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Formulation of *Persea americana* seed extract into tablet dosage.

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ABSTRACT

Formulation of ethanol seed extract of *Persea americana* Mill (Family Lauraceae) into tablet dosage form was investigated. Formulation into tablets was done by direct compression using lactose and microcrystalline cellulose (Avicel) as directly compressible diluent and wet granulation using 4% solutions of gelatin and polyvinyl pyrrolidone (PVP) as binders. Tablets were compressed at 10, 15 and 20 kN and later subjected to tablet quality testing procedures. The mechanical properties of the tablets were assessed using crushing strength (CS) and friability (F) and the crushing strength – friability ratio (CSFR) while drug release properties were evaluated using disintegration and dissolution times. There were statistically significant ($p < 0.01$) differences in the crushing strength (CS) values and drug release properties of *P. americana* prepared by both methods. Tablets containing Avicel produced better mechanical properties than tablets containing lactose. Tablets containing PVP as binder possessed better and more consistent release properties than tablets containing gelatin. Tablets produced by direct compression possessed better mechanical and release properties than tablets produced by wet granulation. The method of preparation of the *P. americana* tablets needs to be carefully selected to ensure the production of tablets with adequate bond strength and the same time release its active contents for pharmacologic action.

Keywords: *Persea americana*, direct compression, wet granulation, tablets, binder.

INTRODUCTION

Herbal or botanical medicine which primarily involves the use of plants or herbs (whole, parts, extract or metabolites) has transversed from the prehistoric age to the modern healthcare known to mankind. It is accompanied in various cultures by other traditional practices such as magic, incantations, rituals, and other inexplicable activities (Sofowora, 2008).

The history of herbal medicine has undisputedly and inextricably been intertwined with that of modern medicine. Of the drugs listed as conventional medications, many were originally derived from plants. The world Health Organization reports that 80% of the world's population presently uses herbal medicine for some aspect of primary healthcare (Majekodunmi *et al*., 2008). Examples of the many conventional medications which were originally derived from plants include salicylic acid, the precursor of aspirin. It was originally derived from white willow bark and the meadowsweet plant. Vincristine, used as an antineoplastic agent, comes from *Catharanthus roseus* (periwinkle). The Cinchona plant is the source of quinine, an effective antimalarial plant; *Papaver somniferum* L. yields morphine, codeines used as analgesic, antitussive and antidiarrhea (The Lancet, 1994; Cox and Balick, 1994).

The characteristics of traditional herbal medicine as a potential source of new drugs and a cheap source of starting material in drug synthesis makes traditional medicine an indispensable field for pharmaceutical researches and core component of modern healthcare. The medicinal values of herbal plants lie in their phytochemical components such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body (Pamplona-Rogers, 1999).

Persea americana Mill. (Avocado) belongs to the family Lauraceae and is widely distributed in Central America but cultivated in tropical and subtropical climates around the world. *Persea americana* knowns as Eben mbakara in Ibibio, Ube bekee in Igbo and Ado in Yoruba is a widely distributed plant in the lowlands and rain forest areas in Nigeria. The fruit is berry, consisting of a single large seed, surrounded by a succulent buttery pulp. The pulp is edible and contains 3-30% oil. It is widely used in Ayurveda and evidence-based phytotherapy. The plant is used in traditional medicine for the treatment of various ailments, such as hemorrhagia, hypertension, stomach ache, bronchitis, diarrhoea, and diabetes (Pamplona-Rogers, 1999; Adeyemi *et al*. 2002). The chemical constituents include peptone, b-galactoside, glycosylated abscisic acid, alkaloids, cellulose, polygalacto urease, polyuronoids, cytochrome *P-450*, and volatile oils were later reported to be present in this plant. The major chemical constituents of the various plant parts of avocado are alkanols, also called aliphatic acetogenins, terpenoids glycosides, various furan ring-containing derivatives, flavonoids and a coumarin.

In spite of their efficacy, herbal medicinal products have been widely criticised due to lack of standardization and poor quality presentation. In traditional medicine, the seed of *P. americana* is usually dried, ground and soaked in water or alcohol and unspecified quantities of the decoction are ingested (Majekodunmi *et al*., 2008).

