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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Full Length Research Paper

Production of bacterial amylases and cellulases using sweet potato (*Ipomoea batatas*. (L.) Lam.) peels

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Peels of sweet potato (*Ipomoea batatas)* were buried in the soil for 14 days and the isolates associated with the degradation of the peels were obtained using standard microbiological procedures. The bacterial isolates obtained were screened for amylolytic and cellulolytic activities under different pH and temperatures as parameters and optimized for enzyme production. Sixteen (16) bacterial isolates were obtained and characterized and screened for amylase and cellulase production. *Bacillus pumilus* has the highest frequency of occurrence (18.75%) followed by *B. subtilis* (12.50%). After 24 to 48 h of incubation, *B. pumilus* produced highest concentration of amylase at 55°C, pH 6 (5.4 U/mL) while *B. subtilis* had the best cellulase production of 0.75 U/mL at 55°C, pH 7*. *B. pumilus* and *Bacillus subtilis* produced the highest amylase and cellulase concentrations and seem to be the potential sources of these enzymes for industrial application.

Key words: Sweet potato peel, amylase, cellulase, bacteria.

INTRODUCTION

Amylases are class of enzymes, which are of important applications in the food, brewing, textile, detergent and pharmaceutical industries. Their most relevant effect is employed during starch liquefaction to reduce their viscosity, production of maltose, oligosaccharide mixtures, high fructose syrup and maltotetraose syrup (Jose and Arnold, 2014). During detergents production, they are applied to improve cleaning effect and are also used for starch de-sizing in textile industry (Aiyer, 2005). α-Amylase is characterized by its random hydrolysis of α-1,4-glucosidic bonds in amylose and amylopectin molecules, while amylopectin α-1,6-bonds are resistant to its cleavage (Parmar and Pandya, 2012). Many micro-organism such as *Bacillus subtilis*, *Bacillus cereus, Bacillus polmyxa, Bacillus amyloliquefaciens, Bacillus coagulans, Lactobacillus, Escherichia, Proteus, Bacillus lincheniformis, Bacillus steriothermophilu, Bacillus megaterium, Strepotmyces* sp., *Pseudomonas* sp. etc. were used in α- and β-amylases production. Although, among bacteria, *Bacillus* sp. was widely used for thermostable α-amylase production so as to meet industrial needs (Parmar and Pandya, 2012).

Cellulose is the most abundant biomass on Earth (Tomme et al., 1995). It is the primary product of photosynthesis in terrestrial environments and the most abundant renewable bioresource produced in the

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biosphere (Jarvis, 2003; Zhang and Lynd, 2004). Cellulose is commonly degraded by an enzyme called cellulase. This enzyme is produced by several microorganisms, mainly bacteria and fungi (Bahkali, 1996; Magnelli and Forchiassin, 1999; Shin et al., 2000; Immanuel et al., 2006). Cellulases from bacteria are more effective catalysts and less inhibited by the presence of material that has already been hydrolyzed. The greatest potential importance of using bacteria for cellulase production is the ease with which bacteria can be genetically engineered, high growth rate as compared to fungi, often more complex and in multi-enzyme complexes providing increased function and synergy, inhabit a wide variety of environmental and industrial niches (Ariffin et al., 2006; Sadhu and Maiti, 2013). However, the application of bacteria in producing cellulase is not widely used (Sonia et al., 2013). Some bacterial species used in cellulase production are *Cellulomonas* species, *Pseudomonas* species, *Bacillus* species and *Micrococcus* species (Nakamura and Kappamura, 1982). Cellulases are used: In the textile industry for cotton softening and denim finishing; in laundry detergents for colour care, cleaning; in the food industry for mashing; in the pulp and paper industries for drainage improvement and fibre modification (Cherry and Fidants, 2003).

Amylase and cellulase yields appear to depend upon a complex relationship involving a variety of factors like inoculums size, pH value, temperature, presence of inducers, medium additives, aeration, growth time, and so forth (Immanuel et al., 2006).

This study was therefore designed to isolate high amylase and cellulase producing bacteria from decaying sweet potato peels and to optimise for enzyme production.

MATERIALS AND METHODS

Samples collection

Sweet potatoes (yellow skin) were purchased from Agbowo Market in Ibadan Metropolis, Oyo State, Nigeria.

Sample preparation

The peels of sweet potatoes were carefully scraped off so that the amount of corker removed was kept to a minimum. The scraped peels were buried inside the soil (14 cm deep) in Botanical Garden, University of Ibadan, Oyo State, Nigeria.

Isolation of organism

The buried scrapped peels were exhumed carefully after 14 days and put in a sterile nylon bag and carried to the laboratory. The adhering sand was shaken off and 1 g of the peel was homogenized aseptically using a sterilized mortar and pestle. Serial dilution was carried out and 1 mL of dilution 10^4 and 10^6 were mixed with 20 mL of plate count agar, poured on plate and allowed to set. This was incubated for 24 h at 37°C and observed for bacterial growth. Colonies with different morphology (shape, texture and colour) were isolated and purified by sub-culturing several times till pure cultures were obtained. Isolation was carried out in triplicates.

Identification of isolates

Organisms were identified based on their macroscopic, microscopic, physiological and biochemical characteristics of the isolates with reference to Bergey's Manual of Systematic Bacteriology (Sneath et al., 1986). The biochemical tests carried out are starch hydrolysis, catalase test, Voges Prokauer test, citrate utilization and endospore test.

Growth on carboxymethylcellulose (CMC)

CMC (2%) was prepared with nutrient agar, sterilized and allowed to cool to 45°C. It was poured into Petri dishes. The plates were inoculated with single streak of test organism and incubated at 37°C for 48 h. Presence of clear zones along line of growth indicates that the organism can utilize or break down cellulose and this was used to screen for cellulase production ability of the isolates.

Growth on starch

Starch agar was prepared by adding 1 g of soluble starch to 100 mL of nutrient agar. The mixture was homogenized and sterilized at 121°C for 15 min. This was then dispensed into sterile plates and allowed to set. A single streak of culture was made on the plate and incubated at 37°C for 48 h. After incubation, the plates were flooded with Gram's iodine. A positive result was indicated by retention of the iodine colour as a clear zone around the growth region indicating starch hydrolysis while unhydrolyzed starch formed a blue and black colouration with iodine. This was used to screen the bacterial isolates for amylase production.

Extraction of enzymes

The medium used was nutrient broth in which soluble starch and CMC (1%) was added respectively. It was sterilized at 121°C for 15 min, allowed to cool and the test organisms inoculated into it. It was then incubated at 30°C for 48 h after which the culture was centrifuged at 10,000 rpm for 15 min using a refrigerated centrifuge (IEC centra, MP4R model). The cell free culture supernatant was then assayed for amylase and cellulase production and activity. One unit (U) of enzyme activity is expressed as the quantity of enzyme, which is required to release 1 mol of glucose per minute under standard assay conditions (Muhammad et al., 2012).

Amylase assay

Amylase assay was determined using DNSA reagent method of Bernfeld (1955) as modified by Giraud et al. (1991). To 1 mL of culture supernatant was added 1 mL of the substrate containing 1.2% w/v soluble starch in 0.1 N phosphate buffer, pH 6.0. The enzyme substrate reaction was incubated at 45°C for 1 h. The reaction was brought to halt by adding a drop of 5 M NaOH. The amount of reducing sugar produced was determined with 3,5-dinitrosalicylic acid (DNS). 1 mL of DNS reagent was added to filtrate-substrate reaction mixture and was heated in a boiling water bath at 100°C for 10 min. It was cooled with distilled water. The absorbance was measured at 540 nm using spectrophotometer

Table 1. Frequency of occurrence of bacterial isolate from decaying sweet potato peels.

Bacterial isolate	No.	Frequency of occurrence (%)
Bacillus species	7	43.75
Pseudomonas species	3	18.75
Flavobacterium rigense	2	12.50
Proteus sp.	1	6.25
Derxia gummosa	1	6.25
Azotobacter vinelandii	1	6.25
Micrococcus luteus	1	6.25
Total	16	100

Table 2. Colonial morphology of *Bacillus* sp. isolated from decaying sweet potato peels.

