Journal of Applied Pharmaceutical Science Vol. 2 (12), pp. 013-016, December, 2012
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2012.21203
ISSN 2231-3354

Antibacterial Activity of Gels with Pomegranate, Apricot and Green Tea Glycolic Extracts

Calixto, Giovana[1]; Vieira, Daniela C.M.[1]; Fiúza, Thalita F.M.[1]; Salgado, Hérida R.[1]; Chorilli, Marlus[1*]
SchoolofPharmaceuticalSciences, DepartamentofDrugsandPharmaceuticals, São Paulo StateUniversity, UNESP, Rodovia Araraquara/ Jaú Km 1, Araraquara, SP, CEP 14801902, Brazil.

ARTICLE INFO

Article history:
Received on: 03/11/2012
Revised on: 21/11/2012
Accepted on: 09/12/2012
Available online: 29/12/2012

Key words:
Antibacterial activity;
Gels; Glycolic extracts,
Pomegranate,
Green Tea.

ABSTRACT

Pomegranate (PGE) and green tea (GTGE) glycolic extracts are being employed in formulations because of their antiseptic and astringent effects. Apricot (AGE) glycolic extract possesses function cooling and antibacterial. The aim was to verify the antibacterial activity of these extracts incorporated in gel base. The antibacterial activity was verified by diffusion in agar method, using cylinder in plate. Plates containing *Staphylococcus aureus* (ATCC 6538p), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 10536) and *Salmonella* sp. (ATCC 19196) were incubated at 37ºC for 24 hours. After incubation, the results were analysed with a pachymeter, observing the bacterial growth inhibition halo diameter and the statistical significance level was determined. PGE presented activity only against *P. aeruginosa*; GTGE presented activity against *S. aureus*, *P. aeruginosa* and *E. coli*; and AGE presented activity against *P. aeruginosa* and *Salmonella* sp. According to the experimental conditions, it is possible to conclude that GTGE presented the greater growth inhibition halo diameter when compared with other extracts, suggesting higher antibacterial action of this extract.

INTRODUCTION

Antibacterial agents are included in pharmaceutical preparations, mainly, to relieve common conditions such as halitosis, body odor and skin infections, including secondary infections associated with acne (Dossey, 2006; Migliato at al., 2009; Prugnolle et al., 2009; Guerrero, 2010). However, the use of these agents has been under constant challenge due to emergence of resistant microorganisms (Arora et al., 2009). The discovery of novel and potential antimicrobial drugs obtained from natural sources, such as plants, is emerging even more (Ganga et al., 2012). Plants have been shown to possess antimicrobial activity holding great potential against resistant microorganisms because the extracts from these natural sources contain molecules with antibacterial properties. Thus, the use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments, and therefore beneficial for human health (Nascimento et al., 2000). In the last few years, the significant growth of the phytotherapy has stimulated the evaluation of activity of several plant extracts (Migliato et al., 2005) and a number of studies have been conducted in different countries to prove such efficiency (Santos et al., 2005; Salvagnini et al., 2006; Iha et al., 2008). Therefore, plants and plant extracts have been widely researched in order to use their active substances as prototypes for drug development and pharmaceutical raw materials made exclusively from plant extracts (Migliato et al., 2008; Hussain et al., 2012) as pomegranate, apricot and green tea glycolic extracts. Pomegranate, *Punicagranatum*, shares its botantical family only with *Punicaprotopunica*, the latter restricted in occurrence to Socotra, an island off the Yemeni coast (Lansky et al., 2007). Pomegranate fruit has historically been identified as a rich source of polyphenolic compounds and hydrolyzable tannins with anti-oxidant properties. Among the variety of polyphenols found in pomegranate, ellagitanninpunicalagin, and its hydrolyzed product ellagic acid, have been shown to have a number of biological activities including, anti-carcinogenic, UV protection and melanin inhibition (Diwakar et al., 2012). Pomegranate glycolic extract is obtained from the fruit of the pomegranate and contain alkaloids and gallic tannins. It has an astringent effect and antiseptic

** Corresponding Author*
Marlus Chorilli, School of Pharmaceutical Sciences – São Paulo State University – UNESP, Rodovia Araraquara-Jaú Km. 01 - Campus Universitário, City: Araraquara State: São Paulo, Zip code: 14801-902.
Ph: +55 16 3301-6998

provided by gallic tannins, being widely used in products for the treatment of acne and seborrhea (Balmé, 2004; Alonso, 2008). Apricot, *Prunusarmeniaca* L., a member of the Rosaceae family; is one of the most important stone fruit of South East Asia. Nutritionally, it is a rich source of sugars, fibers, minerals, bioactive phytochemicals and vitamins like A, C, thiamine, riboflavin, niacin and pantothenic acid (Ali et al., 2011). Furthermore, the apricot fruit also has some pharmacological significance due to having high amounts of antioxidant (Hacıseferoğulları, 2007). The apricot (*Prunusarmeniaca*L.) glycolic extract has astringent action, antiseptic, mucosal protective, refreshing and remineralizing (Coimbra, 1994; Cravo, 1995).