However, to improve patient compliance and acceptability, there is need to formulate the ground seeds of *P. americana* into tablet dosage form. Thus, the aim of the present study is to produce conventional tablets of the extract of the ground seed of*P.americana* for oral administration using direct compression and wet granulation methods for the preparation of the tablets. The mechanical properties of the tablets were assessed using crushing strength, friability and crushing strength-friability ratio while the drug release properties were assessed using the disintegration and dissolution times.

MATERIALS AND METHODS

The materials used were: Avicel PH 102 (FMC International Co. Cork, Ireland), lactose (DMV, Veghel, Netherlands), polyvinylpirrolidine, average molecular weight 360,000 (Aldrich Chemicals Co Limited, Gillingham, Dorset UK), gelatin B.P (Hopkins and Williams, Chadwell, Health, Essex, UK); corn starch B.P, absolute ethanol (BDH Chemicals, Poole, UK), magnesium stearate (Hopkin and Williams, Chadwell, Health, Essex, UK). The seeds of *P. americana* were purchased from the herbal wholesalers at Itam market, Uyo, Nigeria and identified by Mr Etefa in the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo, Nigeria.

Extraction of the powdered seeds

The seeds of *P. americana*were chopped into bits to facilitate drying under shade at room temperature for seven days. The dried seeds were ground to a coarse powder using a laboratory mill (Kenwood UK Ltd, Hertfordshire, UK). One kilograme(1kg) of the powdered sample was exhaustively extracted three times with 2L absolute ethanol for 72h by maceration. The solvent was removed at 30^{o}C under reduced pressure in a vacuum and then evaporated to dryness in a rotary evaporator at 37^{o}C and stored in desiccators prior to analysis. The yield was 9.7%$^{w}/_{w}$.

Preparation of powder Mixtures for Direct compression

In the direct compression method, forty gram batches of 20%, 10% and 70% drug - disintegrant - diluent ratio consisting of *P. Americana* seed extract, corn starch and Avicel or lactose respectively were thoroughly mixed in a mortar for 10 minutes and stored in airtight containers.

Preparation of granule

Batches (200g) of a basic formula of *P. americana* extract (10 or 20% $^{w}/_{w}$), lactose (70 or 80% $^{w}/_{w}$) and corn starch (10% w/w) were dry-mixed for five minutes in a Kenwood planetary mixer and then moistened with the prepared 1%$^{w}/_{w}$ or 4%$^{w}/_{w}$ concentration of binder solution, (PVP or gelatine) to produce granules. Massing was continued for 5min and the wet mass was granulated by passingthem manually through a No12 mesh sieve (1,400μm), dried in hot air oven for 18 h at50^{0}C,and then resieved through a No16 mesh size

(1,000μm) to break aggregates and then dried again in a hot air oven for 24 h. The moisture content of the granule formulations was determined with an Ohaus Moisture Balance (Ohaus Scale Corporation, USA). The granules were stored in airtight containers.

Preparation of tablets

Tablets (500mg) were prepared from 500-1000μm size fractions of granule formulation by compressing them for 30secs with predetermined loads and 10, 15 and 20 kN using a Cadmach Single Punch Tableting Machine (Model: SSF3, Serial No. 193/Z/07-08, Cadmach Machinery Co PVT. Ltd. Ahmedabad-45, India). Before each compression, the die (10.5mm diameter) and the flat-faced punches were lubricated with a $2\%^{w}/_{w}$ dispersion of magnesium stearate in ethanol: ether mixture (1:1 solution). After ejection, the tablets were stored over silica gel for 24 h to allow hardening and elastic recovery.

Evaluation of tablet properties

Crushing Strength and Friability Tests

The load (N) required to diametrically break each tablet (crushing strength, CS) was determined at room temperature using a PTB 301 crushing strength tester (Pharmatest, Switzerland). The friability (F) of the tablets were determined using a Roche friabilator (Model TF 2D, Scientific Equipment Ltd., Bombay, India) operated at 25 revolutions per minute for 4 min.