Isolate	Shape	Elevation	Surface	Colour
SPA1	Circular	Raised	Smooth	Cream
SPA2	Rhizoid	Raised	Shiny	Cream
SPA7	Circular	Raised	Smooth	Cream
SPB2	Rhizoid	Raised	Dull	Cream
SPB3	Oval	Raised	Smooth	Cream
SPB7	Round	Raised	Dull	Cream
SPB8	Circular	Raised	Smooth	Cream

(Unipec 23 D, Uniscope England). One millilitre of uninoculated blank similarly treated was used to set spectrophotometer at zero. Standard maltose concentrations were prepared within the range of 0.2 - 3.0mg/mL maltose into the requisite medium. The results were then used to construct a standard curve. The spectrophotometer values were then extrapolated as maltose equivalent from the standard curve plotted (Bernfield, 1955).

Cellulase assay

Cellulase assay was determined using the method of Mandel et al., (1976). 1 mL of culture supernatant was added to 9 mL of the substrate containing 0.55% w/v of CMC (carboxymethylcellulose) in 0.55 M acetate buffer, pH 5.5. It was incubated at 45°C for 1 h. The reaction was brought to halt by adding a drop of 5 M NaOH. 1 mL of DNS was added to 1 mL of the filtrate in order to estimate the reducing sugar that was released. The mixture was boiled at 100°C for 10 min in water bath. After cooling, the absorbance was determined at 540 nm using Unispec 23D spectrophotometer.

Effect of different temperatures on amylase and cellulase productions

Nutrient broth was prepared and 10 ml each dispensed into screw capped bottles and sterilized at 121°C for 15 min and allowed to cool. *Bacillus* isolates were inoculated into each bottle and incubated at different temperatures (25, 37, 45, 55 and 65°C) for 24 h. Amylase and cellulase activities were then determined as described earlier.

Effect of different pH on amylase and cellulase production

Buffer was used to adjust the pH of nutrient broth to 3.0, 4.0, 5.0, 6.0 and 7.0 accordingly. 10 mL of the adjusted nutrient broth was dispensed into screw capped bottles and sterilized at 121°C for 15 min. After cooling, test isolates were inoculated into each bottle and incubated at 37°C for 24 h. Enzymes activities were determined as earlier described.

RESULTS

Bacillus species had highest occurrence of bacterial isolate from buried potatoes peels after 14 days (Table 1). *Bacillus* sp. recorded 43.75% of occurrence; followed by *Pseudomonas* with 18.75%. Other bacteria isolated were *Flavobacterium rigense*, *Proteus* sp., *Derxia gummosa*, *Azotobacter vinelandii* and *Micrococcus luteus*. The colonial morphologies of *Bacillus* species isolated were represented on Table 2, they all have raised elevations and cream colour while their texture are either smooth, dull or shiny. Also, they exhibit different colony shapes on the plate, *B. pumilus* is circular, *B. licheniformis* is rhizoid, *B. megaterium* is oval and *B. subtilis* is round.

Table 3 shows the biochemical tests for bacillus isolates. The *Bacillus* spp. are Gram positive, rod shaped and endospore positive. All the bacillus isolates have the ability to hydrolyse starch and utilize citrate except *B.*

Table 3. Biochemical characteristics of *Bacillus* sp. isolated from decaying sweet potato peels.

Strain code	Grams reaction	Endospore	Catalase	Starch hydrolysis	Voges-Proskaeur	Citrate Utilisation	Growth at 55°C	Probable identity
SPA1	+	+	+	+	+	-	+	*B. pumilus*
SPA2	+	+	+	+	+	+	+	*B. licheniformis*
SPA7	+	+	+	+	+	-	+	*B. pumilus*
SPB2	+	+	+	+	+	+	+	*B. licheniformis*
SPB3	+	+	+	+	-	-	-	*B. megaterium*
SPB7	+	+	+	+	+	+	-	*B. subtilis*
SPB8	+	+	+	+	+	-	+	*B. pumilus*

Table 4. Amylase and cellulase concentration (U/mL) of *Bacillus* sp. at different temperature.

Code	Probable identity	Amylase					Cellulase				
		27°C	37°C	45°C	55°C	65°C	27°C	37°C	45°C	55°C	65°C
SPA1	*B. pumilus*	2.00	2.40	2.75	2.80	2.70	0.50	0.60	0.60	0.50	0.40
SPA2	*B. licheniformis*	2.00	2.60	2.70	2.70	2.50	0.50	0.60	0.70	0.70	0.40
SPA7	*B. pumilus*	2.70	2.80	2.90	2.90	2.80	0.60	0.65	0.65	0.70	0.40
SPB2	*B. licheniformis*	2.00	2.40	2.50	2.60	2.40	0.50	0.60	0.50	0.60	0.30
SPB3	*B. megaterium*	2.80	2.90	3.00	3.10	2.90	0.50	0.60	0.60	0.70	0.40
SPB7	*B. subtilis*	2.50	3.70	3.80	3.90	3.80	0.60	0.60	0.70	**0.75**	0.45
SPB8	*B. pumilus*	2.20	2.30	5.30	**5.40**	4.90	0.50	0.60	0.65	0.70	0.40

Mean value of triplicate readings. Bold values indicate highest concentration of amylase and cellulase production, respectively at 55°C.

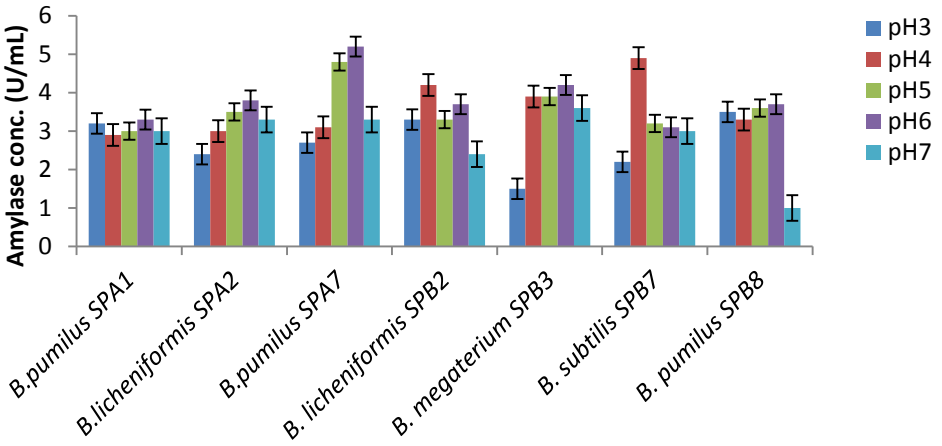

Figure 1. Amylase concentration (U/mL) at different pH.

pumilus which is negative to hydrolysis citrate utilization. *B. subtilis* was positive to Voges-Proskaeur test, citrate utilization but no growth was recorded at 55°C. *B. licheniformis* is positive to Voges-Proskaeur test, citrate utilization and also has the ability to grow at 55°C. *B. megaterium* is negative to both Voges-Proskaeur and citrate utilization test.

