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PROLONGED USE OF COUGH FORMULATIONS AND THE HEALTH RISK FROM THEIR ANTIMICROBIAL ACTIVITY ON SOME NORMAL BACTERIAL FLORA

***Adeleke, O. E., Alabi, O. S and Adetoyi, O. A. Department of Pharmaceutical Microbiology University of Ibadan, Ibadan, Nigeria.**

RUNNING TITLE: COUGH FORMULATIONS AGAINST SOME NORMAL BACTERIAL FLORA.

Correspondence: University of Ibadan, c/o P. O. Box 22039, Orita U. I. Post Office, Ibadan, Nigeria.
E-mail: adelezek@yahoo.com Mobile: 08023896439.

ABSTRACT

Cough formulations were observed to contain some chemical substances that have been associated with antimicrobial property, namely: menthol, honey, citric acid and volatile oils. A prolonged use of such formulations by patients was therefore considered a health risk on the normal bacterial flora. Nine cough formulations denoted by letter codes along with simple syrup B.P., absolute alcohol and sterile distilled water as controls, were investigated for relative antimicrobial activity on some normal flora bacteria by the agar-cup diffusion method. The respective individual single brands of cough formulation with the exception of one brand exhibited inhibitory activity against 5 - 1 2 bacterial isolates including *Escherichia coli, Klebsiella spp, Streptococcus faecalis, Strep. pneumoniae, Strep. viridians, Proteus mirabilis, Staphylococcus aureus and Pseudomonas aeruginosa.*

Simple Syrup B.P. and sterile distilled water did not exhibit any antibacterial activity while the absolute alcohol exerted activity only on *Staph aureus*. The antimicrobial activity recorded for the cough formulations could cause a depletion of the normal bacterial flora following a prolonged use of the formulations tested, hence, the attendant health risk of depressed natural immune system of the body, normally associated with such bacteria.

INTRODUCTION

Cough preparations are drugs, useful for the initiation of cough, serving either as cough suppressants in cases of non-productive cough associated with mucus secretions in the throat. Cough is recognized as an important physiological protective mechanism, particularly, while serving to clear the respiratory passage of foreign materials and excess secretions(1). Cough could however assume a pathological status when it presents as a symptom of an underlying disorder and becomes chronic. In such situations, the use of an appropriate cough formulation becomes highly imperative, though with the attendant implications in the events of a prolonged use. Cough formulations usually contain mixtures of antihistamines, decongestants, antitussives and expectorants (1), reflecting

their forms of activity but so far without established antimicrobial activity. Besides these combinations in cough mixtures, there are other chemical substances that have inhibitory activity on microorganisms, namely: menthol, citric acid, honey and volatile oils e.g. peppermint oil, aniseed and menthol. Ammonium salts and sodium citrate may also be present which along with citric acid would affect the pH of the formulations. In particular, honey and volatile oils (mixtures of esters, aldehydes, alcohols, ketones and terpenes) have been reported by various workers to have pronounced antimicrobial activity[2,3,4]. Certain herbs, also with demonstrable antimicrobial activity are known to be included in cough formulations e.g. bloodroot, eucalyptus, red clover and others. The presentation of cough formulations as oral preparations implicates their intimate contact with the normal bacterial flora against which the antimicrobial property of the relevant components of the formulations may be directed. Such bacteria are recognized for their role in the innate immune response of the body. In spite of this recognition, research effort has hitherto not been directed at a deliberate investigation of the extent of the antimicrobial activity of the cough formulations vis-a- vis its health risk on the normal bacterial flora consequent upon their prolonged administration. It was against this background that this study has been designed.

MATERIALS AND METHODS

BACTERIOLOGY

Bacterial isolates *of Staphylococcus aureus* (4 isolates), *Escherichia coli* (2 isolates), *Klebsiella* sp. 1 isolate), *Streptococcus faecalis* (1 isolate), *Strep. viridians, Strep pneumoniae* (2 isolates), *Proteus mirabilis* (3 isolates) and *Pseudomonas aeruginosa* (3 isolates) were collected from different clinical sources of throat, wound and sputum. They were confirmed by some established conventional methods: Salt tolerance on Mannitol Salt Agar, Catalase, Oxidase and Coagulase tests as well as Gram staining[10]. The bacteria were then preserved as slant cultures at 4°C in a refrigerator.

COUGH FORMULATION AND OTHER CHEMICALS

Nine cough formulations were purchased from some Pharmacy st ores in Ibadan, Nigeria. They were given letter codes as stated along with their respective chemical ingredients in Table 1. **Simple Syrup B.P:** It was prepared as described in the official monograph (B.P. 1980). Absolute alcohol: It was purchased from a local chemical supplier based in Ibadan, Nigeria.

