# EFFECT OF PRESERVATIVE AGENTS ON THE MICROBIAL STABILITY OF SOME INDIGENOUS HERBAL PREPARATIONS.

**ESIMONE C. O., P. O. OLEGHE, E. C. IBEZIM**

*Department of Pharmaceutics, University of Nigeria, Nsukka, Enugu State, Nigeria.*

## ABSTRACT

The efficacy of preservative systems (methyl paraben, propyl paraben, EDTA and sodium benzoate) was evaluated in some liquid herbal medicines. Nine liquid herbal preparations commercially available in Mid West Nigeria were challenged with preservative combinations over a six weeks period. At the end of the six weeks period, the concentrations of viable bacteria and fungi were reduced to various levels by the different preservative combinations. The most effective preservative combinations were that of methyl paraben/propyl paraben, followed by the combinations of EDTA/Methyl paraben/propyl paraben and EDTA/Sodium benzoate. The remarkable effect of the methyl paraben / propyl paraben combinations in preserving the herbal medicines imply they could adequately preserve liquid herbal medicines.

**Keywords:** Pharmaceutical preservatives Liquid herbal preparations

* Corresponding author

## 1. INTRODUCTION

Herbal medicines, if unpreserved, readily become contaminated with adventitious microorganisms leading to spoilage (1). Most herbal medicine practitioners would rather prefer to employ other techniques of preservations to preserve their herbs. These include drying, carbonising, salting, using honey, oils and alcohol. Some others prepare their herbal remedies simply by boiling, believing that there are natural preservatives inherent in the herbs. Much still remains to be done as the methods employed by the practitioners in conducting herbal preparations, generally needs considerable improvement such as simple microbiology, modern sterilization procedures and good manufacturing practice (G.M.P) (1,2).

In recent years, adequate preservation of liquid products has increased in importance. In addition to presenting a health hazard to the user, microbial growth can cause marked effects on product stability (3-7). Non-sterile pharmaceuticals have been reported to contain high levels of microbial contaminations when adequate controls are not implemented during manufacture (8-16).

Preservatives are included in pharmaceutical preparations when it is necessary to combat the effect of contaminating microorganism, which may be inherent in ingredients or introduced during production process or during use by patient. The quality of preservatives included in any formula must be that necessary for assured protection against microbial growth (17-19). For maximum protection of the consumer, the concentration of the preservative shown to be effective in the final packaged product should be considerably below the concentrations of the preservatives that may be toxic to human beings (20). The selection of a preservative system must be done on an individual basis, using published information and 'in house' microbiological studies for guidance. Frequently, a combination of two or more preservatives is needed to achieve the desired antimicrobial effect. (4). This study is designed to evaluate the efficacy of some conventional oral pharmaceutical preservative systems on liquid herbal medicines marketed in Mid West Nigeria. The herbal medicines understudy had hitherto been shown to be grossly contaminated by bacteria and fungi (21)

## 2. MATERIALS AND METHODS

2.1 Collection of herbal

samples

Nine different liquid herbal preparations commercially obtained in Mid West Nigeria were purchased from vendors and used in the study. The product data are presented in Table 1. Water was the vehicle used in the preparation of all the samples

Determination of microbial load of herbal samples:

A modification of the Miles and Misra's technique previously described (21) was adopted. Each sample was shaken vigorously, 1 ml was pipetted and subjected to a 10fold serial dilution in sterile distilled water. 0.02 ml volume of the final dilution was spread plated on well-dried surface of nutrient agar (for bacterial count) and Sabouraud dextrose agar (for fungal count). Each sample was inoculated in triplicate. The Nutrient agar plates were incubated at 37 C for 24 - 48 hours while the S.D.A. plates were incubated at 28 C for up to 5 days. The number of colonies on each plate was determined and the mean for each sample calculated and expressed as mean colony forming units per ml(cfu/ml).

