Effect of compression pressure, preservative, and storage with Potassium Chloride on the microbiological quality of tablets formulated with *Terminalia randii* Gum (Combretaceae)

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Abstracts: Gums are used as binders in tablets and also as emulsion stabilisers, suspending agents and thickeners in syrups. The need for other natural gums apart from the conventional gums to be employed as binding agents in tablets formulation led to this study. A gum obtained from the incised trunk of *Terminalia randii* (Combretaceae) was evaluated for the effect of compression pressure, Methyl Paraben preservative and storage with Potassium Chloride, on the microbial load of tablets formulated with the gum. The microbial load was determined by surface spread method on the processed gum at suitable dilutions, and tablets formulated from the gum at different compression pressures. The formulated tablets were evaluated for microbial load, also when stored in Potassium Chloride for 8 and 12 weeks with and without preservation with 1% Methyl Paraben. In each case the compressed tablets were incubated in 0.1% peptone water as control.

The microbial load recorded reflected generally, reduction in microbial counts in tablets formulated with the gum as a binder both in terms of compression at different pressures and when the different compression pressures were associated with or without 1% Methyl Paraben in the presence of Potassium Chloride. Comparatively, the processed gum showed higher microbial load than the pressure compressed tablets. Besides the different compression pressures, duration of storage was also found to cause reduction of microbial load, particularly in the formulated tablets compressed with Methyl Paraben stored in Potassium Chloride such that after 8 weeks, the microbial load was zero. The studies showed that compression pressures and duration of storage caused marked reduction in microbial load of the tablets formulated with the processed gum of *Terminalia randii* as a binder.

Keywords: Compression pressure, preservative, potassium chloride, microbial quality.

INTRODUCTION

Tablets are compacts drug delivery systems with low water content which usually afford them good protection against microbial contamination. However, there have been substantial numbers of reports of tablets spoilage and clinical infections resulting from microbial contamination, especially under hot and humid conditions of the tropics (Akerele & Ukoh, 2002; Obuekwe *et al.,* 2000; Enayatifard *et al.,* 2010; Mugoyela and Mwabete, 2010). Tablets also undergo deleterious changes when improperly stored. Such changes include discolouration, weakening of tablets matrixes and decreases in the potency of active ingredients (Bos *et al.,* 1989). Potential contamination of tablets may arise from heavy microbiological burden in raw materials, though this is usually drastically reduced by lethal drying stage of wet granulation (Odeku *et al.,* 1999). However, the decreasing use of direct compression in manufacturing of tablets in pharmaceutical industries implies that some contaminants may survive up to the compression stage. The compression of formulation is known to effect some level of microbial destruction but this depends on the compression pressure applied, the properties of the contaminating organisms, and the formulation involved (Blair *et al.,* 1991; Plumpton *et al.,* 1986**)**. The effect of these variables in turn are believed to depend on the mechanism of microbial kill which have been proposed to include to a lesser or greater extent high localized heat shearing forces during compression (Plumpton *et al.,* 1986; Fasihi *et al.,* 1978). The shear stresses manifested during compression depend largely on the principal mode of consolidation of the formulation which can be by fragmentation of plastic flow, with plastic having been shown to be a highly effective mechanism for microbial kill even at low compression pressures (Blair *et al.,* 1991; Comoglu *et al.,* 2002). Binding agents employed in formulations are known to undergo a high degree of plastic deformation during compression and are forced into their inter-particulate spaces where they increase the area of contact between the particles and form strong solid bounds. Previous report had also established that gums when used as binders can cause a high degree of microbial kill during tableting (Odeku *et al.,* 1999). Gums are adhesive substances that are carbohydrate in nature and usually considered to be pathological products

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formed upon injury of the plant (back of trees or shrubs) or owing to unfavourable conditions such as drought, by a breakdown of cell walls (Evans, 2001). On hydrolysis, they yield a mixture of sugars and uronic acids. Plant gums are used in pharmaceuticals as binders in tablets, thickeners in syrups, emulsion stabilizers, suspending agents etc (Exell, 1978). Raw materials account for a high proportion of the micro organisms introduced in the manufacture of pharmaceuticals, and selection of materials of good microbial quality aids in the control of contamination levels in both products and environment (Hugo and Russell, 1992). Untreated raw materials which are derived from a natural source usually support extensive and varied microflora. The microflora of materials of plants origin such as gum acacia and tragacanth agar may arise from that indigenous to plant and may include bacteria such as *Pseudomonas* spp, *Bacillus* spp, and *Lactobacillus spp*. British Pharmacopoecia requires freedom of such materials from *Escherichia coli* and *Salmonella spp* and a total viable count not exceeding 10^4 colony forming units per gram (10^4 cfu/g), before they can be used in the preparation of pharmaceutical products (British Pharmacopoiea, 1998). This study therefore, investigates the microbial quality of *Terminalia randii* gum as a binding agent in tablet formulation.