ANTIMICROBIAL SUSCEPTIBILITY TEST

The agar-cup diffusion as described by Singleton[5] was used for assessing the susceptibility of the bacterial isolates to each of the nine cough formulations and also,

Simple Syrup B.P., absolute alcohol and sterile distilled water as controls. MS-2 that contained only ammonium chloride and ammonium bicarbonate as chemical additives was brought into mixture in ratio 1:1 with each of the other 7 cough formulations. The resulting mixtures were then similarly assessed for antimicrobial activity against the same bacterial isolates. Simply, the method involved digging holes (or wells) in solid culture media that have been seeded with each of the bacterial isolates. The wells were then filled with the respective cough formulations and the controls. After a 24-hrs period of incubation at 37°C, zones of growth inhibition produced were measured to determine bacterial sensitivity or resistance (Tables 3 & 4).

RESULTS

Amongst the 9 cough formulations tested (Table 1), MS - 1 brand in plastic container did not show antimicrobial activity against any one of the bacterial isolates tested, just like the controls except the absolute alcohol which exhibited activity only on one *Staph. aureus* isolate (Table 2). The Zones of growth inhibition produced by the cough formulations varied from 8mm to 30mm. TL brand had the widest spread of antibacterial activity against 13 isolates including at least one isolate of every bacterial sp. tested, followed by CF brand (9 isolates), CT brand (8 isolates), BN brand (6 isolates), EZ and MS - 2 brands (5 isolates each) and, ZP and DK brands (4 isolates each) (Table 2). However, decreased antimicrobial activity was noticeable for the mixtures of 7 cough formulations each with MS - 2 brand. Exceptionally, BN, CF, ZD, CF and DK brands and also absolute alcohol, each in combination with the MS - 2 brand produced higher activity against *Staph. aureus* than when each of the formulations was tested alone. Similar trend obtained with TL and CF brands against one *Proteus mirabilis* isolate (Table 3). Remarkably, Simple Syrup B. P. in combination with the same MS - 2 brand exhibited antistreptococcal activity which was absent when Sterile Syrup B. P. was tested alone.

TABLE 1: THE COUGH SYRUPS (IN LETTER CODES) AND THEIR CHEMICAL CONSTITUENTS AS STATED ON THEIR LABELS.

EZ :	**Diphenhydramine, ammonium Chloride, Sodium citrate and menthol.**
BN :	**Diphenhydramine and ammonium chloride**
CT :	**Ammonium chloride, Ipecacuanha liquid extract, Liquorice extract, pepermint oil and aniseed oil.**
ZD :	**Bromhexine, dextromethorphan, ammonium chloride, menthol, flavoured syrupy base.**
CF :	**Chlorphemiramine, ammonium chloride, Sodium citrate, menthol and ephedrine.**
DK :	**Diphenhydramine, bronhexine, ammonium chloride, Sodium Citrate, and menthol.**
TL :	**Diphenhydramine, ammonium chloride, trisodium citrate, Citric acid, menthol and flavoured Syrup base.**
MS- 1:	**Ammonium Chloride and ammonium bicarbonate (in plastic container).**
MS - 2:	**Ammonium Chloride and ammonium bicarbonate (in glass container).**

TABLE 2: THE ANTIMICROBIAL ACTIVITY OF COUGH FORMULATIONS ON SOME CLINICAL ISOLATES

Zones of growth inhibition (mm)												
ORGANISM	**EZ**	**BN**	**CT**	**ZD**	**CF**	**DK**	**TL**	**MS-1**	**MS-2**	**Syrup BP**	**Alcohol**	**Dist. water**
SA_1	**15***	**-**	**12**	**-**	**-**	**-**	**-**	**-**	**15**	**-**	**-**	**-**
SA_2	**15**	**-**	**16**	**-**	**-**	**-**	**-**	**-**	**10**	**-**	**-**	**-**
SA_3	**-**	**-**	**-**	**-**	**-**	**10**	**12**	**-**	**-**	**-**	**-**	**-**
SA_4 Typed	**17**	**16**	**16**	**-**	**18**	**16**	**17**	**-**	**-**	**-**	**-**	**-**
PA_1	**15**	**-**	**19**	**30**	**-**	**15**	**-**	**-**	**14**	**-**	**-**	**-**
PA_2	**-**	**-**	**-**	**-**	**17 (RM)**	**-**	**12**	**-**	**-**	**-**	**-**	**-**
PA_3 Typed	**-**	**-**	**-**	**-**	**14 (RM)**	**-**	**13**	**-**	**-**	**-**	**-**	**-**
PM_1	**-**	**-**	**12**	**30**	**-**	**-**	**-**	**-**	**12**	**-**	**-**	**-**
PM_2	**-**	**-**	**11**	**30**	**-**	**-**	**-**	**-**	**12**	**-**	**-**	**-**
PM_3	**-**	**-**	**-**	**-**	**-**	**-**	**13**	**-**	**-**	**-**	**-**	**-**
EC_1	**-**	**-**	**-**	**-**	**29 (RM)**	**-**	**13**	**-**	**-**	**-**	**-**	**-**
EC_2	**-**	**10**	**9 (IM)**	**8 (IM)**	**14**	**-**	**13**	**-**	**-**	**-**	**-**	**-**
SP_I	**-**	**-**	**-**	**-**	**9 (IM)**	**-**	**12**	**-**	**-**	**-**	**-**	**-**
SP_2	**-**	**10**	**-**	**-**	**-**	**-**	**13**	**-**	**-**	**-**	**-**	**-**
SV	**9 (IM)**	**9 (IM)**	**10 (IM)**	**-**	**10 (IM)**	**-**	**14**	**-**	**-**	**-**	**-**	**-**
SF	**-**	**9 (IM)**	**-**	**-**	**9 (IM)**	**-**	**13**	**-**	**-**	**-**	**-**	**-**
KL Typed	**-**	**14**	**-**	**-**	**13**	**15**	**16**	**-**	**-**	**-**	**-**	**-**