Disintegration Test

The disintegration times (DT) of the tablets were determined in distilled water at 37 ± 0.5^{0}C using an Erweka disintegration testing apparatus (Model: Copley ZT2, Erweka Apparatebau GMBH, Heusenstamm, Germany). Six tablets randomly selected from each batch were used for the test.

Determination of absorption maxima for *P. americana* seed extract

Extract (1g) was placed in a 100 mL flask and 50mL of 0.1M HCl was added. The flask was agitated in a shaker for about 1h, and then filtered through a Whatman filter paper. The absorption spectrum of the solution of the extract was determined using a UV spectrophotometer (Model Cintra 6, Type GBC UV–Visible, GBC, Scientific Equipment Ltd, Victoria, Australia) and the wavelength for maximum absorption was determined. Various concentrations of the ethanolic extract of *P. americana* were prepared to contain between 0.02 to 0.1mg/mL of the extract in 0.1M HCl. The absorbance of each concentration was taken at 230nm and plotted against the various concentrations to obtain a straight-line graph (Beer Lambert plot) that passed through the origin. The slope formed the basis for the concentration calculations of the study.

Dissolution time test

The dissolution time of the tablets was determined at 37 ± 0.5^{0}C in 900 mL of 0.1 m HCl using a dissolution test apparatus (Model: DA-6D, Veego Scientific Devices Mumbai, India) with the rotating basket positioned 25 mm above the bottom of the round-bottomed flask and operating at 50 rpm. Samples were withdrawn at various time intervals and the amount of drug released was determined spectrophotometrically at 230 nm using the Visible Spectrophotometer replacing the sample with an equal volume of 0.1 m HCl at the same temperature to keep the volume of the dissolution medium constant during the course of the test. The amount released at each time was obtained from the Beer Lambert plot and the % release calculated. All the determinations were made in quadruplicate.

Stability test

P. americana tablets were stored at a temperature of 30 ± 2^{o}C and relative humidity of 75±4% for a period of twelve (12) months. The mechanical and release properties of the tablets after storage were assessed as earlier described.

Statistical analysis

Tukey-Kramers multiple comparison tests were used to compare the effects of the method of preparation and the excipient/binder on the mechanical and release properties of the tablets. At 95% confidence interval, *P* values less than or equal to 0.05 were considered significant.

RESULTS

The results of the mechanical properties of the *P. americana* tablets prepared using direct compression and wet granulation are shown in Table 1. The results indicate that CS increased while F decreased with increase in the compression pressure. The crushing strength values for tablets containing Avicel® was greater thantablets containing lactose as diluent while the reverse was the case for friability. The CS values for tablets prepared by wet granulation using PVP as binder was greater than tablets containing gelatin as binder. The CSFR values also increased with increasing compression force and concentration of diluent/binder employed. The CSFR values for tablets prepared by direct compressionwas greater than tablets containing lactoseas diluent while the tablets containing gelatin had its CSFR greater than tablets containing PVP as binder.

The drug release properties of pharmaceutical tablets are characterized by the disintegration and dissolution times. The result of the spectrophotometric analysis shows that the ethanolic extract of *P. americana* exhibited a principal absorption maximum at 230nm typical for alkaloids (Vinokurova *et al*., 2001).Thus, the calibration curve to assess the release properties of the tablets were determined at a wavelength of 230nm and the linear regression equation for the plot of absorbance versus

Table 1: Crushing strength, friability, crushing strength-friability ratio of *Persea americana* tablets prepared by direct compression and wet granulation (mean ±SD, n=6)

Compression Pressure (kN)	Diluent	CS (kN)	Direct Compression Friability (%)	CSFR	
10			2.53±1.25	0.99±0.04	2.81
15	Lactose		4.10±1.37	0.98±0.03	4.18
20			4.40±0.54	0.69±0.02	6.38
10			3.56±1.12	0.80±0.12	4.45
15	Avicel		4.53±1.62	0.65±0.23	6.85
20			6.00±3.76	0.25±0.04	15.00
Compression Pressure	**Binder**	**CSFR**	**Wet granulation CS (kN)**	**Friability**	
10			3.05±1.65	0.90±0.03	3.33
15	PVP		4.46±1.33	0.85±0.02	5.18
20			5.17±0.73	0.48±0.03	10.63
10			2.34±0.83	1.00±0.04	2.30
15	Gelatin		4.32±0.93	0.97±0.05	4.43
20			4.88±0.43	0.77±0.02	6.23