MATERIALS AND METHODS

Materials

The materials used in this work were Potassium Chloride (BDH), Methyl Paraben, Nutrient agar (LAB M^{TM}), Mannitol salt agar (LAB M^{TM}), Sabouraud Dextrose agar (LAB M^{TM}), Salmonella-Shigella agar (LAB M), McConkey agar (Oxoid), and Peptone water. Raw *Terminalia randii* gum was collected from the wounded Terminalia tree in Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria.

Methods

The raw *Terminalia randii* gum was dried and hydrated in double strength chloroform water for five days with intermittent stirring. The extraneous materials were removed by straining through a calico cloth. The gum was precipitated from solution using absolute ethanol. The precipitated gum was filtered, washed with the ethyl ether and then dried in a hot air oven at 40^{o}C as previously described. (Odeku *et al.,* 1999)

Microbial content of the gum: 0.1gm of the gum was suspended in 20ml of nutrient broth incubated at 37^{o}C in a thermostated Gallen Kamp incubator for 24hours. Six ten-fold serial dilutions were made in sterile distilled water and the last two dilutions (10^{-5} and 10^{-6}) were used for the microbial count using surface viable method. By means of a sterile pipette, 0.2ml of each dilution was placed on the surface of ss dried 20ml nutrient agar plates and spread by means of a sterile glass spreader, the plates were incubated at 37^{o}C for 24 hours. The count was done using a bacteriological digital colony counter, and the results expressed as cfu/g. In addition, 0.2ml of the final dilution was placed each in 2 Petri dishes (20ml) of prepared sterile McConkey agar and incubated for 24hours at 37^{o}C after which they were evaluated for the presence of *Escherichia coli* and *Salmonella* spp. The diluted sample of the gum was also plated into Mannitol salt agar, Sabouraud Dextrose agar, Salmonella-Shigella agar and appropriately incubated. The organisms that grew on the different plates were identified.

Microbial content of gum tablets: Tablets, each weighing 300mg of the gum were panelled on Carver laboratory press (Model C) using a die width of 10.5mm, compression time of 30secs and lubricant composition of magnesium stearate and ethanol. Each set of tablets was compressed at different pressures (0.25, 0.5, 0.75, 1.0 and 1.5 tonnes). Each of the tablets was immediately transferred into 10ml sterile peptone water and dissolved to form a suspension after which 0.1ml of the suspension was placed in 20ml cooled, molten nutrient agar, mixed and poured into sterile Petri dishes under aseptic conditions to prevent contamination from air. The plates were incubated at 37^{o}C for 24hours after which microbial count was performed on them. Two tablets (600mg) each for different pressures were suspended in 10ml sterile peptone water and incubated at 37^{o}C for 48hours (to permit the growth of partially damaged cells) in a thermostated incubator. After incubation, six ten-fold serial dilutions were made in sterile distilled water and 0.2ml of the 10^{-5} and 10^{-6} dilutions were placed and spread on the surface of set and dried sterile nutrient agar plates, and incubated for 24hours at 37^{o}C after which the microbial count was done on each plate and the total viable count expressed as cfu/g per tablet. 0.2ml each of the 10^{-6} dilutions was also placed on both sterile McConkey agar plates and sterile Sabouraud Dextrose agar. McConkey agar plates were incubated at 37^{o}C for 24hours while Sabouraud dextrose agar plates were incubated at 25^{o}C for 7 days to detect the presence of fungi.

Storage studies: Some tablets of the gum were stored for 8 weeks and some for 12 weeks at high relative humidity in a chamber containing Potassium Chloride to mimic tropical humid conditions. The tablets were checked for microbial content at the end of 8 weeks and 12 weeks. Also, a 1% concentration of Methyl Paraben (preservative) was incorporated during compression of some tablets and tablets were checked for microbial count after 8 weeks and 12 weeks storage at a high relative humidity in chamber containing Potassium Chloride.

Identifications of microorganisms: Gram staining was done on the isolated organisms and the slides viewed

under oil immersion lens of the microscope. Biochemical test such as catalase, oxidase, and indole tests were also carried out to confirm the identity of the isolated organisms.

STATISTICAL ANALYSIS

Statistical analysis was done using ANOVA (GraphPad Software Incorporation, San Diego, USA). At 95% confidence interval, p values of ≤ 0.05 were considered significant.

RESULTS

Microbial tests on *Terminalia randii* gum obtained in this study showed the mean viable count to be 2.94×10^{10} cfu/g of material. Microbial content of the compressed tablets immediately after compression was lower than that of gum as indicated in table 1, while the microbial count of compressed tablets after 48hours of incubation was found to be higher than that of the immediately compressed tablets. Microbial content of gum tablets decrease with increase in compression pressure. *Escherichia coli* was found to be present while *Salmonella* spp. was absent. The storage studies showed a sharp reduction in microbial count after 8 weeks of storage with Potassium Chloride without preservative, while there was zero microbial count with preservative (table 2). Twelve weeks storage of the compressed tablets with Potassium Chloride showed zero microbial count with and without preservative (table 3).