## PRESERVATION OF LIQUID PREPARATION

The following preservative combinations were used'.

* EDTA/Methylparaben/ propyl paraben (0.05: 0.1: 0.01 %W/V)Methyl paraben / propyl paraben (0.1:0.01%W/V)EDTA / Sodium benzoate (0.05 0.2%W/V).

Stock solutions (2 %W/V) of the preservatives were prepared and 0.05 ml quantity of this stock incorporated into 100 ml of each of the liquid samples. The resulting preparations were then stored at room temperature (25 C) for a minimum of 7 days before being plated on Nutrient agar plates and Sabouraud dextrose agar plates to check for microbial load of bacteria and fungi respectively. This was repeated weekly for a period of six weeks.

## PHYTOCHEMICAL TEST

The herbal samples were subjected to the following preliminary phytochemical analysis for the presence or absence of the following plant constituents: Alkaloids, saponins, glycosides, resins, flavonoids, tannins, steroids and terpenes. Official methods were employed in all the determinations(22).

## RESULTS

The effects of the preservatives on the microbial contaminants in the herbal preparations over a six weeks period are shown in Figures 1-9 and Tables 2-3. The preservative system containing Methyl paraben and propyl paraben (0.1: 0.0 1 %W/V) was the most effective while that containing EDTA and sodium benzoate (0.05:0.2 %W/V) was least effective against both the bacteria and fungal contaminants of the herbal samples.

From the phytochemical test (Table 3), 44.4% of the samples contain alkaloids, 55.6% saponins, 44.4% flavonoids, 11.1% tannins, 55.6% glycosides, 55.6% resins, 44.4% terpenes and 11.1% steroids, No phytoconstituent was present in sample 7.

## DISCUSSION

It is apparent that these preparations proved a suitable environment for the growth/survival of microorganisms. However, these contaminated liquid preparations were also preserved to varying extent by all the three preservative systems used. The combination of methyl and propyl paraben was most effective while that of EDTA and Sodium benzoate was the least effective. It has been shown that combinations of methyl and propyl paraben are synergistic and are therefore very popular in preserving oral pharmaceuticals (23). The use of more than one ester makes possible a higher total preservative concentration.

For oral liquid products, the USP (1990) requires that for the preservative to be effective in the product examined, the concentrations of viable bacteria are reduced to not more than 0.1% of the initial concentrations by the 14th day while the concentrations of viable yeast and molds remain at or below the initial concentrations during the first 14 days; and the concentration of each test micro-organisms remain at or below these designated levels during the remainder of the 28-day test period. From Figures 1 9 and Table 2, although most of the

values obtained for bacteria were higher than 0.1% concentrations of initial count, they were greatly reduced. That of fungi were below the initial concentrations thus suggesting that the herbal preparations needs to be preserved. The use of preservatives in herbal preparations is necessary, although most herbalists see the incorporation of preservatives in their preparations as adulterations. This should not be so, as adequate preservation is essential not only to prevent deterioration of product, but also to stop the proliferation of contaminating pathogens.

So far, no correlation has been demonstrated between the nature of its phytoconstituents of the various herbal samples and the efficacy of the preservative systems. However, it is possible that certain phytoconstituents like tannins, alkaloids and flavonoid, that have been shown to complex with certain drugs (24) could complex with these preservatives, thus rendering them less active. Conventionally, the choice of a preservative system for a particular product formulation, amongst other things, depends on demonstrable evidence that the preservative does not complex with formulation ingredients.

Our present study shows that the various preservative systems and especially, the methyl paraben/propyl paraben combinations could be adequately used to preserve liquid herbal medicines.