Green tea, obtained from the plant *Camellia sinensis*, are rich in polyphenols, as catechins, especially 3-gallate (-) epigallocatechin, which seems to be the main active. Clinical studies show the green tea as chemoprotective, sunscreen and relate their activity to the retardation of aging by having activities such as antioxidant, anti-free radicals, inhibiting the induction, initiation, promotion and proliferation of carcinogenesis, antiinflammatory, antiangiogenic, inducer of apoptosis of carcinogen cells, metalloproteinase inhibitor, protector of the conversion of benign cells into malignant and inhibitor of DNA damage (Pereira et al., 2009) .

Due to the advantages of these plant extracts, it becomes interesting to incorporate these extracts in gel to be used against topical bacterial infections. Therefore, the aim of this work was to assess and compare the antibacterial activity of the pomegranate glycolic extract (PGE), apricot glycolic extract (AGE) and green tea glycolic extract (GTGE) when incorporated in gel.

MATERIALS AND METHODS

Preparation of the formulations

Firstly, the gel base was prepared using 2.5% (w/w) acrylamidopropylenesulfonic acid/vinylformamide copolymer and dispersed in water at 4000 rpm in Ultra-turrax blender (Turratec TE-102, Brazil). After that, the extracts were incorporated at concentration 10% (w/w) and the negative and positive controls were also prepared. The compositions of all formulations were showed in Table 1.

Table. 1: Composition of the formulations prepared to antimicrobial activity determination.

Formulations	10% Extract	Negative control	Positive control
F1	----	1% Triclosan	----
F2	PGE	----	----
F3	GTGE	----	----
F4	AGE	----	----
F5	-----	----	100% Gel base

These formulations were diluted to be evaluated at concentrations 25 mg/mL, 50 mg/mL and 100 mg/mL of the extracts. It was also tested the negative (F1) and positive (F5) controls diluted in the same concentrations.

Determination of antibacterial activity

Antibacterial activity of the formulations was verified by diffusion in agar Mueller Hinton method, using cylinders in plate (Bauer et al., 1966). It was used in study *Staphylococcus aureus*(ATCC 6538p), *Pseudomonas aeruginosa*(ATCC 27853), *Escherichia coli* (ATCC 10536) and *Salmonella* sp. (ATCC 19196). Inocula of bacteria were adjusted and standardized according to Murray et al. (2003) and according to document NCCLS (2000). Cultures of isolated colonies were obtained in Mueller Hinton (MH) broth for 24 h until obtaining turbidity equal to 0.5 in the McFarland scale equivalent to approximately 1.5 x 106 CFU/mL, and seeded by swab sterile.

The plates were incubated at 37 °C for 24 h. After that, the results were analysed with a pachymeter, observing the diameter of the halo of growth inhibition (mm).

In order to test the efficiency of these gels with extracts, statistical significance was determined by one-way analysis of variance followed by Dunnett's test, with the level of significance set at $P < 0.05$ (Sannomiya el al., 2005).

RESULTS AND DISCUSSION

Table 2 shows the results of the determination of antibacterial activity of the formulations.

Table. 2: Zone of inhibition formed by the formulations

Formulations	Pathogens			
	S. aureus	***P. aeruginosa***	***E. coli***	***Salmonella*sp.**
F1	18	18	17	16
F2	-	6	-	-
F3	9	10	10	-
F4	-	4	-	6
F5	-	-	-	-

(-) = No zone of inhibition

Results show the zones of inhibition in mm, indicating the distance from the border of the disc to the edge of the clear zone.