The effect of different temperatures on amylase and cellulase production of the *Bacillus* species are presented in Table 4, for all the isolates, there was a gradual increase in enzymes activities as the temperature increases with maximum concentration produced at 55°C before a general decline at 65°C. *B. pumilus* SPB8 produced highest concentration of amylase at 55°C (5.4 U/mL) while *B. subtilus* SPB7 produced cellulase best also at 55°C with 0.75 U/mL concentration. Least production of enzymes was noticed at 27°C for all isolates. Figure 1 shows the effect of pH, at pH 6, *B.*

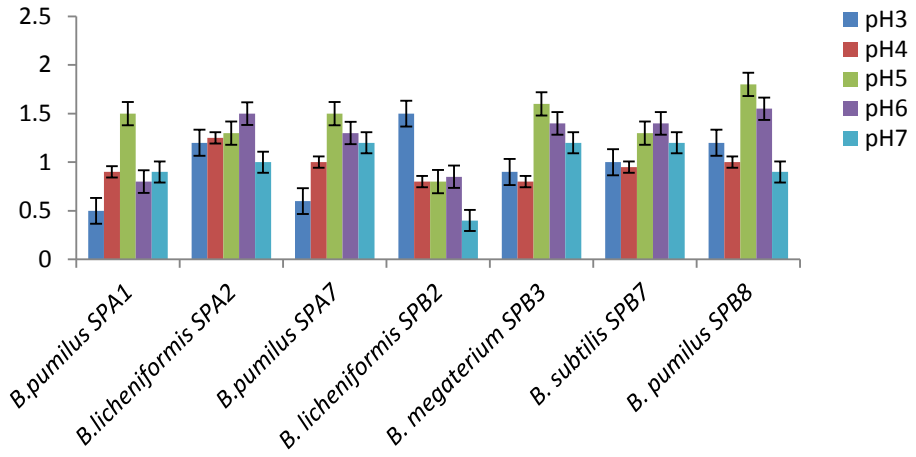

Figure 2. Cellulase concentration (U/mL) at different pH.

pumilus SPA7 produced the highest concentration of amylase (5.2U/mL) followed by *B. megaterium* SPB3 (4.2 U/mL) and the lowest producer at that pH is *B. subtilis* SPB7 (3.1 U/mL). The highest concentration of cellulase was produced by *B. pumilus* SPB8 (1.8 U/mL) at pH 5 followed by *B. pumilus* SPA1 (1.5 U/mL), while *B. licheniformis SPB2* produced the least concentration at this pH (Figure 2). All the organisms recorded their highest cellulase production at either pH 5 or 6 with the exception of *B. licheniformis* SPB2 that recorded its highest cellulase production at pH 3.

DISCUSSION

The most predominant bacterial isolates obtained from decaying sweet potatoes peels were identified as *B. pumulis*, *B. licheniformis*, *B. subtilis* and *B. megaterium*. The prevalence of *B. pumulis* and *B. subtilis* isolated in this work conforms to the findings of Lorena et al. (2001) and Madigan et al. (2005) which states that these two organisms are natural inhabitant of soil.

In this study, *B. pumilus* produced the highest concentration of amylase (5.4 U/mL) at 55°C and pH 6 which was also reported by Andrea et al. (2007) which states that *B. pumilus* produced amylase between the pH of 5.8 and 7.5 and at a temperature of 55°C. Effect of temperature on amylase production was observed by varying growth temperature of isolates and optimum temperature was found to be 55°C. This findings agrees with the behaviour of amylases from *Bacillus* spp. isolated from soils as reported by Cordeiro et al. (2003) and Vipal et al. (2011) who reported 50°C as optimum temperature. The effect of temperature on cellulase production was also observed when temperature of the production medium was varied. Cellulase production was highest in the temperature range of 45 - 55°C, with an optimum temperature of 55°C. Similarly, Shaikh et al. (2013) observed that *Bacillus* sp. produced cellulase optimally at 50°C and affirm that the thermostable property of cellulase has been shown to be of interest for industrial applications. Optimum pH for the production of cellulase by all the organisms used in this study ranged from 5 - 7 with pH 5 been the most predominant. This result was in agreement with the findings of others like Goya and Soni (2011), Azzeddine et al. (2013) and Trinh et al. (2013) who reported pH 5, 6 and 7 respectively as the optimum pH for production of cellulase from *Bacillus* spp.

Conclusion

This study inferred that decaying sweet potato peels harbour amylolytic and cellulolytic *Bacillus* species and the enzymes produced by these bacteria can be harnessed for industrial application. Optimum temperature for amylase and cellulase production was 55°C, whereas optimum medium pH for amylase and cellulase was 6 and 5, respectively. *B. subtilis* and *B. pumilus* produced the highest concentration of amylase (5.4 U/mL) and cellulase (0.75 U/mL), respectively.

Conflict of interest

No conflict of interest among the authors.

REFERENCES

Andrea CB, Mana AEW, Aneh B, Launval AV, Ana CB, Robert D, Emanuel MS, Mana HPF (2007). Structural characterization of the bglH gene encoding a beta-glucosidase-like enzyme in an endophytic Bacillus pumilus strain. Genet. Mol. Biol. 30(1):100-104 http://dx.doi.org/10.1590/S1415-47572007000100018

Aiyer PV (2005). Amylases and their application. Afr. J. of Biotech. 4(8):1525-1529.

Ariffin H, Abdullah N, Umi K, Shirai Y, Hassan MA (2006). Production and characterisation of cellulase from Bacillus pumilus EB3. Int. J.

Eng. Technol. 3:47-53.
Azzeddine B, Abdelaziz M, Estelle C, Mouloud K, Nawel B, Nabila B, Francis D, Said B (2013). Optimisation and partial characterisation of endoglucanase produced by Streptomyces sp. B - PNG 23. J. Arch. Biol. Sci. Belgrade 65(2):549-558. http://dx.doi.org/10.2298/ABS1302549A
Bahkali AH (1996). Influence of various carbohydrates on xylanase production in Verticillium tricorpus. Bioresour. Technol. 57(3):265-268. http://dx.doi.org/10.1016/S0960-8524(96)00074-0
Bernfield P (1955). Methods in Enzymology. Ed by Kaplan, M.O and Colowicty, S.P. Academic Press, N.Y.P.149.
Cherry JR, Fidants AL (2003). Directed evolution of industrial enzymes: an update. Curr. Opin. Biotechnol. 4:438-443. http://dx.doi.org/10.1016/S0958-1669(03)00099-5
Cordeiro CAM, Martinas MLL, Lucaino A (2003). Production and properties of alpha amylase from thermophylic Bacillus species. Braz. J. Microbiol. 33:1-3.
Giraud EA, Keleke S, Lelory B, Rainbault M (1991). Isolation and physiological study of an amylase strains of Lactobacillus plantarum. Appl. Microbiol. Biotechnol. 36:379-383. http://dx.doi.org/10.1007/BF00208160
Goya M, Soni G (2011). Production and characterisation of cellulolytic enzymes by Pleurotus florida. Mycosphere 2. 3:249-254
Immanuel G, Dhanusha R, Prema P, Palavesam A (2006). Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. Int. J. Environ. Sci. Technol. 3:25-36. http://dx.doi.org/10.1007/BF03325904
Jarvis M (2003). Cellulose stacks up. Nature 426:611-612. http://dx.doi.org/10.1038/426611a
Jose LA, Arnold LD (2014). Microbial enzymes: Tools for biotechnological processes. J. Biomol. 4:117-139. http://dx.doi.org/10.3390/biom4010117
Lorena DS, Toshihiko S, Kanefumi K, Yoshihito F, Hayao T (2001). Degradation of cell wall materials from sweet potato, cassava and potato by a bacterial protopectinase and terminal sugar analysis of the resulting solubilised products. J. Biosci. Bioeng. 93:64-72.
Madigan M, Martinko J (2005). Brocks Biology of Microorganisms 11th Ed, Prentice Hall.
Magnelli P, Forchiassin F (1999). Regulation of the cellulase complex production by Saccobolus saccoboloides: induction and repression by carbohydrates. Mycologia 91:359-364. http://dx.doi.org/10.2307/3761382
Mandel M, Andreotti R, Roche C (1976). Measurement of saccharifying cellulose. Biotechnol. Bioeng. Symp. (6): 21 - 23.
Muhammad Irfan, Asma Safdar, Quratulain Syed, Muhammad Nadeem (2012). Isolation and screening of cellulolytic bacteria from soil and optimization of cellulase production and activity. Turk. J. Biochem. 37(3):287 293. http://dx.doi.org/10.5505/tjb.2012.09709
Nakamura K, Kappamura K (1982). Isolation and identification of crystalline cellulose hydrolyzing bacterium and its enzymatic properties. J. Ferment. Technol. 60(4):343-348.
Parmar Dipali, Pandya Ajit (2012). Characterization of amylase producing bacterial isolates. Bull. Environ. Pharmacol. Life Sci. 6:42-47.
Sadhu S, Maiti TK (2013). Cellulase production by bacteria: A review. Brit. Microbiol. Res. J. 3:235-258. http://dx.doi.org/10.9734/BMRJ/2013/2367
Shaikh NM, Patel AA, Mehta SA, Patel ND (2013). Isolation and Screening of cellulolytic bacteria inhabiting different environment and optimization of cellulase production. Univ. J. Environ. Res. Technol. 3:39-49
Shin CS, Lee JP, Lee JS, Park SC (2000). Enzyme production of Trichoderma reesei rut C-30 on various lignocellulosic substrates. Appl. Biochem. Biotechnol. 84-86:237-245. http://dx.doi.org/10.1385/ABAB:84-86:1-9:237
Sneath PHA, Nair NA, Sharpe ME (1986). Bergeys manual of systematic bacteriology vol. 2. The Williams and Wilkin Co., Baltimore.
Sonia Sethi, Aparna Datta, Lal Gupta B, Saksham Gupta (2013). Optimization of cellulase production from bacteria isolated from soil. Hindawi Publishing Corporation, ISRN Biotechnology, Article ID 985685). http://dx.doi.org/10.5402/2013/985685
Tomme P, Warren RAJ, Gilkes NR (1995). Cellulose hydrolysis by bacteria and fungi. Adv. Microbial Physiol. 37:1-81. http://dx.doi.org/10.1016/S0065-2911(08)60143-5
Trinh DK, Quyen DT, Do TT, Ndhiem NM (2013). Optimisation of culture conditions and medium components for carboxymethyl cellulase production by a novel Basidiomycete strain Peniophora sp. NDVNO1. Iran. J. Biotechnol. 11:251-259
Vipul V, Mrigank SA, Abhishek RG, Monika S, Akhilesh K (2011). Amylase production and purification from bacteria Isolated from a waste potato dumpsite in District Farrukhabad U.P State India. Eur. J. Exp. Biol. 3:107-113
Zhang YH, Lynd LR (2004). Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplexed cellulase systems. Biotechnol. Bioeng. 88:797-824. http://dx.doi.org/10.1002/bit.20282

