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In vitro Evaluation of Lozenges Containing Extracts of Roots of *Zapoteca portoricensis* (FAM: Fabaceae)

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Abstract: The aim of this research was to formulate *Zapoteca portoricensis* root extract as Lozenges and to evaluate some of their antimicrobial and tablet properties. The root extracts were formulated into Lozenges using either Sodium Carboxy Methyl Cellulose (SCMC) or Carboxy Methyl Cellulose (CMC) as binders. Uniformity of weight, crushing strength, microbial sensitivity and pre-extinction time studies (using *E. coli*, *S. aureus* and *Candida albicans*) were conducted on three Lozenges formulated with either SCMC (Batch A), CMC (Batch B) and a reference standard, Dequadin^{R}, containing dequalinium hydrochloride (Batch C). Results showed that Batches B and C passed the weight uniformity test. The three batches had mean crushing strengths of 4.86±0.043, 3.9±0.03 and 13.1±0.43 KgF, respectively for A, B and C. *S. aureus* and *Candida albicans* were sensitive to the test lozenges whereas *Escherichia coli* was not. *Candida albicans* was minimally sensitive to the standard lozenge, while *S. aureus* was not. Both the test and the standard samples showed extinction times greater than 30 min.

Key words: Lozenges, *Zapoteca portoricensis*, antimicrobial, microorganisms, herbal formulation

INTRODUCTION

Pathogenic micro-organisms constitute menace to human health and contribute about 30-40% of microbial infections suffered in the tropics (Aguwa, 1996). An antimicrobial agent is one that interferes with the growth and activities of micro-organism. This may be directed against specific groups of organisms such as bacteria, fungi, protozoa or viruses. Antimicrobial agents could be synthetic, semi synthetic or of natural origin (Okore, 2005). Antimicrobial properties are found to reside in lower and higher plant forms (Ojo *et al*., 2007; Ebi and Ofoefule, 1997; Njoku *et al*., 1999). There is increasing interest in natural antimicrobial agents due to toxicity and problems of resistance crowding the existing antimicrobial agents (Olaniyi, 2005).

Zapoteca portorcensis, family-Fabaceae is a woody herb widely distributed in tropical rain forest. Aqueous and alcoholic extracts of the leaf have been reported to be used in the treatment of gastro intestinal disorders, spasmodic and in the treatment of tonsillitis (Nwakile, 2004). Reported phytochemical analysis of the roots indicated the presence of a number of secondary metabolites such

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as saponins, resins, glycosides, flavonoids, alkaloids, terpenoids and steroids (Nwodo and Uzochukwu, 2006). Recently, the antimicrobial and anti-inflammatory activities of *Zapoteca portoricensis* have been authenticated in experimental models (British Pharmaceutical Codex, 1973). These preliminary results, coupled with the folkloric use of the plant in the treatment of tonsillitis suggest that *Zapoteca portoricensis* is a good candidate for formulation into lozenges for the possible treatment of mouth and throat infections. One of the commonest brands of throat Lozenge found in pharmacy shops in Nigeria is Dequalinium Chloride. Its formulation form includes other excipients that promote better taste or delayed disintegration (Encyclopedia of Pharmaceutical Technology, 2002), while possessing anti-bacterial (*Borrelia vincentii*) and anti-fungal (*Candida albicans*) properties . Dequalinium Lozenge (0.25 mg) is used in the treatment of infections of the gum, mouth and throat. Because of the high risk of misuse, this Lozenge is suspected to be liable to microbial resistance. Moreover, because it is sweetened, several people sometimes use it as sweet or candy.The objective of this study was to explore the formulation potentials of *Zapoteca portorcensis* as a suitable alternative to Dequalinium chloride Lozenges. Its physical and antimicrobial properties shall be evaluated.

Experimental

Materials

Methanol (Analar grade) (Fluka, Spain), Sucrose (Merck, Germany), Sodium Carboxy Methyl Cellulose (SCMC) (Merck,Germany), Carboxyl Methyl Cellulose (CMC) (Merck, Germany), Poly Vinyl Pyrollidone (Merck, Germany), Lactose (Merck, Germany), Sodium Chloride(BDH, England), Saliva (donated by one of the authors), nutrient agar (Fluka, Spain), Sabourand dextrose agar (Flukka, Spain), Nutrient broth (Fluka, Spain), Sabourand dextrose broth (LBBM™, UK). Stock cultures of *E. coli*, *S. aureus* and *Candida albicans* were obtained from the Microbiology Unit of the Department of Pharmaceutics, University of Nigeria, Nsukka, in September 2006.