KEY

SA = *Staph. aureus*
PA = *Pseud. aeruginosa*
PM = *Proteus mirabilis*
EC = *Esch. coli*
SP = *Strep. Pneumoniae*
SV= *Strep. viridians*
SF = *Strep. faecalis*
KL = *Klebsiella sp.*
Rm = Resistant mutant (few discrete colonies within the Zone)
IM = Intermediate
\- = Resistant (No Zone of growth inhibition)
* = Zone of growth inhibition in mm.

TABLE 3: ANTIMICROBIAL ACTIVITY OF COUGH FORMULATIONS EACH IN COMBINATION WITH MS - 2 BRAND ON SOME CLINICAL ISOLATES

Zones of growth inhibition (mm)										
ORGANISM	**EZ**	**BN**	**CT**	**ZD**	**CF**	**DK**	**TL**	**Syrup BP**	**Alcohol**	**Dist. Water**
SA_1	**15***	**14**	**-**	**14**	**12**	**15**	**-**	**-**	**10**	**-**
SA_2	**15**	**18**	**14**	**16**	**14**	**20**	**-**	**-**	**13**	**-**
SA_3	**-**	**-**	**-**	**-**	**-**	**-**	**10**	**-**	**-**	**-**
SA_4	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
PA_1	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
PA_2	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
PA_3 Typed	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
PM_1	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
PM_2	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
PM_3	**-**	**-**	**-**	**-**	**24**	**-**	**25**	**-**	**-**	**-**
EC_1	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
EC_2	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
SP_1	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**8 (IM)**	**-**	**-**
SP_2	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
SV	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**9 (IM)**	**-**	**-**
SF	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**9 (IM)**	**-**	**-**
KL Typed	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

KEY

SA = *Staph. aureus*
PA = *Pseud. aeruginosa*
PM = *Proteus mirabilis*
EC = *Esch. coli*
SP = *Strep. pneumoniae*
SF = *Strep. faecalis*
SV = *Strep. viridians*
KL = *Klebsiella* sp.
IM = Intermediate
- = Resistant (No Zone of growth inhibition)
* = Zone of growth inhibition in mm.

DISCUSSION

The inhibitory effect of certain chemical substances - menthol, citric acid, honey, volatile oils and others, contained in cough formulations on microorganism is well recognized [6,7,8,9] yet, a deliberate effort directed at determining the extent of such antimicrobial activity offered by whole cough formulations and its health risk with respect to the human body normal bacterial flora in the event of their prolonged use by patients, has hitherto been lacking. It is interesting to note in this study the varying levels of antimicrobial activity recorded for the cough formulations tested individually but remarkably for TL, CF, CT and BN brands. Of further interest was the observation that 5 cough formulations (DK, CF, ZD, CT and BN brands) had their antistaphylococcal activity increased when combined each with MS - 2 brand. This improved combination antibacterial effect was also observed for TL and CF brands against *Streptococcus pneumoniae* as well as for Sterile Syrup B.P. Remarkably none of *Pseud.* and *Esch.* spp.

was sensitive to any one of the cough formulations in combination with MS - 2.

The bacteria used in this study are among the normal bacterial flora of man, occurring variously in mouth, throat, pharynx and gut within which they perform host body defense against microbial infections[5]. This indication suggests a depletion of these microbes by the antimicrobial activity of cough formulations, particularly, when subjected to prolonged use by patients. The health risk involved becomes worrisome when cough formulations have to be taken concomitantly with some antibiotics usually in the conditions of microbial infections that may present cough as a symptom. The awareness generated in this study on the health risk in the prolonged use of cough formulations should form part of health education to patients.

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