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Full Length Research Paper

Comparative effects of peel extract from Nigerian grown citrus on body weight, liver weight and serum lipids in rats fed a high-fat diet

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The effects of ethanolic extracts of five different citrus peels on mean body and liver weight and serum lipid content were investigated in albino rats. Six groups (n=8 each) were fed with a high fat diet for seven days *ad libitum* before oral daily administration of the peel extracts of orange (OR), lemon (LE), lime (LI), tangerine (TA), grapefruit (GR) and synergistic combination of equal ratios (w/w), that is, (SY), respectively at a dose of 500 mg / kg body weight for 14 days. The positive control group received only the high fat diet (HFD), while the negative control group received only a standard diet (STD). The body weights of the animals were monitored every two days and the animals were sacrificed after the 7th and 14th days of or following the administration of the extracts. All the parameters increased in the positive control group (HFD) compared to the negative control (STD) group. Body and liver weights decreased in all treated groups, as well as serum cholesterol and triglycerides, which decreased significantly in SY and GR groups, $p < 0.05$. All extracts contained mainly flavonoids and alkaloids while the grapefruit peel extract contained additional saponins that could contribute to the reduction in both body weight and serum lipid content. Conclusively, peel extract from different types of Nigerian citrus which ordinarily serve as waste may synergistically be used to control and manage obesity and associated pathologies.

Key words: Citrus peel, high fat diet, obesity, serum lipids, liver and body weight.

INTRODUCTION

Excessive body weight or obesity has in the last few decades become an emerging serious health concern throughout all cultures, especially when the diets tend towards western type. Obesity is generally associated with an increased risk of excessive fat – related metabolic and chronic diseases such as type two diabetes mellitus, hypertension and dyslipidemia (Bays et al., 2006). Excessive weight gain is also generally linked to the onset of cardiovascular disease, cancers fibroid, renal disease and psychosocial incapacity, amongst others (Abu-Abid et al., 2002; WHO, 2002; Hossain et al., 2007). There is also evidence that obesity is associated with increased morbidity and mortality (Huang et al., 2009). In order to reduce the prevalence of these excessive weight-related diseases, several measures, which include production of low fat diets, dietary restriction, use of

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leptin, induction of thermogenesis and liposuction have been tried out. Most recent studies on the treatment of overweight have focused on the potential role of plant constituents, including polyphenols found in citrus (Sindler, 2001; Murase et al., 2002; Aoki, 2007).

Citruses, belonging to the family *Rutaceae*, are one of the main fruit crops grown throughout the world. Citrus fruits have been used by man for centuries for agricultural, medicinal and herbal purposes (Tomar et al., 2013). Several pharmacological properties have been attributed to various members of the citrus species, ranging from anticancer, (Jacob et al., 2000; Silalahi, 2002; Enterazi et al., 2009) antimicrobial, (Nannapaneni et al., 2008; Tao et al., 2009; Dhanavade et al., 2011; Kumar et al., 2011; Lawal et al., 2012), antifungal, (Valezquez-Nunez et al., 2013) antityphoid, (Kumar et al., 2011), antioxidant, (Duda-Chodak and Tarko, 2007), anti-inflammatory, (Galati, 1994; Karaca et al., 2008), antiulcer, (Nagaraju et al., 2012), hypolipidemic (Khan et al., 2010), hepatoprotective (Karaca et al., 2008; Kangralkar et al., 2009) and antidiabetic, (Daniels, 2006; Parmar and Kar, 2007), among others. The peels of the citrus fruits, especially grapefruit and bitter orange, which are rich in flavonoid glycosides, polyphenols and volatile oils have been used in several cultures for weight control, amongst other pharmacological uses (Fujioka et al., 2006; Stohs and Shara, 2007). Previous studies have demonstrated the effects of these flavonoids on lipid and glucose metabolism in experimental animals and humans (Jung, 2004; Miwa, 2005), specifically on lipid catabolism, glucose transport, the insulin-receptor function, and peroxisome proliferator-activated receptors (PPARs) activation, all of which play essential roles in weight control (Shisheva, 1992; Liang, 2001; Kim, 2003; Lee, 2003).

Nigeria is richly blessed with an all year round availability of a number of citrus fruits, most of which form a huge economic asset to both rural dwellers who cultivate the fruits and the urban vendors. The most commonly sold citrus, sweet oranges (*C. sinensis*), are often sold in the peeled form, thus leaving huge amounts of peels as waste. As part of an ongoing search for local herbal drugs for weight control, this work investigated the comparative and synergistic effects of five locally grown citrus fruits, namely, sweet orange (*C. sinensis* L.), lemon, (*C. limon* L.), lime (*C. aurantifolia* L.), tangerine (*C. reticulate* L.) and grapefruit (*C. paradisi* L.) on mean liver and body weights and serum lipids in albino rats fed a high fat diet.

MATERIALS AND METHODS

Materials

Plants

Citrus species used were orange (*C. sinensis* L. (OR), lemon (*C. limon* L. (LE), grape (*C. paradise* L. (GR), tangerine (*C. reticulate* L. (TA) and lime (*C. aurantifolia* L. (LI) obtained from Eke Awka local market and private compounds in Awka, Anambra State. The samples were authenticated by Professor Clement Okeke, Department of Botany, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria..

Table 1. Percentage composition of experimental diets.

Component	STD	HFD
Carbohydrate	73	56
Protein	16	16
Palm oil	3	20
Crude fiber	5	5
Mineral salt[a]	2	2
Vitamins[b]	1	1

[a]The mineral mix above consisted of calcium (0.8 g), phosphorous (0.6 g), manganese (50 mg), zinc (30 mg), sodium (0.15 g). [b]The vitamins consisted of vitamin A (8000 iu), vitamin D3 (2400 iu), vitamin E (15 mg), vitamin B2 (4 mg), vitamin C (50 mg).

Chemicals: All chemicals used in this work are of analytical grade, and products of BDH, (Poole, England), Merck (Germany) and others. Kits by Randox, (UK) were used for the estimation of total serum cholesterol and triacylglycerol.