Methods

Extraction

Cleaned roots were sun-dried and cut into smaller pieces prior to milling. Using cold maceration method, 100 g of the ground roots was soaked in 500 mL of methanol for two days with regular agitation and residue eluted twice using another 100 mL of methanol each time. Extracts were filtered with filter paper and filtrate evaporated to obtain a solid mass of extract.

Anti-Microbial Tests

Antimicrobial and antifungal properties of the extracts were evaluated using standard methods (Esimone *et al*., 2003, 2006).

Preparation of Lozenges

The formulation formular is as shown in Table 1. Exactly 200 mg of the moistened extract was mixed with sucrose, lactose and SCMC or CMC paste. This was triturated into a homogenous mass, filled into holes of a plastic mould and oven-dried at 40°C.

Uniformity of Weight/Crushing Strength

Ten Lozenges were randomly selected from each batch and tested for weight uniformity and crushing strength using a weighing balance and a Monsanto Hardness Tester (Manesty, England), respectively. The same was done for the reference standard.

Release of Extracts from the Lozenges

Release of extracts from the lozenges was evaluated via an *in vitro* antimicrobial pharmacodynamic protocol. From the three batches, a lozenge was placed inside a flask containing 25 mL of sterile saline-

saliva solution and shaken through out the experiment with a mechanical shaker (Griffin Gerald Co., England). About 1 mL of the solution was withdrawn at time intervals of 5, 10, 20, 30, 60 and 120 min. This was introduced into pre-bored holes or cups in nutrient agar (for bacteria) or sabouraud dextrose agar (for fungi) plates previously seeded with a standardized inoculum of bacteria (*S. aureus* or *E. coli*) or fungi (*C. albicans*). This was left in the cup for 15 min at room temperature (to allow for pre-diffusion of extracts) and later incubated for 24 h at 37°C for bacteria and 27°C for fungi. A fresh normal saline-saliva mixture was similarly introduced into appropriate cup as a negative control. The zones of inhibition were later observed visually and measured.

Pre-extinction Time Studies

A lozenge was introduced into a flat-bottomed flask containing 25 mL of sterile saline-saliva solution and shaken for 30 min in a mechanical shaker. Using a sterile 2 mL eedle/syringe, 1 mL was sampled out in duplicate. While 1 mL was mixed with a reaction mixture containing *S. aureus* and 2 mL of nutrient broth, the other 1 mL was mixed with a reaction mixture containing *Candida albicans* suspension and Sabourauds dextrose broth. A loop-full was taken from each of these mixtures and transferred to 5 mL of the appropriate recovery medium (nutrient broth or Sabouraud dextrose broth) in a test tube at various time intervals ranging from 2 to 30 min. The recovery media were incubated for 24 h (*S. Aureus*) or for 48 h (*Candida albicans*) after which the tubes were observed for signs and degree of microbial growth.

RESULTS AND DISCUSSION

The percentage yield of the extract was calculated to be 6.2%.

Uniformity of Weight

Table 1 shows the weight uniformity test results. According to the British Pharamacopoiea (B.P) (2004), not more than two of the individual weights should deviate from the average by more than 5% and none should deviate by more than twice that percentage. It was only batch B (Lozenges formulated with CMC) that passed this test while A and C failed, based on B.P specifications (British Pharmacopoiea, 2004). This may be attributed to formulation, drying, or trituration/mixing deficiencies. Weight Uniformity is an important quality control parameter that ensures that the formulation weight is actually the intended weight. This guarantees that the ingredients were not only accurately weighed but well blended into a homogenous mass. It is probable that tablet batch that fails weight uniformity test may fail content uniformity test. In the production of antibacterial lozenges, negative deviation from the mean may imply suboptimal quantity of the antibacterial agent which may introduce drug resistance.

Table 1: Weight uniformity test for lozenges formulated with SCMC or CMC and the standard dequadin®

	SCMC total weight = 11200 mg mean weight = 1120 mg A		CMC total weight = 12080 mg mean weight = 1208 mg B		Dequadin total weight = 11300 mg mean weight = 1130 mg C	
Lozenges	Weight (mg)	Deviation (%)	Weight (mg)	Deviation (%)	Weight (mg)	Deviation (%)
1	1200	7.1	1200	0.7	1050	7.1
2	1100	1.8	1200	0.7	1100	2.7
3	1300	16.1	1150	4.8	1100	2.7
4	1200	7.1	1200	0.7	11200	6.2
5	1000	10.7	1260	4.3	1150	1.8
6	1100	1.8	1200	0.7	1150	1.8
7	1200	7.1	1200	0.7	1200	2.7
8	950	15.2	1200	0.7	1200	6.2
9	980	12.5	1250	3.5	1050	7.1
10	1100	1.8	1200	0.7	1200	6.2

Crushing Strength

As shown in Table 2, the highest crushing strength was recorded by batch C (Dequadin®), followed by A. The high crushing strength exhibited by the standard lozenge may be attributed to the compression method adopted as against the mould method employed in our investigation. A crushing strength of 4 kg F is an industrially acceptable value (14).

