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Evaluation of Stability study of Selected Preservatives in Aluminium Hydroxide Gel- USP

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ABSTRACT

Plan: *The aim of present study is to find out new preservatives synthesized from natural sources, which may have better efficiency and stability than the existing synthetic preservatives. The derivatives of naturally occurring gallic, p-coumaric and ferulic acids were subjected to pharmaceutical product for their stability study. Their preservative efficiency was evaluated and compared with the standard parabens.*
Preface: *Deterioration of pharmaceutical preparations due to growth of microorganisms is a great challenge and need of preservation becomes very important. Literature reports about various problems associated with the existing synthetic preservatives such as lack of stability, development of microbial resistance (in due course of time) and several serious side effects.*
Methodology: *The selected amide, anilide and ester derivatives of gallic, p-coumaric and ferulic acids were subjected to stability testing in an official antacid preparation, (aluminium hydroxide gel-USP) against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Candida albicans and Aspergillus niger as representative challenging microorganisms as per ICH guidelines.*
Outcome: *The selected derivatives were found to be effective against all selected strains and showed preservative efficacy comparable to that of standard. The 8- hydroxy quinoline ester derivative of gallic, p-coumaric and ferulic acids showed better stability than other derivatives and may be used as an alternative to existing preservatives in the pharmaceutical preparations.*
Key words: *Gallic acid, p-Coumaric acid, Ferulic acid, Amides, Esters, Preservative*

1. INTRODUCTION

Non-sterile products such as pharmaceuticals, cosmetics, food items etc. with a high degree of water availability may be contaminated with microorganisms which may cause spoilage of the product with loss of therapeutic properties and, if they are pathogenic, serious infections can arise [1]. To inhibit the growth of contaminating microorganism, antimicrobial preservative systems have been developed and introduced into the pharmaceutical, cosmetic or food products during manufacturing process and/or throughout its use by consumers [2].

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In several cases, the microorganisms became resistant to antimicrobials and are able to degrade many commonly used preservatives especially *p*-hydroxybenzoates [3]. Microbial resistance has been reported to some of the commonly used chemical preservatives like benzalkonium chloride, dibromodicyanobutane, chloramine, chlorhexidine, cholorophenol, benzoic acid, dimethyl oxazolidine, dimethyl dithiocarbamate, dimethoxy dimethyl hydantoin, formaldehyde, glutaraldehyde, hydrogen peroxide, iodine, methylene bischlorophenol, methylparaben, propylparaben, phenylmercuric acetate, mercuric salts, povidine-iodine, sorbic acid and quaternary ammonium compounds [4]. The preservative potential of natural organic acids is well established in the literature *viz.* caprylic acid, veratric acid, 2, 4-hexadienoic acid and anacardic acid [5-8].

Literature reports reveals that the ferulic acid possesses antimicrobial, antioxidant and preservative activities [9-10]. The gallic acid and its derivatives possess wide spectrum of biological activities like antimicrobial, anticancer, antiviral, anti-inflammatory, analgesic and anti-HIV activities [11-15]. Also, the *p*-coumaric acid and its derivatives possess wide spectrum of biological activities like antimicrobial and antioxidant [16].

In the present study, we have planned to evaluate the selected test preservatives from gallic, *p*-coumaric and ferulic acids in aluminium hydroxide gel-USP for their stability study as per ICH guidelines.

2. EXPERIMENTAL

2.1.1 Materials

Nutrient agar, nutrient broth, sabouraud dextrose agar and sabouraud dextrose broth were obtained from Himedia, Mumbai. Mannitol, methyl and propyl paraben were obtained from CDH, Mumbai.

2.1.2 Methods

Aluminium Hydroxide Gel USP was used as the pharmaceutical product for evaluation of stability over a period of six months.