DISCUSSION

The British Pharmacopoeia (1998) requires that natural gums should meet some microbiological standards such as the absence of pathogens such as *Escherichia coli* and *Salmonella* spp. The value of 2.94×10^{10} cfu/g for *Terminalia randii* gum is significantly ($p<0.05$) higher than the official value of 1×10^{4} viable cells per gram of material specified for acacia. The reduction in the microbial content of freshly prepared tablets of the gum may be associated with the effect of compaction leading to inactivation of the micro-organisms on the surfaces of the tablets (Blair *et al.,* 1991; Ohiri *et al.,* 1997). Some level of microbial destruction seen to be effected by the compression on the formulation depends on the compression pressure applied in line with earlier reports (Plumpton *et al.,* 1986; Blair *et al.,* 1991). This was evident in each case of the results as the microbial content of the compressed tablets of the gum decreased with increased compression pressure. The microbial content of the gum tablets immediately after compression was significantly ($p<0.05$) reduced from 2.10×10^{4} cfu/g (0.25tonnes compression pressure) to 0.7×10^{4}cfu/g (for 1.5 tonnes compression pressure) which is below the official limit of 10^{4} cfu/g. This shows the efficacy of the compression pressure used in this study to reduce microbial counts in tablets. The higher values of microbial content of tablets incubated in peptone water for 48 hours than those tablets evaluated immediately after compression may be because the 48 hours incubation in peptone water permitted the growth of partially

Table 1: Mean microbial viable count of compressed tablets of *Terminalia randii* gum (cfu/g)

Tablet treatments	Compression Pressure (tonnes)				
	0.25	0.50	0.75	1.00	1.50
Tablets immediately after compression	2.10×10^{4}	1.33×10^{4}	1.20×10^{4}	1.10×10^{4}	0.7×10^{4}
Tablets after 48hrs incubation	2.2×10^{10}	0.52×10^{10}	0.47×10^{10}	0.33×10^{10}	0.13×10^{10}

Table 2: Microbial content of *Terminalia randii gum* tablets stored with Potassium chloride for 8 weeks

Tablet treatments	Compression Pressure (tonnes)				
	0.25	0.50	0.75	1.00	1.50
Mean viable count of tablets stored without preservative (cfu/g)	3.60×10^{3}	3.00×10^{3}	2.50×10^{3}	2.33×10^{3}	2.17×10^{3}
Mean viable count of tablets stored with preservative (cfu/g)	–	–	–	–	–

Table 3: Microbial content of *Terminalia randii* gum tablets stored with Potassium chloride for 12 weeks

Tablet treatments	Compression Pressure (tonnes)				
	0.25	0.50	0.75	1.00	1.50
Mean viable count of tablets stored without preservative (cfu/g)	–	–	–	–	–
Mean viable count of tablets stored with preservative (cfu/g)	–	–	–	–	–

damaged cells in the course of the compression of the tablets. However the reduction in microbial content of tablets stored with potassium chloride for 8 weeks may be due to concentration gradient between the tablets of the gum and Potassium Chloride since diffusion will always proceed whenever a concentration gradient exists as previously reported (Roberts, 1994). Thus, water molecules in the tablets of the gum diffuse out towards the Potassium Chloride because the potential energy of the water molecules (water potential) in the tablets of the gum (with lower osmotic pressure) is greater than the potential energy of the water molecules in the Potassium Chloride (with higher osmotic pressure). Diffusion will continue until eventually the particles of the molecules are uniformly distributed throughout the system, at which time equilibrium is said to be reached. This is evident after 12 weeks of storage in which case the water content of the gum at equilibrium was not high enough to support the growth of micro-organisms, thus resulting in completely sterile tablets of the gum at all pressures. It has been reported that tropical conditions characterised by high relative humidity and temperatures could affect the microbial stability of tablets (Fasihi *et al.,* 1978; Bos *et al.,* 1989). The availability of water, in combination with elevated temperatures, and the presence of suitable substances created a favourable condition for microbial growth. The removal of water molecules with Potassium Chloride and storage at room temperature in this study discouraged the growth of micro-organisms thereby favouring microbial stability of the tablets. The addition of preservative (methyl paraben) in some batches of the gum tablets, together with the effect of the compression pressures and the storage with Potassium Chloride have apparently contributed to the destruction of the pathogens contained in the raw gum and in prevention of microbial growth, resulting in completely sterile batches of tablets after 8 weeks and 12 weeks of storage. However the choice of preservative will depend on such factors like compatibility with drug and other excipients (Ohiri *et al.,* 1997).

CONCLUSION

From the foregoing, it is recommended that *Terminalia randii* gum could be used as binding agent in tablet formulations, if it is freshly processed and adequately protected. This could be achieved by the addition of a suitable preservative into the granules prepared with the gum. Methyl paraben was found to be an effective preservative for the tablets. Reduction of the moisture content of the gum (before use) and/or the formulation could also favour the microbial content. Furthermore, tablets compressed with the gum should be well packaged to prevent absorption of water during storage.

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