Formulation optimization of an improved traditional medicine from the stem bark extract of *Mangifera indica* L. using Design of Experiments (DOE) strategy.

Abstract

Introduction: Improved Traditional Medicines (ITMs), a recent concept by the World Health Organization (WHO) was introduced to promote the rational use of herbal medicine for primary health care in developing countries. The ITM by WHO andAIPO have categorized into 4 categories with respect to the quality of the active ingredient. However, this category needs more research in finding a greater variety of acceptable dosage forms. There is a need to account for formulation and process variables in these dosage forms to maintain product properties hence performance of plant extract, ensuring consistent quality. One of the methods to account for formulation and process variables is by using the Design of Experiments (DoE) approach.

Objective: The main objective of this work was to optimize the formulation of a category 2 Improved Traditional Medicine containing *Mangifera indica* L. stem bark aqueous extract using Design of Experiments.

Method: *Mangifera indica* L. stem bark was collected and identified at the National herbarium. It was dried, ground and the powder used for extraction using digestion method using water as solvent (at 70°C). Phytochemical screening was done on the extract. The extract then proceeded unto pharmaceutical development. The formulation optimization of *Mangifera indica* aqueous stem bark extract (MIABE) started with the definition of the Quality Target Product Profile (QTPP) that was expected for the final product; which is an orodispersible tablet that will facilitate patient compliance and promote a rapid disintegration. These QTPPs formed the basis of the Critical Quality Attributes (CQAs) which were identified (as hardness, disintegration time and mass uniformity) and used for all experiments. The experimental part was divided into 2 main manufacturing processes; direct compression and wet granulation techniques. Each process was investigated for drug product optimization.

A risk assessment was undertaken to identify the formulation variables that impact product quality. For direct compression, a 3^2 full factorial Design of Experiment (DoE) was used to investigate the effect of superdisintegrant (25%) and lubricant level (0.25-5%) on powder flow characteristics. For wet granulation, a 2^2 full factorial DoE was used to investigate the effect of superdisintegrant (2-5%) and binder (5-10%) on flow properties and tablet properties.

Results: The design and evaluation of the formulations in this study resulted in successful formulation optimization of an Improved Traditional Medicine. DoE proved to be an excellent method to optimize formulations of ITMs, providing several tools that increase a much better understanding of the formulation and manufacturing process. Further studies on this formulation DoE are needed to evaluate the effect of more process variables (compression force and speed) and more formulation variables such as palatability.

Conclusion: Optimization models were developed for the various responses (disintegration time, wetting time and hardness) showing the influence of formulation variables on these responses. Therefore, the formulation optimization of a category 2 ITM containing *Mangifera indica* L. stem extract using Design of Experiment is a suitable approach to save time, money and improve drug product understanding.

Key words: Improved Traditional Medicines, Design of experiments, orodispersible tablets, Quality by

Design

INTRODUCTION

In the last decade traditional medicine has become very popular in Cameroon, partly due to the long unsustainable economic situation in the country. The high cost of drugs and increase in drug resistance to common diseases like malaria, bacterial infections and other sexually transmitted diseases has caused the approach to the alternative traditional medicine as an important option for a concerted search for new chemical entities (NCE). WHO in collaboration with the Cameroon Government has put in place a strategic platform for the practice and development of TM in Cameroon [1, 2].

To protect Intellectual Property and ensure proper regulation of these traditional medicines, WHO and the African Intelligence Property Organization (AIPO) developed a categorization system of Improved Traditional Medicines (ITMs) into 4 categories [3]. In order to bring ITMs through the pipeline, reduction of early formulation development time and costs is crucial. Approaches that might shorten and improve drug development timeline are much sought-after [4]. In many cases, the value of the design phase is often underestimated in the rush to start development and get products to the market quickly [5].