**Table 1: Product data of the herbal preparations**

| Sample no. | Therapeutic claim (s) | Route of administration | Local means of preservation |
|---|---|---|---|
| 1. | Anti Typhoid fever` | Oral | Boiling |
| 2. | Effective against Arthritis, Rheumatism, Gout, Muscular Pains and Anaemia | Oral | Boiling |
| 3. | Anti malaria | Oral | Alcohol |
| 4. | Anti malaria | Oral | Honey |
| 5. | Effective against Asthma | Oral | Honey |
| 6. | Effective against general fever | Oral | Dark oil |
| 7. | Effective against difficulty in urinating and stooling | Oral | Alcohol |
| 8. | Effective against all forms of illness | Oral | Alcohol |
| 9. | Anti Insomnia | Oral | Boiling |

**Table 2: Comparative effect of the preservative systems.**

**Preservative systems (Increasing order of organisms survival)**

| SAMPLE CODE | BACTERIA | FUNGI |
|---|---|---|
| 1 | 3< 1 <2 <C | 2<1 <3<C |
| 2 | 2<3 or 1<C | 3<2< 1<C |
| 3 | 3<1 or 2<C | 2<1 or 3<C |
| 4 | 2<1 or 3<C | 2 or3<1 <C |
| 5 | 2<1 <3<C | 2<1or3 <C |

| | | |
|---|---|---|
| 6 | 2<1 or 3<C | 1 or 2 or3 <C |
| 7 | 2<1 or 3<C | 2<1<3 <C |
| 8 | 1< 2 <3<C | 1<3<2 <C |
| 9 | 2<1 <3 <C | 1or 3<2<C |
| Total | 2<1<3<C | 2<1<3<C |

## KEYS

C = Control
1 = EDTA/Methyl paraben/Propyl paraben
2 = Methyl paraben /Propyl paraben
3 = EDTA/Sodium benzoate
1,2,3=Preservative systems

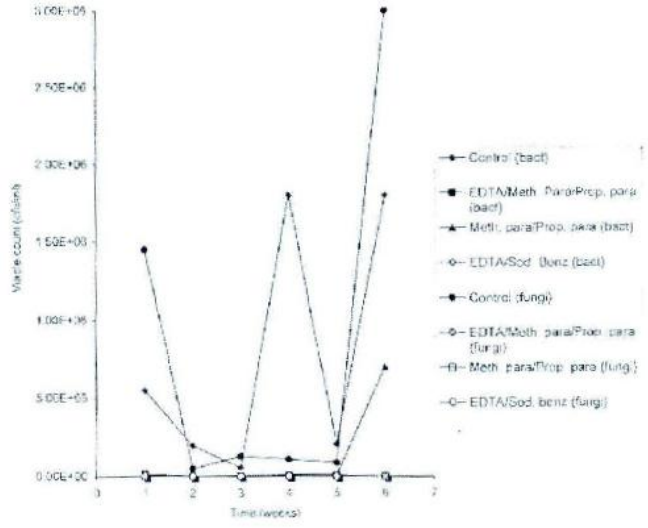


Fig. 1: Effect of preservative systems on the bacterial and fungal loads of herbs medicines (Sample 1)

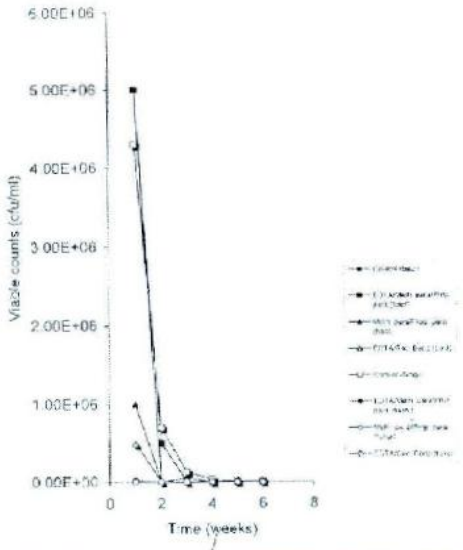


Fig. 1: Effect of preservative systems on the bacterial and fungal loads of herbs medicines (Sample 2)