Antimicrobial Evaluation

From Inhibition Zone Diameter (IZD) values obtained, as shown in Table 3, *Zapoteca portoricensis* had more antifungal activity (against *Candida albicans*) than antibacterial (against *S. aureus*), this was markedly evident in batch A Lozenges and least in batch C. Batch C (Dequadine®)) showed no antibacterial activity and little antifungal effect. Since Dequalinium is known to have activity against gram negative and positive bacteria and *Candida albicans* (Nwodo and Uzochukwu, 2006), the above results obtained may be attributed to very minimal release of active drug below the MIC. The slow or no release in turn may be due to the high mean crushing strength of 13.1 recorded by this batch of Dequadine® lozenges. High crushing strength has been reported to cause slow or delayed release of drug (Obitte, 2001).The poor or no zone of inhibition recorded may also be due to resistance or inactivity against *S. aureus* and *Candida albicans*. Batch B, containing CMC, recorded peak zone of inhibition within 20-30 min, while batch A did so at the 120th min. The later (A) that was formulated with SCMC experienced a little delayed release of drug approximately

Table 2: Crushing strength test results for lozenges formulated with SCMC or CMC and the standard lozenges (dequadin®)

	Crushing strength (kg f^{-1})		
Lozenges	SCMC A	CMC B	Dequadin C
1	5.0	3.8	10.8
2	4.9	4.0	13.5
3	4.8	4.0	12.5
4	5.0	3.9	14.5
5	4.6	3.7	10.8
6	4.9	3.9	13.0
7	4.7	4.0	14.0
8	5.0	3.9	14.0
9	4.8	3.9	14.5
10	4.8	3.9	13.0
Mean	4.86±0.043	3.9±0.03	13.1±0.43

Table 3: Inhibition zone diameter test for test drug

	Mean inhibition zone diameter (mm)	
	Lozenges formulated with SCMC	
Time (min)	*Staph aureus*	*Candida albicans*
5	10.00±0.14	12.25±0.32
10	10.75±0.45	14.50±0.21
20	11.75±0.35	14.45±0.80
30	12.00±0.28	14.75±0.95
60	12.25±0.42	14.40±0.21
120	13.00±0.71	16.00±0.33
Lozenges formulated with CMC		
5	12.00±0.23	12.00±0.43
10	13.25±0.48	14.75±0.99
20	14.50±0.86	15.75±0.78
30	14.50±0.62	17.00±0.19
60	13.50±0.55	14.50±0.59
120	13.00±0.28	13.50±0.83

Table 4: Inhibition zone diameter results for the standard drug (dequadin®)

	Mean inhibition zone	
	Diameter of standard drug (mm)	
Time (min)	*Staph aureus*	*Candida albicans*
5	NA	FAINT
10	NA	8.00±0.12
20	NA	8.75±0.17
30	NA	8.50±0.24
60	NA	7.50±0.18

NA: No activity

Table 5: Preextinction time results of test and standard drug

	TIME (min) of contact organism with drug									
	Test lozenges (*Z. portoricensis*)									
	0	2	4	6	8	10	15	20	25	30
Test organisms										
Staph aureus	+++	+++	+++	+++	+++	+++	++	++	++	+
Candida albicans	+++	+++	+++	+++	++	++	++	++	+	+
	Standard lozenges (dequadin®)									
Staph aureus	+++	+++	+++	+++	+++	+++	+++	+++	+++	++
Candida albicans	+++	+++	+++	+++	+++	+++	++	++	++	+

+++: Highly turbid (showing growth), ++: Moderately turbid, +: Turbid

evidenced by the peak IZD probably due to polymer property of SCMC. Upon hydration of the tablet, SCMC forms a gel layer which appears to restrict further permeation of fluid (Obitte, 2001; Chukwu, 1994). Present observation therefore is that *Zapoteca portoricecsis* lozenges formulated with CMC may ensure a more rapid release of the active plants extract.