An ITM formulation is composed of several composition factors and process variables. These factors and variables do not only affect the characteristic properties of the dosage form but also render formulation difficult [6]. This implies the need to account for formulation and process variables to consistently maintain product properties hence properties and performance of extract. One approach to study the effect of formulation and process variables is to use Design of Experiment (DoE), a systematic approach introduced by the International Conference on Harmonization (ICH) and the Food and Drug administration (FDA) in the year 2002 under the canopy of Quality by Design (QbD) [7, 8].The main objective of this work was the optimization of an Improved Traditional Medicine containing *Mangifera indica* aqueous stem bark extract using the Design of Experiments approach.

METHODS

Study type

The study was experimental factorial design based on the Quality by Design approach. The study was from 10th November 2016 to 5th May 2017. The raw material (Mango stem bark) was harvested at Mbangassina (Centre Region, Cameroon) and identified at the National Herbarium, Yaoundé with a voucher number **(**18646/SRFCam). Research took place in the Institute of Medical Research and the Studies of Medicinal Plants (IMPM) Yaoundé, at the phytochemistry laboratory and the Pharmaceutical Technology laboratory (LaboTEP).

Excipients

Talc, Magnesium stearate, gelatine, lactose, corn starch, methyl parahydroxybenzoate, sucrose, crospovidone and povidone were used in the manufacture of the orodispersible tablet and were gift samples provided by IMPM magazine. Strawberry powder was obtained commercially.

Herbal drug processing

Collection of the bark of *Mangifera indica* L. was collected from Mbangassina, in January 2017. It was dried under shade in an aerated room for 2 weeks. It was then ground with an electrical mill and stored in plastic bag.

Extraction

Ground bark was extracted by digestion method at 70°C with water used as solvent. 50kg of water was used for 3kg of dried powder and the process was repeated to increase yield. The extract, together with starch was dried in plates placed in an oven at 70°C to obtain a powder. The dried crude extract and starch was ground and stored in air tight plastic bags till further use. Percentage of extract in starch extract mix after drying was calculated as per equation 2 and percentage yield of extraction as per equation 3 [9, 10].

$$\%\ \textbf{extract in Starch extract mix} = \frac{\textbf{mass of extract in grams}}{(\textbf{starch extract mix})} \times 100$$

Equation 1: Percentage of extract in starch-extract mix

$$\%\ \textbf{Yield} = \frac{\textbf{Mass of dry extracts in grams}}{\textbf{Intitial mass of dry unprocessed powder}} \times 100$$

Equation 2: Percentage yield of extraction

Phytochemical Analysis

i. Test for alkaloids

1g of extract was mixed with 10ml distilled water in a test tube. To this mixture, 2% sulphuric acid was added. This was later split into 2 different test tubes in equal proportions [11]

a. **Meyer:** 3 drops of Meyers reagent was added to one of the test tubes. The presence of alkaloids is confirmed by the formation of white precipitates
b. **Draggendorf**: 3 drops of draggendorf reagent were added to the other test tube. Alkaloids are confirmed if formation of a brown precipitate [12].

ii. Test for phenolic compounds

To 0.5g of extract mixed in 5ml distilled water, a few drops of iron chloride solution were added. A resulting violet, greenish blue or black solution indicates the presence of phenolic compounds [13].

iii. Test for sterols and triterpenes

In a test tube containing 0.5g extract dissolved in 5ml distilled water, 3 drops of 10% potassium hydroxide is added. The solution was then heated in a water bath for 10minutes. It was left to cool; 3 drops of ether were added. This mixture is agitated, left to rest, and 3 drops of Libermann-Buchard reagent were added. The presence of triterpenes is confirmed by a violet coloration and sterols by a bluish green coloration [14].

iv. Test for flavonoids

A test tube containing the 0.5g extract and 5ml distilled water solution was heated for 10minutes, a few drops of methanol was added. The resulting solution was then added 3 drops of concentrated hydrochloric acid and a piece of magnesium. The presence of flavonoids is confirmed with effervescence and the brick red coloration [15].

Test for tannins

A mixture of 0.5g extract and 5ml distilled water was heated in a water bath and filter. To the filtrate was added 3 drops of iron chloride solution 3%. The appearance of a blue, blue black or black coloration indicates the presence of gallic tannins, a dark green indicates the presence of catechins [11, 16].