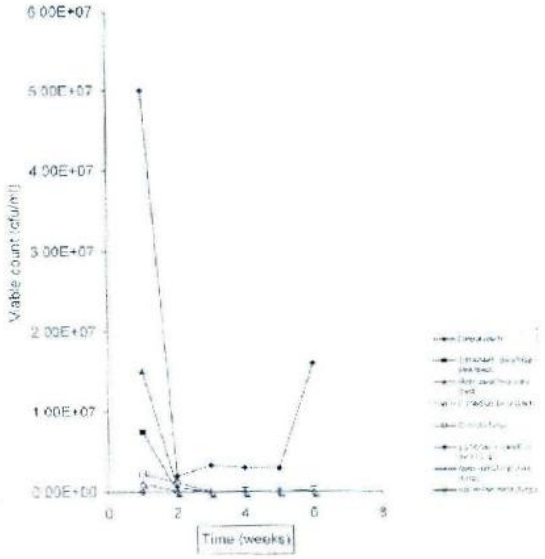


Fig. 3: Effect of preservative systems on the bacterial and fungal loads of herbs medicines (Sample 1)

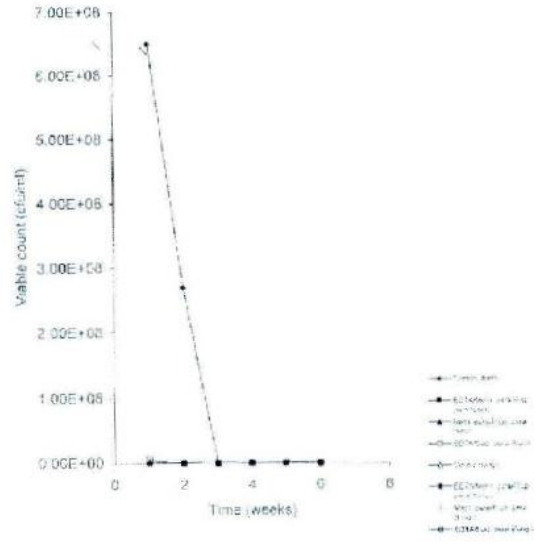


Fig. 1: Effect of preservative systems on the bacterial and fungal loads of herbs medicines (Sample 4)

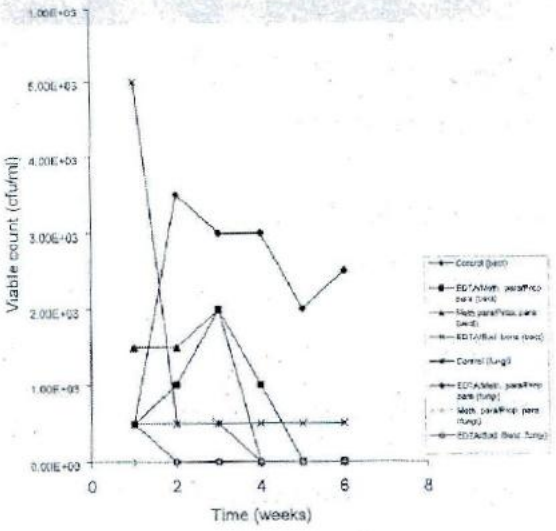


Fig. 3: Effect of preservative systems on the bacterial and fungal loads of herbs medicines (Sample 5)

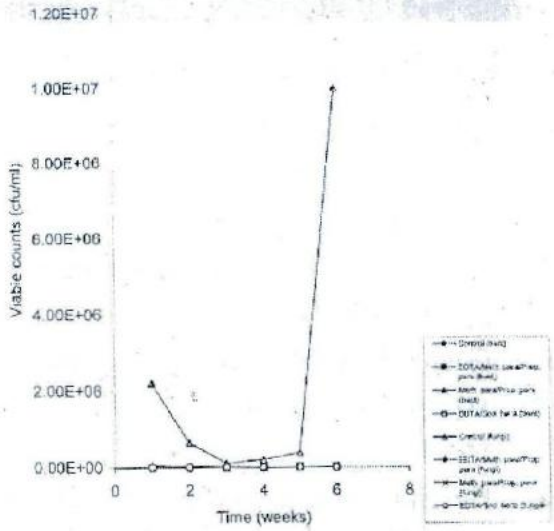


Fig. 1: Effect of preservative systems on the bacterial and fungal loads of herbs medicines (Sample 6)