2.1.3 Formula for preparation of Aluminium Hydroxide Gel USP 2004:

Aluminium hydroxide gel, 36 g; Mannitol, 7 g; Methyl paraben, 0.2 g; Propyl paraben, 0.02 g; Saccharin, 0.05 g; Peppermint oil, 0.005 ml; Alcohol, 1 ml; Purified water q.s., 100 ml.The weighed quantity of aluminum hydroxide gel and mannitol were triturated with 50 ml of water in a mortar. Methyl paraben, propyl paraben, saccharin and peppermint oil were dissolved in alcohol and added to above mixture and triturated well. The volume was made up to 100 ml with purified water followed by its sterilization by autoclaving.

For stability testing, the aluminium hydroxide gel was prepared using the preservatives mentioned in Table 1 by replacing methyl paraben and propyl paraben with selected preservatives (Fig 1) with equimolar amount of methyl paraben (0.0013 mol) and added into aluminum hydroxide gel [17].

2.1.4 Strains:

Staphylococcus aureus MTCC 2901, Bacillus subtilis MTCC 2063, Escherichia coli MTCC 1652, Candida albicans MTCC 227 and *Aspergillus niger MTCC 8189* were used in this study were common contaminants and prescribed in USP for preservative efficacy testing in pharmaceutical preparations.

2.1.5 Preparation of inoculums

The representative microorganisms were inoculated in nutrient agar I.P. (*S. aureus, B. subtilis, E. coli*) and sabouraud agar I.P. (*C. albicans, A. niger*). The seeded plates were incubated at 37 ^{o}C for 24 h (*S. aureus, B. subtilis, E. coli*), 37^{o}C for 48 h (*C. albicans*) and 25 ^{o}C for 7 d (*A. niger*). After the incubation period, suspensions of microorganisms were prepared in sterile saline solution (0.9% w/v NaCl) to give a microbial count of 1x 10^4 CFU/ml [7].

2.1.6 Determination of CFU/ml

Aluminium Hydroxide Gel-USP in their final container was used in the challenge test. The preparation was inoculated with the microbial cell suspension with a cell count of 1 x 10^4 CFU/ml. The inoculum never exceeded 1% of the volume of the product sample. Inoculated samples were mixed thoroughly to ensure homogeneous microorganism distribution and incubated. The CFU/ml of the product was determined at an interval of 0, 1, 2, 3, 4, 5 and 6 months on agar plate (ICH guidelines).

2.1.7 Determination of pH

The pH of samples of Aluminium hydroxide gel containing different preservatives was determined using digital pH meter at an interval of 0, 1, 2, 3, 4, 5 and 6 month (ICH guidelines).

3. RESULTS AND DISCUSSION

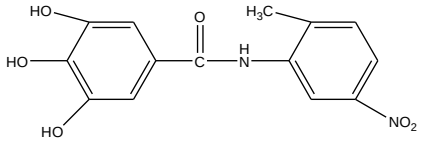
The results of stability testing were performed in triplicate and were reported as mean values in Table 2 to Table 4. As per the results of Table 2 the pH of Aluminium hydroxide gel samples were in range of 7.3-9.6 which indicated that the selected preservatives were stable over the six months period as the change was comparable to that of the standard preservative (Table 2).

The results of microbial study indicated that the fungal growth was observed in samples containing the preservatives gallic *N*,*N*-dimethyl amide, gallic naphthylamide, *p*- coumaric *N*,*N*-diphenyl amide and ferulic naphthyl amide after 4 month while the *p*- coumaric -2-chloro 4-nitro anilide and ferulic-morpholino amide after 5 month whereas in all other samples there was no evidence of fungal growth during six months period (Table 3).

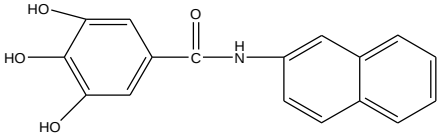
Table 1. Amount of selected preservatives added in Aluminum Hydroxide Gel – USP [18].