Test for saponins:

Foam Test: 0.5g of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins [17]

Quality Target Product Profile

The pharmaceutical development of MIABE ODTs began with the identification of the desired dosage form and performance attributes through the target product profile. *Mangifera indica* ODTs are being developed for clinical trial purposes.

The pharmaceutical target profile for MIABE is an orodispersible tablet that will facilitate patient compliance and promote a rapid onset action. The manufacturing process for the tablet should be robust and reproducible, and should result in a product that meets the appropriate drug product critical quality attributes. The expected quality profile for drug product is shown in (Table 1).

Table 1: Quality Target Product Profile of *Mangifera indica* L. orodispersible tablet [18]

Quality Target Product Profile element		Target	Justification
Dosage form		Tablet	Ease of production
Dosage design		Orodispersible tablets without a score or coating	Increased patient compliance
Route of administration		Oral	Ease of administration
Dosage strength		240mg	Suitable for clinical trial for antianaemia properties
Drug product quality attributes	Physical attributes	The colour and shape are acceptable to the patients. No unpleasant odour, no visible defects	Patient compliance
	Content uniformity	USP standards	USP standards
	Disintegration	Less than 3 minutes	European pharmacopoeia standards
Hardness		28- 60N Robust tablet able to transport and handling.	USP standards

Critical Quality Attributes

The QTPP forms the basis for determining the CQAs, critical process parameters (CPPs), and Control Strategy.

From the target product profile, the initial CQAs which were used to define satisfactory quality were identified. The CQAs definition was based on empirical evidence derived from previous experimentation as well as similar experiences with other products. Table 2 indicates which quality attributes were classified as CQAs.

Table 2: Critical Quality Attributes for *Mangifera indica* L. orodispersible tablets [19]

Quality Attributes of Drug Product	Target	CQA	Justification
Physical attributes			

Appearance	Colour and shape acceptable to patients. No visual tablet defects observed.	Yes	Changes in colour, shape and appearance can be an indication of physical and chemical degradation linked to safety and efficacy. Therefore, they are not critical. Target is set to ensure acceptability.
Odour	No unpleasant Odour	No	Neither extract nor the excipients have an unpleasant odour. No organic solvents is used in Tablet manufacturing process; therefore, considered critical
Size and Shape	Round 12mm diameter	No	For comparable ease of swallowing as well as patient acceptance and compliance with treatment regimens
Disintegration	Less than 3mins	Yes	The faster the disintegration the faster rate of dissolution.
Mass uniformity	USP Standards	Yes	Variability in mass uniformity will affect safety and efficacy.

Design of experiments (DoE)

For DoE, two factors three variables (level) (3^2) factorial was used for direct compression which requires 9 experiments. The two factors X1 (level of disintegrant) and X2 (level of lubricant) are represented by –1, 0, and +1, corresponding to the low, middle and high values respectively. These are represented on table 3 below.

Table 3: Coded Design of Experiments for Direct compression [19]

Factor	Level		
	-1 (low)	**0 (medium)**	**1 (high)**
X1 = Disintegrant (crospovidone) %	2	3.5	5
X2 = Lubricant (Magnesium stearate) %	0.25	2.5	5

Table 4 elaborates the design into 9 formulations possible description under superdisintegrant crospovidone and lubricant magnesium stearate (% w/w).

Table 4. 3^2 factorial design used for optimization of orodispersible tablets by direct compression [20]

Formulation	Superdisintegrant Crospovidone	Lubricant Magnesium stearate (%w/w)
F1	-1	1
F2	0	-1
F3	-1	0
F4	1	0
F5	0	1
F6	-1	-1
F7	1	1
F8	1	-1
F9	0	0

Table 5 gives the general formulation of the 9 different formulations under ingredient, function, percentage and quantity (mg).