Table 2: Disintegration (DT) and dissolution times (t_{50}, t_{80}) of *P. americana* tablets prepared by direct compression and wet granulation (mean±SD, n=6)

Compression pressure (kN)	Diluent	Direct compression Disintegration time (min)	t_{50} (min)	t_{80} (min)
10		1.05 (0.02)	0.70(0.05)	0.90(0.32)
15	Lactose	1.37 (0.03)	0.95(0.04)	1.43(0.23)
20		1.53 (0.02)	1.07(0.02)	1.87(0.14)
10		1.08 (0.04)	68.65(0.12)	168.12(0.26)
15	Avicel	1.33 (0.020	63.66(0.04)	158.26(0.24)
20		2.33(0.13)	61.43(0.05)	147.44(0.23)
Wet granulation				
Compression Pressure (kN)	**Binder Conc.(%$^{w}/_{w}$)**	**Disintegration Time(min)**	**DissolutionTime t_{50} (min)**	**t_{80} (min)**
10		8.43±0.03	12.53±0.03	72.16±0.12
15	PVP	9.54±0.05	19.27±0.04	74.36±0.13
20		11.42±0.12	21.34±0.05	78.32±0.15
10		3.20±0.05	20.38±0.02	83.43±0.23
15	Gelatin	5.37±0.06	23.21±0.03	85.23±0.13
20		7.12±0.07	31.43±0.04	87.13±0.17

concentration was y = 2.5382x + 0.0004 with coefficient of determination, r^2 = 0.998. The amount of drug released was plotted against time and representative plots for tablets containing Avicel® as direct compression excipient and gelatin as binder are presented in Fig. 1. The dissolution time (t_{50} and t_{80} - time required for 50% and 80% of *P.americana* to be released respectively) were obtained from the dissolution profiles of the tablets. The disintegration and dissolution times of the tablets are shown in Table 2. The disintegration and dissolution times exhibited by tablets prepared by direct compression containing Avicel® was greater than tablets containing lactose, while the tablets prepared by wet granulation containing PVP as binder had their disintegration and dissolution times greater than tablets containing gelatin.