The formulation F1 presented activity against all the microrganisms tested. The formulation F2 presented activity only against *P. aeruginosa.* The formulation F3 presented activity against *S. aureus*, *P. aeruginosa*and *E. coli*. The formulation F4 presented activity against *P. aeruginosa*and *Salmonella* sp. Finally, the formulation F5 not presented activity against the microrganisms tested. The gel containing triclosan (F1) resulted in inhibition of the bacterial growth for all the microorganisms. This result was expected because the triclosan, according to the literature, has a spectrum of action against gram-positive and gram-negative bacteria (Adolfsson-Erici et al., 2002). The gel without preservative (F5), in turn, not showed action against the microorganisms in question. With regard to the formulations containing plant extracts, it was observed that a statistically higher antibacterial activity for GTGE (F3). Possibly, this higher activity is due to the presence of polyphenols present in GTGE. Several authors have demonstrated high effective antibacterial plant extracts containing polyphenols (Guedes et al., 2009; Rotava et al., 2009). Specific antioxidant polyphenols, called catechins, play an

important role in green tea's inhibition of bacterial growth (Nakayama et al., 2012). These catechins are the key ingredients of green tea and have various functionalities, including anti-bacterial action (Chan et al., 2011). Catechins demonstrate stronger action against Gram-positive bacteria, including *Staphylococcus aureus*(Mbata et al., 2008), and they can cause deactivation of the toxins produced by bacteria (Kumar et al., 2012; Sharma et al., 2012), which may have been the cause of the best performance of formulation F3. Therefore, the results obtained in this study showed that the plant extracts, mainly, green tea extract, incorporated in gels have great antibacterial activity and they can be used as a possible natural source for novel pharmaceutical preparations to kill some human pathogenic bacteria.

CONCLUSIONS

According to the experimental conditions, it is possible to conclude that the formulation with green tea glycolic extract (GTGE) presented greater growth inhibition halo diameter when compared with other extracts, suggesting bigger antibacterial action of this extract.

ACKNOWLEDGMENTS

The authors are grateful to CNPQ and PADC/ FCF – UNESP from Brazil.

REFERENCES

Adolfsson-Erici M., Pettersson M., Parkkonen J., Sturve J. Triclosan, a commonly used bactericide found in human milk and in the aquatic environment in Sweden. Chemosphere.2002; 46:1485–89.

Ali S., Masud T., Abbasi KS. Physico-chemical characteristics of apricot (*Prunusarmeniaca*L.) grown in Northern Areas of Pakistan. Sci. Hortic. 2011; 130:386-392.

Alonso JR. Tratado de Fitomedicina – Bases Clínicas e Farmacológicas. ISIS Ediciones SRL, Buenos Aires (1998) 1039

AroraDS.,Gurinder JK., Kaur H. Antibacterial Activity of Tea and Coffee: Their Extracts and Preparations, Int J Food Prop. 2009; 12:286-294

Balmé F. Plantas Medicinais. Helmus, São Paulo (1994) 398

Bauer AW., Kirby E., Sherris EM., Turk M. Antibiotic by standarized single disk method. Am. J. Clin. Path.1996; 45:493-96.

Chan EWC.,Soh EY., Tie PP., Law YP. Antioxidant and antibacterial properties of green, black, and herbal teas of Camellia sinensis.Pharmacognosy Res. 2011; 3:266–272

Coimbra R. Manual de Fitoterapia. 2nd ed. CEJUP, Belém (1994) 438

Cravo AB. Frutas e ervas que curam – Panacéia Vegetal. 4 ed. Helmus, São Paulo (1995)

Diwakar G., Rana J., Scholten J. D. Inhibition of melanin production by a combination of Siberian larch and pomegranate fruit extracts. Fitoterapia.2012; 83:989-895.

Dossey L. Listerine's Long Shadow: Disease Mongering and the Selling of Sickness. Explore (NY). 2006; 2:379-385.

Ganga B., Rao P., Rao U., Sambasiva RE., Mallikarjuna RT., Praneeth VS. Evaluation of in-vitro antibacterial activity and anti-inflammatory activity for different extracts of *Rauvolfiatetraphylla L.* root bark. AsianPac J Trop Biomed. 2012; 2:818-821

Guedes RCM., Nogueira, NGP., Fusco-Almeida AM., Souza CRF., Oliveira WP. Atividade antimicrobiana de extratos brutos de *Petiveriaalliacea*L. Lat. Am. J. Pharm. 2009; 28:520-524.

Guerrero D. Dermocosmetic approach to acne by the dermatologist. Ann DermatolVenereol. 2010; 137:29-33.

Hacıseferoğulları H., Gezer İ., Özcan MM., Asma BM. Post-harvest chemical and physical–mechanical properties of some apricot varieties cultivated in Turkey.J **Food** Eng. 2007; 79:364-373.

Hussain W., Hussain J., Ali R., Khan I., Shinwari Z., Nascimento IA., Lope WA. Tradable and Conservation Status of Medicinal Plants of Kurram Valley, Parachinar, Pakistan.Journal of Applied Pharmaceutical Science.2012; 2:066-070.