Animals

Male albino rats of about 6 weeks of age (weighing between 200 – 230 g) were purchased from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were left to acclimatize for one week under ambient conditions before the experiments.

The animals were handled in accordance with the guidelines of the Ethics Committee on Animal Research of the Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria.

Methods

Extraction and phytochemical analyses

Around 500 g of each peel were air dried, ground into coarse powder, extracted exhaustively with 70% ethanol using the soxhlet apparatus and concentrated *en vacuo* at 40°C. The yields of the extracts were calculated and phytochemical analysis was carried out on the citrus peel extracts according to the method of Harborne (2003).

Experimental procedure

Diets used consisted of corn flour, rice husk, crayfish, palm oil, multivitamins and mineral salts in the percentage combinations (w/w) as stipulated in Table 1:

Rats were divided into eight groups (n = 8 each). All groups, except

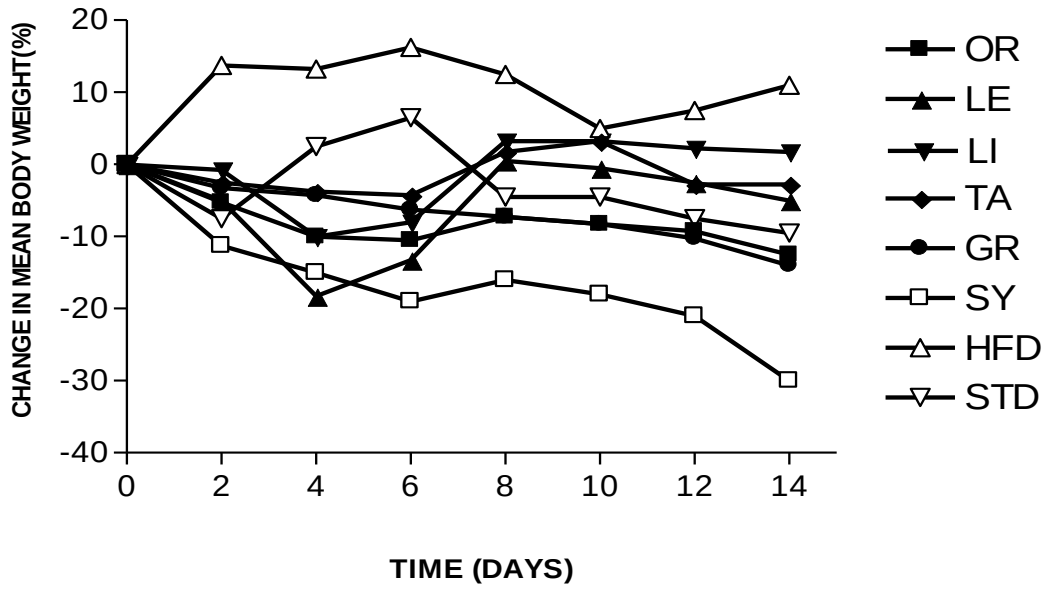

Figure 1. Percentage change from initial mean body weight with time.

the negative control, were initially allowed access to the high fat diet (HFD, Table 1) and water *ad libitum* for seven days. At the end of the 7 days the individual weights of the animals were taken and a daily oral dosage regimen of citrus peel extracts (500 mg/kg body weight of animal) was administered for 14 day as follows:

Group one was fed with HFD and orange peel extract (OR)
Group two was fed with HFD and lemon peel extract (LE)
Group three was fed with HFD and lime peel extract (LI)
Group four was fed with HFD and tangerine peel extract (TA)
Group five was fed with HFD and grapefruit peel extract (GR)
Group six was fed with HFD and synergistic (equal) combinations of the extracts (w/w) (SY)
Group seven was fed with HFD only (HFD; positive control)
Group eight was fed with standard diet only (STD, Table 1; negative control).

The weights of the animals were taken at 2 days interval after overnight fasting, the rats were sacrificed after the 7th and the 14th day of administration of citrus peel extract by cardiac puncture and the serum was collected. The total serum cholesterol and triacylglycerol were determined spectrophotometrically using Randox kits supplied by Randox, (UK). The liver weights at necropsy were also determined.

Statistical analyses

All statistical analyses were performed using Graph Pad Prism (version 4.0). A level of $p < 0.05$ was considered significant. Data were presented as mean ± SEM. The data were tested by ANOVA, followed by Bonferroni's pair-wise comparison test.

RESULTS AND DISCUSSIONS

There was high significant increase ($p < 0.05$) in the mean body weight, as expected, in the rats fed on a high fat diet (Figure 1). The body weight was, however, significantly reduced ($p < 0.05$) by concurrent administration of a daily oral dose of citrus peel (500 mg/kg body weight) for all test groups in the 1st week. The groups LE, LI and TA regained weight in the 2nd week, however, OR, GR and SY groups, significantly and consistently lost weight ($p < 0.05$). The relative liver weight increased significantly ($p < 0.05$) for the high fat fed group by the 14th day. The citrus peel extract caused a general non-significant decrease in liver weight by the 7th day when compared with the HFD group ($p > 0.05$), but there was no further significant loss except only for the OR group by the 14th day (Figure 2). The serum cholesterol levels decreased significantly in all the groups when compared with HFD group, by the 7th day, but the decrease was more in the GR, SY, and OR groups by the 14th day at $p < 0.05$ (Figure 3). Similarly, serum triglycerides also reduced significantly in all test groups, especially for SY, GR, OR and LE groups by the 14th day ($p < 0.05$). The least changes were observed for TA and LI groups (Figure 4).

There was also noticeable reduction of appetite and the animals were observed to show signs of tremor and exfoliation of fur for SY, LI, OR and GR groups in the 2nd week but not for LE and TA groups.

On a comparative basis, these results showed that grapefruits (GR) peel extract followed by orange (OR) were the most effective single remedies, while TA was the least effective for weight loss and for the reduction of cholesterol and triglycerides. A synergistic combination of all extracts was most beneficial.

Phytochemical analysis of the citrus peel extracts used in this work showed the presence of mainly flavonoids, alkaloids and additionally saponins in grapefruits (GR) (Table 2). The relative quantities and identities of the

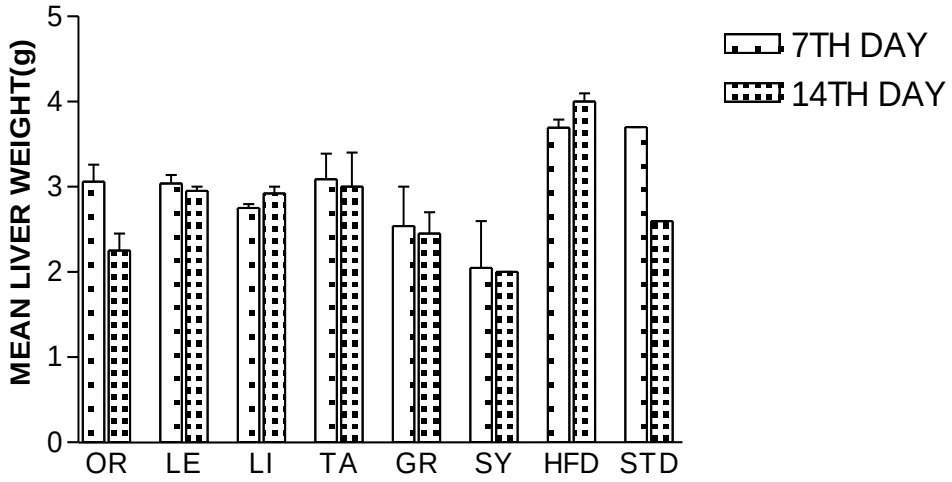

Figure 2. Mean liver weight after 7 and 14 days of administration of extracts.