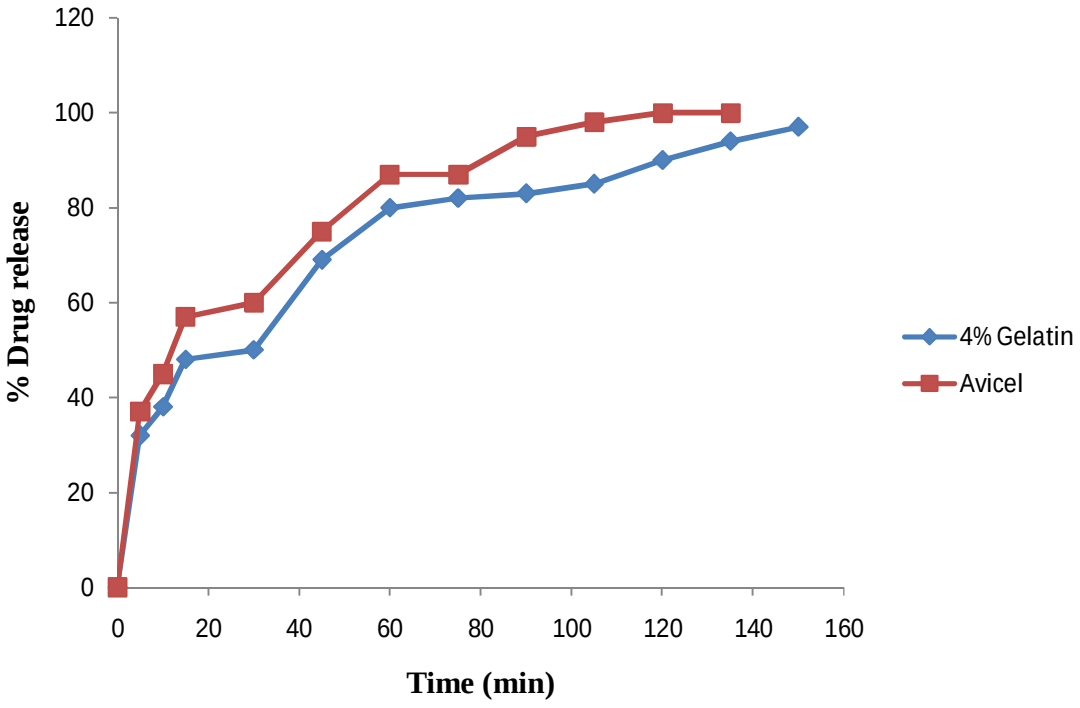

Figure 1: Representative plot of the release profile of *P. americana* tablets containing the Avicel as direct compression excipient and 4% gelatin as binder compressed at 10kN (Error bars = mean ± SD)

DISCUSSION

The mechanical properties of pharmaceutical tablets are important tests for pharmaceutical tablets that often form part of a manufacturer's own specification which are quantifiable by the crushing strength (CS) and the friability (F) of the tablets. The CS provides a measure of tablet strength while F is a measure of tablet weakness (BP,1998; Majekodunmi *et al*., 2008). Both parameters indicate the ability of tablets to withstand fracture and abrasion during production and subsequent use (BP, 1998; Odeku and Itiola, 1998). The Pharmacopoeial requirements (BP, 1998) for the crushing strength is largely dependent on the intended use of the tablet, while for friability, conventional compressed tablets that lose less than 1%w/w of their mass during the friability test are generally considered acceptable (BP,1998; Majekodunmi *et al*., 2008; Odeku and Fell, 2006). All the tablets prepared passed the test for friability by losing less that 1% of their weight. This indicates that all the excipients at the concentration used appear suitable for the production of *P.americana* tablets since the tablets possessed high friability values. Tablets prepared by wet granulation using all the binders on the other hand, showed acceptable CS and F values at the concentrations employed, indicating the suitability of wet granulation method for the production of *P.amercana* tablets. Furthermore, there were statistically significant ($p<0.05$) differences in the CSFR values of *P.americana* tablets prepared by both methods. The CSFR values of the tablets prepared by wet granulation were significantly ($p<0.05$) higher than those prepared by direct compression except for formulation containing Avicel®. Thus, the mechanical properties of the tablets were affected by the type and concentration of diluent/binder employed. The differences depended on the type and concentration of excipient and binder employed in the formulation. This result is similar to previous findings (Odeku and Fell, 2006).

All formulations of *P.amercana*tablets complied with the official requirement on disintegration (i.e. disintegration within 15 minutes). Statistical analysis showed that tablets prepared using 90%$^{w}/_{w}$ Avicel® as excipient had significantly ($p<0.05$) higher disintegration and dissolution times than those prepared using the other excipients. The dissolution times of the tablets prepared by wet granulation also varied and depended on the type and concentration of the binding agent employed. All the tablet prepared by wet granulation met the official requirement on dissolution for uncoated tablets (i.e release of 75%$^{w}/_{w}$ of the active drug within 45min) (BP, 1998).There were generally significant ($p<0.05$) differences in the dissolution times of tablets prepared by both methods. Theresults indicate the importance of the inclusion of excipients such as disintegrant in the formulation of *P.amercana* tablets.

Wet granulation appears to produce tablets of acceptable mechanical and drug release properties than those prepared by direct compression.

Thus, the methods of preparation of the *P.amercana*tablets need to be carefully selected to ensure the production of tablets with adequate bond strength to withstand the rigors of handling and at the same time release the active compound (s) for biological action. Furthermore, the type and concentration of excipient and binder employed in the formulation of *P.amercana* tablets need to be carefully chosen to enable the production of suitable tablets.

CONCLUSION

The result obtained shows that the methods of preparation of the *P.americana*tablets need to be carefully selected to ensure the production of tablets with adequate bond strength to withstand the rigors of handling and at the same time release the active compound (s) for biological action. Furthermore, the type and concentration of excipient and binder employed in the formulation of *P.americana* tablets need to be carefully chosen to enable the production of suitable tablets.

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