Pre-Extinction Time

Significant kill, evidenced by growth reduction was witnessed with the test Lozenge against *S. aureus* at the 15th min and Candida albican at the 8th min. For Dequadine®) (reference drug) it was at the 30th min and 15th min, respectively against *S. aureus* and *Candida albican*. The extinction times of test drug against *S. aureus* and *Candida albicans* were predictably above 30 min. The reference drug only had extinction time above 30 min against *Candida albican*. Based on the antimicrobial studies, we did establish that the reference drug had no activity against *S. aureus*, although there was minimal activity against *Candida albican*. This result is being reinforced by the >30 min extinction time against *Candida albican* and moderate turbidity showing reduced activity against *S. aureus*. For the test drug higher activity against Candida albicans and *S. aureus* is also being corroborated and reinforced (more than the reference drug) by the extinction times of >30 min. Since within the experimental period the highest activity only indicated growth reduction instead of absence of turbidity, it is obvious that the test lozenge showed bacterio/fungistatic activity against the organisms unlike the reference lozenge that showed only fungistatic activity against *C. albicans*.

CONCLUSION

The results of this work show that the extract of *Zapoteca portoricensis* can favourably compete with Dequadin^{R}, a branded Lozenge in the market, when formulated into lozenges for intended antimicrobial and antifungal effect against susceptible organisms associated with buco-laryngo-oesophagal infections. It is therefore recommended that pharmaceutical manufacturers could go into the commercialization *Zapoteca portoricensis* root extract as formulated Lozenges since the plant can easily be sourced and processed.

REFERENCES

Aguwa, C.N., 1996. Therapeutic Basis of Clinical Pharmacy in the Tropics. 2nd Edn. Enugu, SNAAP press (Nig.) Ltd, pp: 135.

British Pharmaceutical Codex, 1973. London. The Pharmaceutical Press, pp: 145: 730-734.

British Pharmacopoiea, 2004. The staionery office, UK., IV: A444-447.

Chukwu, A., 1994. Studies on Detarium microcarpium gum, investigation as a prolonged release matrix for encapsulated Chlorpheniramine maleate. STP Pharma. Sci., 4 (6): 401-405.

Ebi, G.C. and S.I. Ofoefule, 1997. Investigation into the Folklore Antimicrobial Activities of Landolphia Owerriensis. Phyt. Res., 11: 149-151.

Encyclopedia of Pharmaceutical Technology, 2002. Marcel Dekker inc. USA. Swarbrick, J. and J.C. Boylan (Eds.). 2nd Edn., 2: 1686-1688.

Esimone, C.O., I.M. Ebebe, K.F. Chah and C.G. Onyeka, 2003. Comparative antibacterial effects of Psidium guajava aqueous extract. J. Trop. Med. Plants, 4 (2): 185-189.

Esimone, C.O., N.J. Nwodo, I.R. Iroha and N.R. Ogbuefi, 2006. Antifungal Screening of the Methanolic Extract of the Lichen *Ramalina farinacea* (L.) Ach. Afri. J. Pharmaceut. Res. Develop., 2 (2): 1-6.

Njoku, O.U., C.O. Ezugwu and A.A. Ezeilo, 1999. Preliminary investigation on the physicochemical and antimicrobial properties of tetra pleura tetraptera fruit oil. J. Pharm. Res. Dev., 4 (1): 25-30.

Nwakile, C.O., 2004. Antimicrobial Evaluation of Successive Extracts with different solvents of Root of *Zapoteca portoricensis*. Undergraduate Degree project (unpublished), Department of Pharmaceutical Chemistry,University of Nigeria, Nsukka.

Nwodo, N.J. and I.C. Uzochukwu, 2006. Studies on Anti-inflammatory and Antimicrobial activities of crude methanol extracts of *Zapoteca portoricensis* Jacq. H. Hernandez. Rec. Prog. Med. Plts., 19: 61-69.

http://www.ix.uga.edu/main/home/dstrong/phm4120/Tablets2006.htm (Accessed on 20/6/07)

Obitte, N.C., 2001. *In vitro* properties of encapsulated theophyline granules prepared with *Tacca involucrata* starch, M. Pharm Dissertation, Pharm. Tech. Industrial Pharmacy, University of Nigeria, Nsukka.

Ojo, O.O., A.O. Ajayi and Anibijuwon, 2007. Antibacterial potency of methanol extracts of lower plants. J. Zheij. Univ. SC., 8 (3): 189-191.

Okore, V.C., 2005. Pharmaceutical Microbiology; Principles of the Pharmaceutical Application of Anti microbial Agents. 1st Edn. EL Memak, Enugu (Nig), pp: 36.

Olaniyi, A.A., 2005. Essential Medicinal Chemistry, 3rd Edn. Ibadan Shaneson CI Ltd., pp: 346-349.