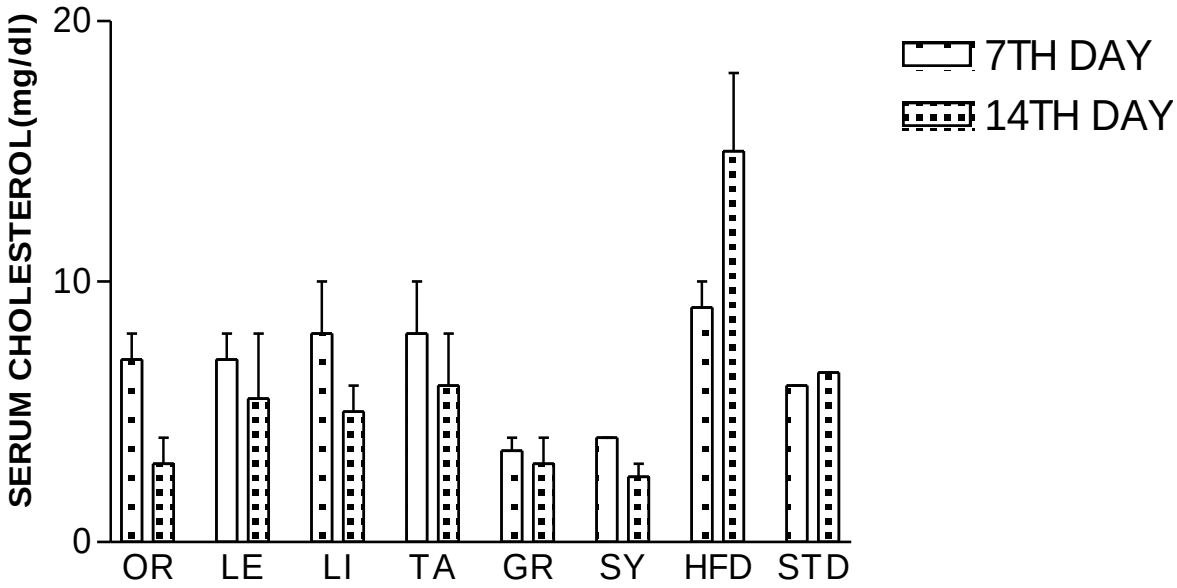

Figure 3. Serum cholesterol levels after days 7 and 14 of administration of extracts.

individual components were however not determined. Polymethoxylated flavones (PMFs - tangeretin, nobiletin, hesperidin, sinensten and naringin) found in the peels and in smaller in amounts in the juices of a variety of citrus fruits have been isolated from tangerine, orange, grapefruit (Rouseff and Ting, 1979). PMFs showed effects in reducing cholesterol (especially LDL cholesterol, by 30 to 40%, although treatment did not appear to have any effect on levels of HDL cholesterol) and to suppress appetite in previous animal studies, suggesting health benefits in cardiovascular health (Hakim and Harris, 2004; Kurowska and Manthey, 2004).

There was noticeable reduction of appetite for SY, LI, OR and GR group\s in the second week, observed by the increasing amount of leftover food per day (actual weight not determined). This may have been due to the presence of polyphenols, as well as pectin in the peel extracts. Pectin, though not soluble in pure alcohol, is extractible in the hydroacholic solution (70%, (v/v) used in this work. Pectin reduces appetite by swelling into a gel in the stomach to give a feeling of fullness for at least 4 h (Rayburn et al., 1998). Another mechanism for weight loss may include stimulation of β - 3 cell receptors, thus eliciting thermogenesis, leading to increased lipolysis and metabolic rate (Preuss et al., 2002). It has also been determined that PMFs also help reduce cortisol levels.

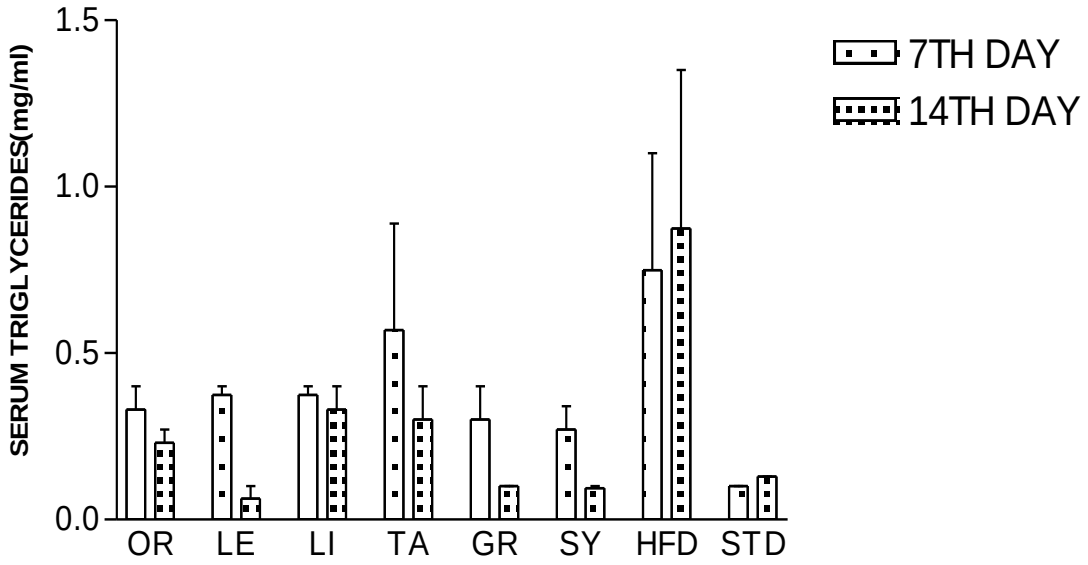

Figure 4. Serum triglycerides after days 7 and 14 of administration of extracts.

Table 2. Yield and phytochemical analyses of citrus peel extracts.

Sample yield (%)	Tannins	Saponins	Glycosides	Flavonoids	Alkaloids
OR (4.63%)	-	-	-	+	+
LE (4.52%)	-	-	-	+	+
LI (3.68%)	-	-	-	+	+
TA (3.50%)	-	-	-	+	+
GR (5.54%)	-	+	-	+	+

-Absent; + Present.

Cortisol is a stress hormone, higher levels of which have been linked to weight gain. The use of PMFs to reduce systemic and local cortisol concentrations (liver and adipose tissue), has been beneficial in promoting blood sugar control and weight loss (Talbot, 2009). Grapefruit peels have been reported to promote weight loss by reducing insulin spike after a meal, thus the body processes more food for use as energy and less is stored as fat. Grapefruit extract was the most effective single extract in this work.

The mechanisms by which cholesterol and triglycerides were reduced could be due to interaction of extract with bile acids thus preventing reabsorption of the bile acid, and therefore, cholesterol or by inhibition of β HMG CoA reductase and acyl CoA cholesterol acyl transferase (ACAT), thereby preventing de novo synthesis, or by increased lipase activity (Bok, 1999). Hesperidin and naringin, and their aglycones hesperetin and naringenin, have been reported to decrease plasma and hepatic cholesterol and triacylglycerol by inhibiting these hepatic enzymes in experimental animals (Lee, 1999; Lee, 2003; Kim, 2003). A study also demonstrated that hesperidin and naringin were beneficial for improving hyperlipidemia and hyperglycemia in type-2 diabetic animals by partly regulating the fatty acid and cholesterol metabolism and affecting the gene expression of glucose-regulating enzymes; they also markedly enhanced hepatic and adipocyte PPARγ protein expression (Jung et al., 2006). Furthermore, naringenin increased hepatic fatty acid oxidation through up-regulation of the gene expression of enzymes involved in peroxisomal β-oxidation and white adipose tissues in mice (Huong, 2006; Hamendra and Anand, 2007; Fukuchi et al., 2008).

Other positive effects of citrus extracts reported include antiviral, antiulcer, anticancer, antioxidant, diuretic, anti - allergy, antihypertensive, antimutagenic, relief of stomach upset, distension and asthma (Kim et al., 2000; Murakami et al., 2000; Kanaze et al., 2008).

Other constituents of citrus peels include essential oils which have lipolytic, antimicrobial, antioxidant and anti-inflammatory effects; (Hyang-Sook, 2006; Kanaze et al., 2008; Oliveira et al., 2014) and also vitamin C which contributes to effective digestion and weight loss by increasing acidity thereby increasing calcium assimilation

and replacement of fat in cells.