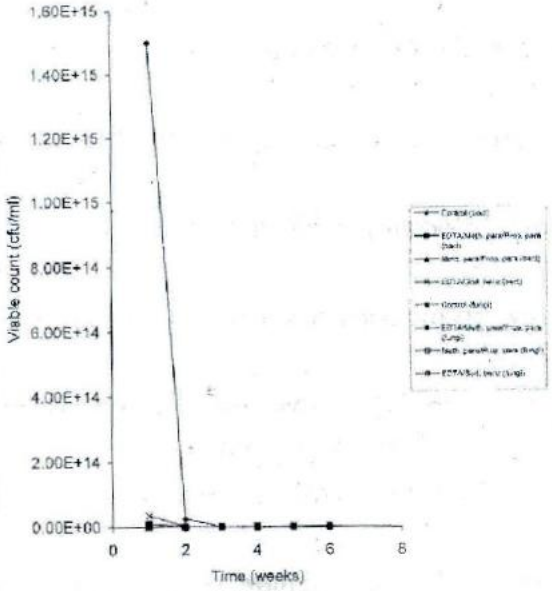


Fig. 8: Effect of preservative systems on the bacterial and fungal loads of herbs medicines (Sample 7)

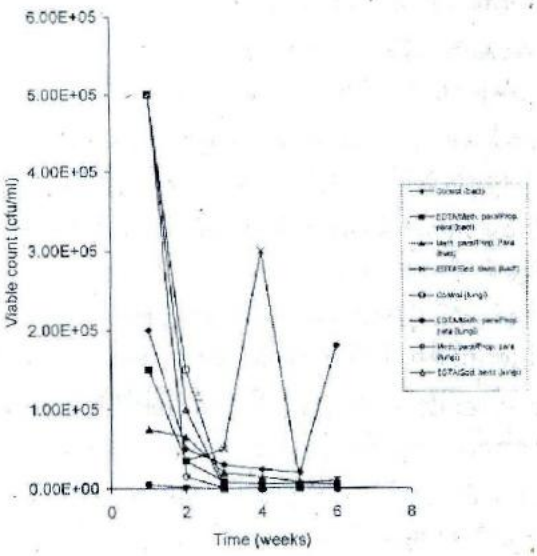


Fig. 8: Effect of preservative systems on the bacterial and fungal loads of herbs medicines (Sample 8)

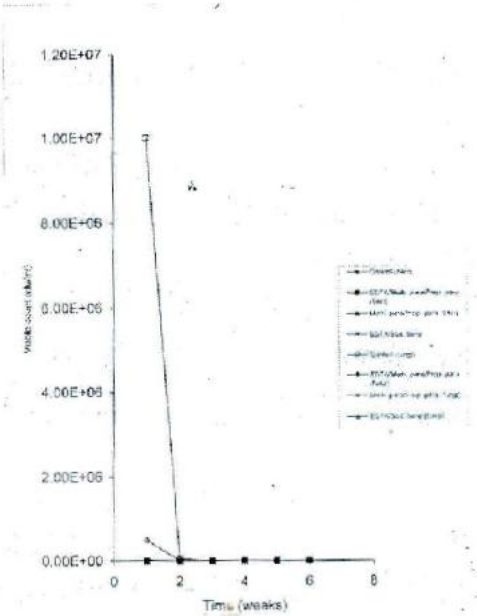


Fig. 9: Effect of preservative systems on the bacterial and fungal loads of herbs medicines (Sample 9)

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