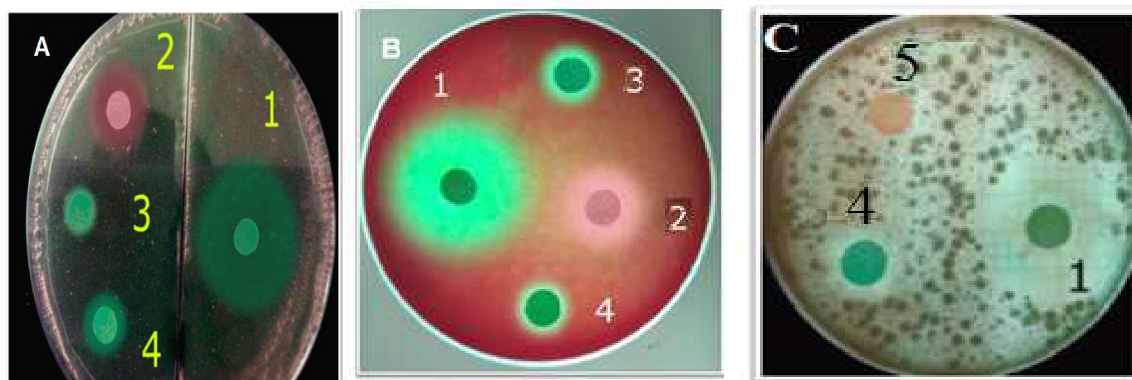

Fig. 2 Disk diffusion test of myrrh mouthwash (M9) on different types of pathogens. (A) : *Staph. aureus,* (B): *Strep. mutans* , (C) : *C. albicans* , (1) : myrrh mouthwash, (2): a brand of chlorhexidine gluconate mouthwash, (3): a brand of benzydamine HCl mouthwash, (4): blank formulation (5) : a brand of nystatin oral suspension

3.4.3. Isothermal stress stability

Within 9 weeks of storage of myrrh tincture (F9) in three different conditions, the formulation exhibited great stability when its corresponding mouthwash was tested against *Strep. mutans*. Table 5 shows the inhibition zones observed within various intervals form 0 -9 weeks. Besides, signs of physical change in color or odor were neither observed in the tincture nor in its corresponding mouthwash. Moreover, palatability of the mouthwash was accepted. Coefficients of variation in pH in mouthwash during the whole storage period were 1.2%, 0.5 % and 0.72 % at 8 °C, 35 °C and 70 °C, respectively. These findings might predict a proper stability of the product on shelves at room temperature.

Table 5. Data of Antibacterial activity of myrrh mouthwash (M9) after 9 weeks storage of myrrh tincture (F9)

Storage temperature	Period of storage (Weeks)			
	1st	2nd	4th	9th
	Inhibition zone (mm) mean ± SD ; (CV%)			
8 ºC	31.331± 0.263 ; (0.839 %)	31.327 ± 0.306 ; (0.971 %)	31.312 ± 0.252 ; (0.800 %)	31.200 ± 0.100 ; (0.321 %)
35 ºC	31.307 ± 0.493 (1.574)	29.400 ± 0.361 (1.226)	28.267 ± 0.208 (0.736)	27.333 ± 0.208 ; (0.762 %)
70 ºC	30.070 ± 1.153 ; (3.834 %)	27.367 ± 0.379 ; (1.383 %)	26.500 ± 0.300 ; (1.132 %)	26.367 ± 0.153 ; (0.579 %)

CONCULSION

Based on evidences obtained in this study, it could be concluded that a cosolvent of ethanol: phosphate buffer pH 7 (85:15) is an excellent solvent system for extracting the antimicrobial constituent of Yemeni myrrh. Besides, the Yemeni myrrh mouthwash presnted by this study to be preparad, by 1 to 50 dilution with water, from a tincture, containing 2.6 % w/v extracted constituents of myrrh and 9.5 % w/v sodium lauryl suphate sulfate, is a very promising formulation for large-scale production owing to its remarkable antimicrobial activity, accepted short-term stability, optimum pH, viscosity and palatability though long-term stability study remains to be established.

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