No negative side effects were reported in previous work in the animals that were fed with PMFs. However, in this work, the animals showed signs of tremor and exfoliation of fur for SY, OR and GR groups, both in the second week. Synephrine, the major alkaloid of C. aurantium, similar in structure to epinephrine was reported to exhibit milder ephedrine-like effects, which range from CNS stimulation, energy boost, appetite suppression to increased fat metabolism without the cardiovascular side effects of nervousness, dry mouth and high blood pressure (Pellati et al., 2002). A number of adrenergic alkaloidal amines (synephrine, n-methyl tyramine, hordemine, octopamine and tyramine) have been reported in the Mediterranean citrus, *C. aurantium*, as major ingredients of dietary supplements for weight loss (Pellati et al., 2002). The citrus peels used in this work contained alkaloids, although not classified.

Results obtained in this work and other previous reports that assessed the effects of these citrus flavonoids on lipid and glucose metabolism have led to the conclusion that the extracts from peels of Nigerian grown citrus could prevent the development of obesity through the modulation of lipid and glucose metabolism, with grapefruit peels being the single most effective peels while synergistic effect gave best results. Grapefruit peels have been reported to contain the highest total phenolics and the highest total antioxidant activity, followed by sweet orange peels, while tangerine peels had the least (Li et al.,2006; Londono-Londono et al., 2010).

Conclusively, peels from different types of Nigerian citrus which ordinarily serve as waste may synergistically be used to control and manage weight problems and associated pathologies. However, despite the positive effects of citrus extracts in weight reduction in this work, there may be risk of cardiovascular toxicity, due to the possible presence of adrenergic amines such as synephrine, n-methyl tyramine etc, which may have epinephrine-like action. Dosage control may be required to reduce the adverse effects. It has, however, been reported that different extraction processes may result in different products with varying concentrations and ratios of PMFs. The extraction process can therefore be selected and modified as desired to shift the ratios of the component PMFs (Kawaii et al., 1999) to enhance the beneficial and reduce the untoward effects, although the soil composition may have an effect on the concentrations of constituents. However, additional studies are needed to validate these conclusions.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Abu-Abid S, Szold A, Klaustner J (2002). Obesity and Cancer. J. Med. 33(1-4):73-86.

Aoki F (2007). Suppression by licorice flavonoids of abdominal fat accumulation and body weight gain in high-fat diet-induced obese C57BL/6J mice. Biosci. Biotechnol. Biochem. 71:206-214. http://dx.doi.org/10.1271/bbb.60463

Bays H, Blonde L, Rosenson R (2006). Adiposopathy: how do diet, exercise and weight loss drug therapies improve metabolic disease in overweight patients? Expert Rev. Cardiovasc. Ther. 4:871-895. http://dx.doi.org/10.1586/14779072.4.6.871

Bok SH (1999). Plasma and hepatic cholesterol and hepatic activities of 3-hydroxy-3-methyl-glutaryl-CoA reductase and Acyl CoA cholesterol transferase are lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids. J. Nutr. 129:1182-1185.

Daniels S (2006).Citrus peel extract shows benefit for diabetes. Life Sci. 79: 365-373.

Dhanavade MJ, Jalkate CB, Ghosh JS, Sonawane KD (2011). Study antimicrobial activity of lemon (Citrus lemon L.) peel extract. Br. J. Pharmacol. Toxicol. 2(3):119-122.

Duda-Chodak A, Tarko T (2007). Antioxidant properties of different fruit seeds and peels., Acta Sci. Pol. Technol. Aliment. 6(3):29-36.

Enterazi M, Majd A, Falahian F, Mehrabian S, Hasheni M, Layimi A (2009). Antimutagenic and anticancer effects of Citrus medical.fruit juice. Acta Med. Iran. 47(5):373-377.

Fujioka K, Greenway F, Sheard J, Ying Y (2006). The effects of grapefruit on weight and insulin resistance: relationship to the metabolic syndrome. J. Med. Food 9(1):49-54. 16579728. http://dx.doi.org/10.1089/jmf.2006.9.49

Fukuchi Y, Hiramitsu M, Okada M, Hayashi S, Nabeno Y, Osawa T, Naito M (2008). Lemon polyphenols suppress diet-induced obesity by up-regulation of mRNA levels of the enzymes involved in β-oxidation in mouse white adipose tissue. J. Clin. Biochem. Nutr. 43(3):201-209. http://dx.doi.org/10.3164/jcbn.2008066

Galati EM (1994). Biological effects of hesperidin, a citrus flavonoid. (Note I): antiinflammatory and analgesic activity. Farmaco. 40:709-712.

Hakim IA, Harris RB (2001). Joint effects of citrus peel use and black tea intake on the risk of squamous cell carcinoma of the skin. BMC Dermatol. 1:3. http://dx.doi.org/10.1186/1471-5945-1-3

Hamendra SP, Anand K (2007). Antidiabetic potential of Citrus sinensis and Punica granatum peel extracts on alloxan treated male mice. Bio Factors 31: 17-24. http://dx.doi.org/10.1002/biof.5520310102

Harborne JB (2003). Phytochemical Methods – A Guide to ModernTechniques of Plant Analysis Chapman and Hall, London

Hossain P, Kawar B, El-Nahan M (2007). Obesity and diabetes in the developing world – a growing challenge. J. New Engl. Med. 356(3):213-215. http://dx.doi.org/10.1056/NEJMp068177

Huang Y-W, Liu Y, Dushenkov S, Ho C-T, Huang M-T (2009). Anti-obesity effects of epiepigallocatechin -3-gallate, orange peel extract, black tea extract, caffeine and their combinations in a mouse model. J. Funct. Foods 1: 304-310. http://dx.doi.org/10.1016/j.jff.2009.06.002

Huong DT (2006). Activity and mRNA levels of enzymes involved in hepatic fatty acid oxidation in mice fed citrus flavonoids. Nutrition 22:546-552. http://dx.doi.org/10.1016/j.nut.2005.11.006

Hyang-Sook C (2006). Lipolytic effects of citrus peel oils and their components. J. Agric. Food Chem. 54(9):3254-3258. http://dx.doi.org/10.1021/jf052409j

Jacob R, Hasegawa S, Manners G (2000). The potentials of citrus limonoids as anticancer agents. Perishable handling Quarterly 8: 6-8.

Jung UJ (2004).The hypoglycemic effects of hesperidin and naringin are partly mediated by hepatic glucose-regulating enzymes in C57BL/KsJ-db/db mice. J. Nutr. 134:2499-2503.

Jung UJ, Lee MK, Park YB, Kang MA, Choi MI (2006). Effect of citrus flavonoids on lipid metabolism and glucose-regulating enzyme mRNA levels in type-2 diabetic mice. Int. J. Biochem. Cell Biol. 38(7):1134-1145. http://dx.doi.org/10.1016/j.biocel.2005.12.002

Kanaze FI, Termentzi A, Gabrieli C, Niopar I, Georgarakis M, Kokkalou E (2008). The phytochemical analysis and antioxidant activity of orange peel (Citrus sinensis) cultivated in Greece-Crete indicates a new commercial source of hesperidin. Biomed. Chromatogr. 23:239-249. http://dx.doi.org/10.1002/bmc.1090

Kangralkar VA, Patil AM, Gravimath CC, Kundargi R, Vidya K (2009).