Table 5. General composition of MIABE ODT by direct compression [21]

Ingredient	Function	Percentages %	Quantity (mg)
MIABE	Active ingredient	60	240
Cornstarch	Superdisintegrant	20	80
Kollidon CL (crospovidone) #	Superdisintegrant	2-5	8.8-22
Magnesium stearate #	Lubricant	0.25-5	1.1-22
Talc	Glidant	1	4.4
Kollidon (Povidone)*	Binder	2	8.8
Aerosil**	Drying agent	2	8.8
Sucrose	Sweetener	5	22
Paraben	Conservative	0.03	0.13
Strawberry flavor	Flavoring agent	0.04	0.18
Lactose	Filler		40.49-74.59
Total		100	440

As per coded value per formulation

*Used in F3-F9 **Used in F1 only

The level provided for each excipient is consistent with previous experience and based on literature. The formulation has a final mass of 440mg. Table 6 gives the details of formulation for all 9 formulations.

Table 6: Detailed formulations for direct compression [22]

Raw materials (%)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
MIABE	60	60	60	60	60	60	60	60	60
Cornstarch	20	20	20	20	20	20	20	20	20
Kollidon CL	2	3.5	2	5	3.5	2	5	5	3.5
Magnesium stearate	5	2.5	2.5	2.5	5	0.25	5	0.25	2.5
Talc	1	1	1	1	1	1	1	1	1
Kollidon	0	0	0	2	2	2	2	2	2
Sucrose	5	5	5	5	5	5	5	5	5
Strawberry powder	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Aerosil	2	0	0	0	0	0	0	0	0
Lactose	4.93	8.18	9.43	4.43	3.43	9.68	1.93	6.68	5.93
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Design of experiments

A 2^2 factorial design was implemented for the optimization of MIABE orodispersible tablet. The dependent response measured were disintegration time, hardness, friability, wetting time and water absorption ratio. Two independent factors, the concentration of crospovidone and concentration of gelatine were set at two different levels. High and low levels of each factor were coded +1 and –1, respectively. Experimental design 2^2 used for optimization of orodispersible tablets by wet granulation is shown in table

7.

Table 7: Experimental design 2^2 used for optimization of orodispersible tablets by wet granulation [15]

Factor	Level	
	-1 (low)	1 (high)
Disintegrant (crospovidone) %	2	5
Binder (gelatine) %	5	10

Table 8 gives the coded design for the 4 formulations (F10-F13) of wet granulation. The superdisintegrant Crospovidone ranged from -1 to +1.

Table 8: Coded Design of Experiments for wet granulation [23]

Formulation	Superdisintegrant Crospovidone	Binder: gelatine
F10	-1	-1
F11	1	1
F12	1	-1
F13	-1	1

The general formulation of the 4 batches has been represented in table 9. MIABE showed the highest (54.54%), role as active ingredient, while corn starch as superdisintegrant was 18.18%.

Table 9. General formulation for wet granulation

Raw material	Role	Percentages %
MIABE	Active ingredient	54.54
Corn starch	Superdisintegrant	18.18
Crospovidone	Superdisintegrant	2-5
Sucrose	Sweetener	5
Gelatin	Binder	5-10
Aerosil	Drying agent	2
Magnesium Stearate	Lubricant	0.5

Talc	Glidant	1.5
Strawberry powder	Flaovring agent	0.04
Paraben	Conservative	0.05
Microcrystalline cellulose	Filler /disintegrant	Qsp 100

Formulation for wet granulation. The raw material percentages for F10-F13 were of two phases (internal/intragranular phase and external/extragranular phase. The MIABE and corn starch showed no significant difference for F10-F13.Table 10. below gives the detailed formulation for the 4 batches of wet granulation.

Table 10: Detailed formulation for wet granulation.