Code	*Preservative*	*Amount (g)*
G-1	Gallic-8-hydroxy quinoline ester	0.386
G-2	Gallic 2-methyl 5-nitro anilide	0.395
G-3	Gallic *N*,*N*-dimethyl amide	0.256
G-4	Gallic naphthyl amide	0.383
PC-1	*p*- Coumaric -8-hydroxy quinoline ester	0.378
PC-2	*p*- Coumaric 3-chloro 4-nitro anilide	0.413
PC-3	*p*- Coumaric *N*,*N*-diphenyl amide	0.326
PC-4	*p*- *C*coumaric naphthyl amide	0.375
F-1	Ferulic-p-amino ester	0.370
F-2	Ferulic-morpholino amide	0.341
F-3	Ferulic 8-hydroxy quinoline ester	0.417
F-4	Ferulic naphthyl amide	0.414
Standard	Methyl Paraben	0.2

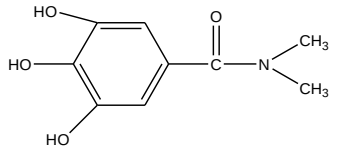
Fig.1 Structures of selected preservatives for aluminium hydroxide gel samples



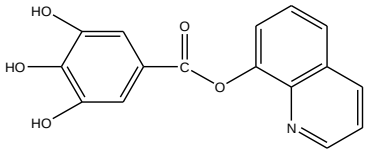
Gallic 2-methyl 5-nitro anilide (G-1)



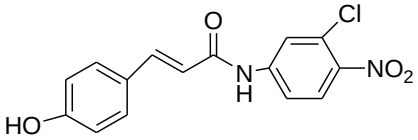
Gallic naphthyl amide (G-2)



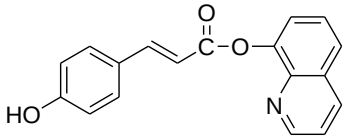
Gallic *N,N*-dimethyl amide (G-3)



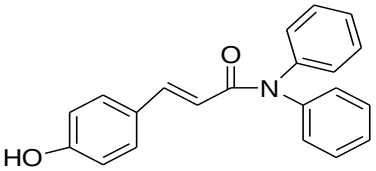
Gallic-8-hydroxy quinoline ester (G-4)



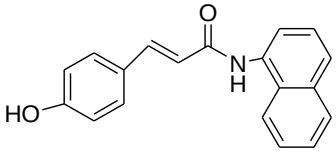
p-coumaric 3-chloro-4-nitro anilide (PC-1)



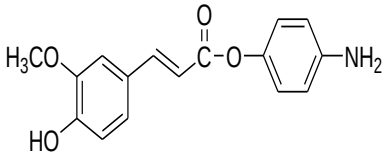
p-coumaric naphthyl amide (PC-2)



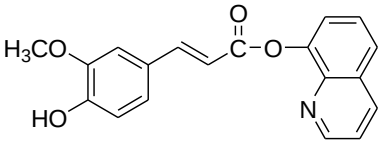
p-coumaric N,N-diphenyl amide (PC-3)



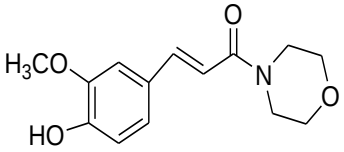
p-coumaric-8-hydroxy quinoline ester (PC-4)



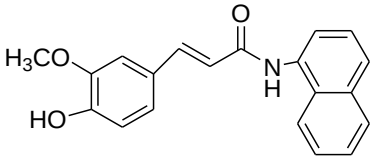
Ferulic-p-amino ester (F-1)



Ferulic naphthyl amide (F-2)



Ferulic-morpholino amide (F-3)



Ferulic 8-hydroxy quinoline ester (F-4)

The results of microbial study indicated that the bacterial growth was observed in samples containing the preservatives gallic *N*,*N*-dimethyl amide, *p*- coumaric -2-chloro 4-nitro anilide, *p*- coumaric naphthyl amide after 3 months while gallic naphthyl amide, *p*- coumaric *N*,*N*-diphenyl amide and ferulic naphthyl amide after 4 month and gallic 2-methyl 5-nitro anilide and ferulic morpholino amide after 5 month whereas in all other samples there was no evidence of bacterial growth during six months period (Table 4). These results indicated that the products are stable over a period of 6 month with added preservatives.

