





**Table 1:** Results of the biochemical tests performed with skin isolates

Isolate→	1	2	3	4	5	6	7	8	9	10
<b>Test↓</b>										
<b>IMViC tests</b>										
Indole	-	-	-	-	-	-	-	-	+	-
Methyl Red	-	-	-	-	-	-	-	-	-	-
Voges Proskauer	-	-	+	+	+	+	+	+	+	-
Citrate utilization	+	+	-	-	+	+	+	+	+	+
<b>Nitrate reduction</b>										
Nitrate reduction	+	+	-	+	+	-	+	+	+	-
Catalase	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	-	-	-	-	-	-	-	+
Urease	-	-	-	-	+	+	-	-	-	-
Gelatin	+	+	+	+	-	-	+	+	-	+
Starch hydrolysis	-	-	-	+	+	-	+	+	-	-
<b>Sugar Fermentation</b>										
Lactose	-	-	A	-	A	AG	-	-	AG	A
Glucose	-	-	-	A	A	A	A	A	A	-
Sucrose	-	-	AG	A	A	A	A	A	A	A

‘-’ negative for the test ; ‘+’ for the test  
 A- acid production; AG-acid & gas production.

**Antimicrobial Activity (AMA) of personal care products on Skin isolates:** The antimicrobial test was performed on the 10 isolated bacterial samples with the three personal care products talc-cum-powder, fairness cream and deodorant spray and the results are shown in Table 2.

Isolates 1,2,5, 6 &10 have shown AMA with both talc and deodorant. Isolates 4&5 have not shown AMA with both talc and deodorant. The experimental results indicate that the topical application of the three personal care products - talc-cum-powder and deodorant spray on the body can restrict the colonization of skin microbiota.

**Table 2 :**AMA of talc-cum-powder, fairness cream and deodorant spray with the bacterial samples isolated from the skin.

Isolate No.	Diameter of inhibition zone (in cms) on Muller-Hilton agar medium		
	Talc-cum-powder	Fairness cream	Deodorant spray
1	1.2	#	1.3
2	1.2	#	1.5
3	#	#	0.8
4	#	#	0.8
5	1.0	#	1.8
6	2.0	#	2.0
7	#	#	#
8	#	#	#
9	1.5	#	#
10	2.0	#	1.8

‘#’ – indicates that no zone of inhibition was observed.

The products talc-cum powder and fairness cream were analyzed for their preservative compounds by HPLC and found that the talc contained triclosan (0.71%) while the fairness cream contained phenoxyethanol (0.417%), methylparaben(0.354%) & propyl paraben (0.137%). This finding endorses that the combination of phenoxyethanol, methyl paraben and propyl paraben at very lower concentrations are used to maintain the cream stability and

reduce the spoilage without showing any inhibitory effect on the skin isolates.

**The Minimum Inhibitory concentrations** of the six preservatives were determined based on the turbidity and the color change, but the results are completely not accurate since it is based on a visual definition of an endpoint. The values determined for each preservative against the test organism are shown in Table 3 in the percentage of their concentration taken:

**Table 3:** showing Minimum inhibitory concentrations of preservatives with the 3 test cultures

	<i>P.aeruginosa</i>	<i>M.luteus</i>	<i>S.epidermidis</i>
Phenoxyethanol	0.3	0.3	0.3
Methyl paraben	0.5	0.6	0.4
Propyl paraben	0.4	0.5	0.4
Sorbic acid	0.2	0.2	0.2
Potassium sorbate	0.6	0.4	0.4
Sodium benzoate	0.5	0.4	0.4

The challenge tests were carried out to assess the effect of the six selected preservatives on the three test organisms using a basic cream. The desired concentrations of the six preservative are summarized in Table 4:

**Table 4:** Desired concentrations of preservatives

	PE	MP	PP	SA	PS	SB
<i>P.aeruginosa</i>	C <sub>0.4</sub>	C <sub>0.6</sub>	C <sub>0.5</sub>	C <sub>0.3</sub>	C <sub>0.5</sub>	C <sub>0.6</sub>
<i>M.luteus</i>	C <sub>0.4</sub>	C <sub>0.5</sub>	C <sub>0.5</sub>	C <sub>0.2</sub>	C <sub>0.5</sub>	C <sub>0.5</sub>
<i>S.epidermidis</i>	C <sub>0.4</sub>	C <sub>0.5</sub>	C <sub>0.5</sub>	C <sub>0.3</sub>	C <sub>0.5</sub>	C <sub>0.8</sub>

**Combinations of preservatives:** No colony formation was detected from 7 to 28 days of incubation with all combinations except for the combination with potassium sorbate.

**Optimum dose of organic acids for the three test organisms at pH 5.5:**

Generally , the three test organic acids- sorbic acid, potassium sorbate and sodium benzoate are used as preservatives in the personal care products like shampoos, face wash and other washing products in which the pH will be less because they work best below pH of 6.5 The optimum working pH of these three organic acids is 5.5. Hence, the growth patterns of the three organisms were observed with the three organic acids at pH of 5.5.

No colonies were recovered from the cream inoculated with the three test organisms for 7, 14, 21, and 28 days when each of the three preservatives used at 0.1% concentrations. This may be possibly due to

- 1) Retardation in the growth of bacteria in cream at pH 5.5.(Essodolom, 2013).
- 2) The three organic preservatives might have worked efficiently in controlling the growth of bacteria.
- 3) The combined effect of both pH at 5.5 and activity of organic acids.

The other three preservatives phenoxyethanol, methyl paraben and propyl paraben exhibit their antimicrobial activity at a wide pH range from 4.5 -7.5(Soni et al., 2002.).

Hence, the pH of the cream was adjusted to 6.0 so as to observe the growth patterns of the three bacteria in the presence of six preservatives under similar conditions.

The skin surface pH acts as an antimicrobial barrier. At pH 5.0 the growth of pathogenic bacteria is inhibited. Species of the normal resident flora are positively affected by the physiological, acidic milieu (Kurabayashi, 2002). Further, the dissociation of endogenous bacteria from skin surface is enhanced at alkaline conditions (Lambers, 2006). The superficial layers of the skin are naturally acidic (pH 4-4.5) due to lactic acid in sweat and produced by skin bacteria. At this pH, mutualistic flora grows but not the transient flora. Another factor affecting the growth of pathological bacteria is that the antimicrobial substances secreted by the skin are enhanced in acidic conditions. In alkaline conditions, bacteria cease to be attached to the skin and are more readily shed. It has been observed that the skin also swells under alkaline conditions and opens up allowing move to the surface (Schauber & Gallo, 2008). Hence, lowering the pH of personal/skin care products will not only be beneficial for the control of microbial growth in the products but also bring down the pH of the skin to the recommended levels.

#### 4. Conclusions

The study reveals that the six preservatives-Phenoxyethanol, Methyl paraben, Propyl paraben, Sorbic acid, Potassium sorbate and Sodium benzoate shown antimicrobial activity with the three test organisms at various concentrations and time periods. The data also supports that combinations of preservatives are more effective than individual preservatives used at higher concentrations. Lowering of pH to 5.5 in personal/skin care products not only addressing the microbial growth reduces the pH of the skin to recommended levels by US and EU pharmacopeia.

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