Hepatoprotective activity of essential oil of Citrus reticulate against paracetamol induced hepatic damage in albino rats. Pharmacol. Online 3:505-508.
Karaca M, Ozbeck H, Him A, Tutuncu M, Akkan HA, Kaplanoglu V (2008). Investigation of anti-inflammatory activity of Bergamot oil. Eur. J. Gen. Med. 5(4):176-179.
Kawaii S, Tomono Y, Katase E, Ogawa K, Yano M (1999). Quantification of flavonoid constituents in citrus fruits. J. Agric. Food Chem. 47:3565-3571. http://dx.doi.org/10.1021/jf990153+
Khan Y, Khan RA, Syeda A, Afsan S (2010). Evaluation of hypolipidemic effect of citrus lemon. J. Basic Appl. Sci. 6(1):39-43.
Kim HK (2003). Lipid-lowering efficacy of hesperetin metabolites in high-cholesterol fed rats. Clin. Chim. Acta 327:129-137. http://dx.doi.org/10.1016/S0009-8981(02)00344-3
Kim DH, Song MJ, Bae EA, Han MJ (2000). Inhibitory effect of herbal medicines on rotavirus infectivity. Biol. Pharm. Bull. 23(3):356-358. http://dx.doi.org/10.1248/bpb.23.356
Kumar KA, Narayani M, Subanthini A, Jayakumar M (2011). Antimicrobial activity and phytochemical analysis of citrus fruit peels utilization of fruit waste. Int. J. Eng. Sci. Technol. 3:5414-5421.
Kurowska E, Manthey J (2004). Hypolipidemic effects and absorption of citrus polymethoxylated flavones in hamsters with diet-induced hypercholesterolemia. J. Agric. Food Chem. 52:2879-2886. http://dx.doi.org/10.1021/jf035354z
Lawal HO, Adewuyi GO, Fawehinmi AB, Adeogun A, Etatuvie SO (2012). Bioassay of herbal mosquito repellent formulated from the essential oil of plants. J. Nat. Prod. 5(2012):109-115.
Lee MK (2003).Naringenin 7-O-cetyl ether as inhibitor of HMG-CoA reductase and modulator of plasma and hepatic lipids in high cholesterol-fed rats. Bioorg. Med. Chem. 1: 393-398. http://dx.doi.org/10.1016/S0968-0896(02)00441-8
Lee SH (1999). Cholesterol-lowering activity of naringenin via inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase and acyl coenzyme A: cholesterol acyltransferase in rats. Ann. Nutr. Metab. 43:173-180. http://dx.doi.org/10.1159/000012783
Li BB, Smith B, Hossain M (2006). Extraction of phenolics from citrus peels 11: Enzyme-assisted extraction method. Sep. Purif. Technol. 46:189-196. http://dx.doi.org/10.1016/j.seppur.2005.07.019
Liang YC (2001). Suppression of inducible cyclooxygenase and nitric oxide synthase through activation of peroxisome proliferator-activated receptor-gamma by flavonoids in mouse macrophages. FEBS Lett. 496:12-18. http://dx.doi.org/10.1016/S0014-5793(01)02393-6
Londono-Londono J, Rodriguez de Lima V, Lara O, Andres G, Crecsynski Pasa TB, Arango GJ, Ramirez Pineda JR (2010). Clean recovery of antioxidant flavonoids from citrus peel: optimizing an aqueous ultrasound- assisted extraction method. Food Chem. 119(1):1 -87. http://dx.doi.org/10.1016/j.foodchem.2009.05.075
Miwa Y (2005). Glucosyl hesperidin lowers serum triglyceride level in hypertriglyceridemic subjects through the improvement of very low-density lipoprotein metabolic abnormality. J. Nutr. Sci. Vitaminol. (Tokyo). 51:460-470.http://dx.doi.org/10.3177/jnsv.51.460
Murakami A, Nakamura Y, Torikai K (2000).Antiproliferative effects of citrus flavonoids on human squamous cell carcinoma in vitro. Cancer Res. 60:5059-5066.
Murase T, Nagasawa A, Suzuki J, Hase T, Tokimitsu I (2002). Beneficial effects of tea cathechins on diet –induced obesity, stimulation of lipid metabolism in liver. Int. J. Obes. Relat. Metab. Disord. 26:1459-1464. http://dx.doi.org/10.1038/sj.ijo.0802141
Nagaraju B, Anand SC, Ahmed N, Narendra JN, Chandra S, Ahjmed F, Padmarathi GV (2012).Antiulcer activity of extracts of Citrus medica Linn. fruit against ethanol-induced ulcer. Adv. Biol. Res. 6(1):24-29.
Nannapaneni R, Muthaiyan A, Crandell PG, Johnson MG, Bryan CA, Chalova VI, Callaway TR, Carroll JA, Arthington JD, Nisbet DJ, Ricket SC (2008). Antimicrobial activity of commercial citrus-based natural extracts against Escherichia coli 0157:H7 isolates and mutant strains. Foodborne Pathog. Dis. 5(5):695-699. http://dx.doi.org/10.1089/fpd.2008.0124
Oliveira SAC, Zambrana JRM, Di Iorio FBR, Pereira CA, Jorge AOC (2014).The antimicrobial effects of Citrus limonum and Citrus aurantium essential oils on multi-species biofilms. Braz. Oral Res. (Sao Paulo). 28(1):1-6.http://dx.doi.org/10.1590/1807-3107BOR-2014.vol28.0035
Parmar HS, Kar A (2007). Antidiabetic potential of Citrus sinensis and Punica granatum peel extracts in alloxan treated male mice. Biofactors 31(1):17-24. http://dx.doi.org/10.1002/biof.5520310102
Pellati F, Benvenuti S, Melegari M, Firenzuoli F (2002).Determination of adrenergic agonists from extracts and herbal products from Citrus aurantium L. var amara by LC. J. Pharm. Biomed. Anal. 29(6):1113-1119. http://dx.doi.org/10.1016/S0731-7085(02)00153-X
Preuss HG, Di Ferdinando D, Bagchi M, Bagchi D (2002).Citrus aurantium as a thermogenic weight reduction replacement for ephedria, an overview. J. Med. 33(1-4):247-264.
Rayburn K, Martinez R, Escobedo M (1998). Glycemic effects of various species of nopal (Opuntia sp.) in type 2 diabetes mellitus. Texas J Rural Health 26:68-76.
Rouseff R, Ting S (1979).Tangeretin content of Florida citrus peel as determined by HPLC. Proc. Fla State Hort. Soc. 92:145-148.
Shisheva A (1992).Quercetin selectively inhibits insulin receptor function in vitro and the bioresponses of insulin and insulinomimetic agents in rat adipocytes. Biochemistry 31:8059-8063.
http://dx.doi.org/10.1021/bi00149a041
Silalahi J (2002).Anti-cancer and health protective properties of citrus fruit component. Asia Pacific J. Clin. Nutr. 11:79-84. http://dx.doi.org/10.1046/j.1440-6047.2002.00271.x
Sindler BH (2001).Herbal therapy for management of obesity: observations from a clinical endocrinology practice. Endocr. Pract. 7(6):443-447. http://dx.doi.org/10.4158/EP.7.6.443
Stohs SJ, Shara M (2007). A review of the safety and efficacy of Citrus aurantium in weight management. In: (ed.) Bagchi D, Preuss H. Obesity: Epidemiology, Pathophysiology and Prevention. Boca Raton, FL: CRC Press. pp. 371-382. http://dx.doi.org/10.1201/9781420005479.ch28
Talbot S (2009). The cortisol connection: Why stress makes you fat and ruins your health and what you can do about it. Chapter 8. The cortisol connection.com/ch8_6.php. Cited on March 10th, 2015.
Tao Ng, Liu Y, Zhang MI (2009).Chemical composition and antimicrobial activities of essential oils from the peel of bingtang sweet orange (Citrus sinensis Osbeck). Int. J. Food Sci. Technol. 44:1281-1285. http://dx.doi.org/10.1111/j.1365-2621.2009.01947.x
Tomar A, Mall M, Rai P (2013). Pharmacological importance of citrus fruits. Int. J. Pharm. Sci. Res. 4(1):156-160.
Valezquez-Nunez MJ, Avila-Sosa R, Palou E, Lopez-Malo A (2013). Antifungal activity of orange (Citrus sinensis var valencia) peel essential oil applied directly or vapor contact. Food Control 31(1):1-4. http://dx.doi.org/10.1016/j.foodcont.2012.09.029
WHO (2002). Traditional Medicine Strategy 2002-20056. Geneva, Switzerland. WHO.

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