4. CONCLUSION

The stability studies of selected preservatives using aluminium hydroxide gel was analyzed for pH and cfu/ml and the results indicated that the change in pH was comparable to that of standard and the microbial growth was observed in samples containing the preservatives gallic *N*,*N*-dimethyl amide, gallic naphthyl amide, *p*- coumaric *N*,*N*-diphenyl amide, ferulic naphthyl amide, *p*-coumaric-2-chloro-4-nitro anilide, ferulic morpholino amide and *p*- coumaric naphthyl amide in last two months whereas in all other samples there was no evidence of fungal growth during six months period.

No microbial growth in samples of Aluminium hydroxide gel containing the 8- hydroxy quinoline derivative of gallic acid, *p*- coumaric acid and ferulic acid and the *p*- amino ester derivative of ferulic acid was observed and hence these derivatives were stable over a period of six month and may be used as an alternative to the existing chemical preservatives. Even though the newly prepared preservatives were synthesized from the acids obtained from natural sources which are used in routine as food like rice, cereals and fruits, further research is warranted to generate the toxicity profile, for the safe use of these acids in pharmaceutical preparations.

Table 2: Stability studies of sample of Aluminium hydroxide gel for pH.

Code	*0 month*	*1 month*	*2 month*	*3 month*	*4 month*	*5 month*	*6 month*
G-1	8.3	8.6	8.5	8.6	8.3	8.4	8.7
G-2	9.1	9.3	9.6	9.5	9.4	9.2	9.5
G-3	7.9	7.6	7.5	7.3	7.4	7.8	7.6
G-4	8.4	8.6	8.5	8.7	8.2	8.4	8.6
PC-1	9.2	9.1	9.6	9.5	9.2	9.3	9.5
PC-2	9.3	9.0	9.2	9.0	9.1	9.2	9.0
PC-3	9.0	9.3	9.2	9.2	9.1	9.4	9.3
PC-4	8.5	8.2	8.3	8.1	8.5	8.3	8.6
F-1	8.7	8.9	8.6	8.7	8.8	8.9	8.9
F-2	7.6	7.5	7.6	7.5	7.8	7.6	7.5
F-3	8.2	8.0	8.0	8.2	8.5	8.3	8.6
F-4	8.8	8.4	8.8	8.6	8.5	8.8	8.4
Standard	8.6	8.3	8.6	8.7	8.5	8.7	8.6

Table 3: Stability studies of Aluminium hydroxide gel samples for fungal count (cfu/ml).

Code	*0 month*	*1 month*	*2 month*	*3 month*	*4 month*	*5 month*	*6 month*
G-1	-	-	-	-	-	-	-
G-2	-	-	-	-	-	-	-
G-3	-	-	-	-	-	13	23
G-4	-	-	-	-	-	10	17
PC-1	-	-	-	-	-	-	-
PC-2	-	-	-	-	-	-	7
PC-3	-	-	-	-	-	4	8
PC-4	-	-	-	-	-	-	12
F-1	-	-	-	-	-	-	-
F-2	-	-	-	-	-	-	11
F-3	-	-	-	-	-	-	-
F-4	-	-	-	-	-	15	23
Standard	-	-	-	-	-	-	-

Table 4: Stability studies of Aluminium hydroxide gel samples for bacterial count (cfu/ml).

Code	*0 month*	*1 month*	*2 month*	*3 month*	*4 month*	*5 month*	*6 month*
G-1	-	-	-	-	-	-	-
G-2	-	-	-	-	-	-	6
G-3	-	-	-	-	7	15	20
G-4	-	-	-	-	-	4	12
PC-1	-	-	-	-	-	-	-
PC-2	-	-	-	-	5	13	19
PC-3	-	-	-	-	-	8	18
PC-4	-	-	-	-	7	12	21
F-1	-	-	-	-	-	-	-
F-2	-	-	-	-	-	-	8
F-3	-	-	-	-	-	-	-
F-4	-	-	-	-	-	12	16
Standard	-	-	-	-	-	-	-

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