Raw material %	**F10**	**F11**	**F12**	**F13**
Internal /intragranular Phase				
MIABE	54.54	54.54	54.54	54.54
Corn starch	18.18	18.18	18.18	18.18
Gelatin	5	10	5	10
Parabens	0.05	0.05	0.05	0.05
External/extragranular phase				
Kollidon CL	2	5	5	2
Magnesium stearate	0.5	0.5	0.5	0.5
Talc	1.5	1.5	1.5	1.5
Aerosil	2	2	2	2
Sucrose	5	5	5	5
Strawberry powder	0.04	0.04	0.04	0.04
Microcrystalline cellulose	11.19	3.19	8.19	6.19

Statistical analysis

Raw data on measured variables were collected and entered in Microsoft Excel 365. The GraphPad Instat version 5.1 software was used for comparison between the groups which were analyzed using one-way analysis of variance, the ANOVA test followed by Turkey's Kramer post hoc test 13]. The results were expressed in terms of mean ± standard deviation. P-values ≤0.05 were considered as statistically significant. Means were used in plotting of graphs for results presentations.

Ethical considerations

Ethical clearance for this work was obtained from the Institutional Review Board (IRB) of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I. Furthermore, we obtained administrative authorisations from the Institute of Medical Research and the Studies of Medicinal Plants (IMPM) to carry out all the required laboratory work and procedures.

RESULTS

A mean percentage yield 42.96% was obtained with water extraction by digestion. Greater percentage yield was observed when the water: drug extract was increased from 12.5:1 (batch 1) to 16.7:1 (batches 2-7). The extraction of *M indica* by digestion is shown in Table 11.

Table 11: Extraction of *Mangifera indica* L. by digestion

Batch	Starting mass (Kg)	Amount of extract (g)	Yield (%)
1	4	1555.9	38.9
2	3	1301.9	43.40
3	3	1294.0	43.13
4	3	1593.6	53.12
5	3	1284.5	42.81
6	3	1070.4	35.68
7	3	1309.5	43.65
Total	22	9409.8	**42.96**

Phytochemical screening

Phytochemical screening provided plants with colour, flavour and natural protection against pests. A phytochemical screening was performed on the *Mangifera indica* stem bark aqueous extract and the results have been shown in table 12. Phytochemical screening showed the absence of alkaloids and triterpenes and detected the presence of active pharmaceutical components such as tannins, saponins, phenols, flavonoids and coumarins.

Table 12: Phytochemical screening of aqueous stem bark extract of *Mangifera indica* L.

Phytochemical	MIABE Mbangassina
Alkaloids	-
Saponins	++
Phytosterols and triterpenes	-
Phenols	+++
Tannins	++++
Flavonoids	+++
Reducing sugars	+++
Coumarins	++

+= Detectable presence ++= slightly abundant +++= abundant ++++ = very abundant

Pre-compression studies

To determine the flow properties of our powder blends, bulk and tapped densities, Carr's index (1), Hausner's ratio (2), and the angle of repose (3) were analysed before compression into tablets. Table 13 shows pre-compression parameters of powder mixture.

Table 13: Pre-compression parameters of powder blends

Method	Formulation	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle of repose (°)	Carr's index	Hausner's ratio
Direct compression	**F1**	0.68	0.88	33.33 ± 0.88^{ab}	23.0	1.29
	F2	0.65	0.90	35.67 ± 0.33^{ab}	27.8	1.38
	F3	0.67	0.94	29.33 ± 0.88^{a}	29.0	1.40
	F4	0.67	0.94	33.00 ± 3.00^{ab}	25.5	1.40

	F5	0.63	0.86	35.33 ± 1.85^{ab}	26.7	1.36
	F6	0.63	0.85	36.67 ± 1.76^{bc}	27.0	1.37
	F7	0.66	0.86	36.00 ± 0.01^{ab}	23.0	1.30
	F8	0.59	0.81	43.67 ± 0.67^{c}	27.0	1.37
	F9	0.56	0.89	36.67 ± 0.67^{bc}	37.0	1.59
Wet granulation	**F10**	0.67	0.80	27.33 ± 0.88^{b}	16.3	1.19
	F11	0.72	0.79	18.63 ± 1.13^{a}	8.9	1.09
	F12	0.63	0.79	28.47 ± 0.78^{b}	20.3	1.25
	F13	0.67	0.76	20.97 ± 2.91^{ab}	11.8	1.13

***Legend** : Values carrying the same letter for the same method are not statistically different ($p \geq 0.05$)*

Summary of pre-compression parameters

The figure 1 below shows a summary comparing direct compression and wet granulation technique and the pre-compression parameters of the powder blends.