Iha SM., Migliato KF., Vellosa JCR., Sacramento, LVS., Pietro RCLR., Isaac VLB., Brunetti IL., Corrêa MA., Salgado HRN. Estudo fitoquímico de goiaba (*Psidiumguajava*L.) com potencial antioxidante para o desenvolvimento de formulação fitocosmética. Rev Bras Farmacogn. 2008; 18:387-93.

Kumar A., Kumar A., Thakur P., Patil S., Payal C., Kumarand A., Sharma P. Antibacterial activity of green tea (Camellia sinensis) extracts against various bacteria isolated from environmental sources. Recent Research in Science and Technology.2012; 4:19-23.

Lansky E. P., Newman R.A. *Punicagranatum*(pomegranate) and its potential for prevention and treatment of inflammation and cancer.J Ethnopharmacol. 2007; 109:177–206

Migliato KF., Chorilli M., Michelin DC., Aquino NC., Corrêa MA., Scarpa MV., Marona HRN. Avaliação da atividade anti-séptica de sabonete líquido contendo n-carboximetil quitosana. Int J PharmCompd, 2005; 7:49-53.

Migliato KF. (2008). Syzygiumcumini (L.) Skeels: validação do extrato, análises farmacognósticas, estudo da atividade antimicrobiana, avaliação genotóxica e citotóxica. PhD Thesis. Faculdade de Ciências Farmacêuticas de Araraquara, Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP.

Migliato KF., Chorilli M., Scarpa MV., Moreira RRD., Corrêa MA., Isaac VLB., Salgado HRN. Verificação da atividade antibacteriana de sabonete líquido contendo extrato glicólico de *Dimorphandramollis*Benth. Rev. ciênc. farm. básicaapl. 2009; 30:69-74.

Mbata TI., Debiao LU., Saikia, A. Antibacterial activity of the crude extract of Chinese green tea (*Camellia sinensis*) on *Listeria monocytogenes*. Afr. J. Biotechnol. 2008; 7:1571-1573.

Murray PR., Barron EJ.,Pfaller MA., Tecnover FC., Yolken RH. Manual of clinical microbiology.8.ed. ASM, Washington (2003) 1102-1106

Nakayama M., Shigemune N., Tsugukuni T., Jun H., Matsushita T., Mekada Y., Kurahachi M., Miyamoto T. Mechanism of the combined anti-bacterial effect of green tea extract and NaCl against Staphylococcus aureus and Escherichia coli O157:H7. FoodControl. 2012; 25:225-232.

Nascimento GGF.,Locatelli J., Freitas PC., Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria.Braz. J. Microbiol. 2000; 31:247-256.

National Committee for Clinical Laboratory Standards (NCCLS).Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2000.

Pereira AV, Almeida TC, Beltrame FL, Costa ME, Garrido LH. Determinação de compostos fenólicos em amostras comerciais de chás verde e preto - *Camelliasinensis*(L.) Kuntze, Theaceae. Actasci health sci. 2009; 31:119-124.

Prugnolle F., Lefèvre T., Renaud F., PapeMøller A., Missé D., Thomas F., Infection and body odours: Evolutionary and medical perspectives. Infect. Genet.Evol.2009; 9:1006-1009.

Rotava R., Zanella I., Silva LP., Manfron MP., Ceron CS., Alves SH., Karkow AK., Santos JPA. Atividade antibacteriana, antioxidante e tanante de subprodutos da uva. Cienc. Rural. 2009; 39:941-944.

Salvagnini LE.,Migliato KF., Isaac VLB., Correa MA., Salgado HRN., Pietro RCLR. Evaluation of efficacy of preservatives associated to *Achilleamillefolium*L. extract against *Bacillus subtilis*. Br. J. Microbiol. 2006; 37:75-77.

Sannomiya M., Fonseca VB., da Silva MA., Rocha LRM., dos Santos LC., Hiruma-Lima CA., Souza Brito ARM., Vilegas W. Flavonoidsandantiulcerogenicactivityfrom*Byrsonima crassa*leavesextracts. J Ethnopharmacol. 2005; 97:1-6.

Santos PRV., Oliveira ACX., Tomassini TCB. Controle microbiológico de produtos fitoterápicos. Rev Farm BioquímUniv S Paulo. 1995; 31:35-38.

Sharma A., Gupta S., Sarethy IP., Dang S., Gabrani R. Green tea extract: Possible mechanism and antibacterial activity on skin pathogens. Food Chem. 2012; 135:672-675.

How to cite this article:

Calixto, Giovana; Vieira, Daniela C.M; Fiúza, Thalita F.M; Salgado, Hérida R; Chorilli, Marlus., Antibacterial Activity of Gels with Pomegranate, Apricot and Green Tea Glycolic Extracts. J App Pharm Sci. 2012; 2 (12): 013-016.