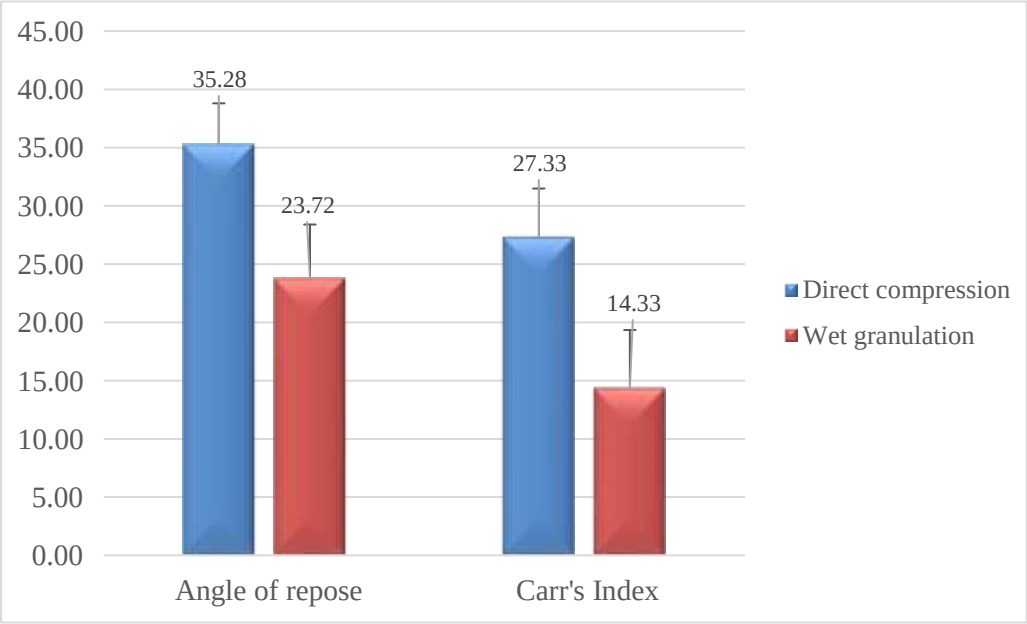

Figure 1: Comparison of Angle of repose and Carr's index for direct compression and wet granulation

From the pre-compression studies done on the powder blends for both tablet manufacturing processes (direct compression and wet granulation), we made the following observations as detailed in table 14

below. From these results, we observed that the powder blend for wet granulation had better flow characteristics than with direct compression.

Table 14: Observations made from powder flow of formulations

Method Formulati	n Observation	
Direct compression	**F1**	Passable and good
	F2	Very poor and poor
	F3	Good and very poor
	F4	Passable and poor
	F5	Very poor and poor
	F6	Very poor and poor
	F7	Very poor and good
	F8	Very poor and poor
	F9	Very poor and very poor
Wet granulation	**F10**	Good and good
	F11	Excellent and excellent
	F12	Good and good
	F13	Good and excellent

Modelling of disintegration time.

The ANOVA table partitions the variability in Disintegration time (DT) into separate pieces for each of the effects (table 15). It then tests the statistical significance of each effect by comparing the mean square against an estimate of the experimental error. In this case, 3 effects have P-values less than 0.05, indicating that they are significantly different from zero at the 95,0% confidence level.

Table 15: Analysis of variance for disintegration time

Parameter	Sum of squares	Df	Mean square	F-Ratio	p-Value
Superdisintegrant	4458.31	1	4458.31	4118.53	0.000 level
Binder level	54149.8	1	54149.8	50022.88	0.000

Superdisintegrant level x Binder level	2394.19	1	2394.19	2211.72	0.000
Total error	8.66	8	1.08		
Total (corr.)	61010.9	11			

The R-Squared statistic indicates that the model as fitted explains 99,98% of the variability in Disintegration time. The adjusted R-squared statistic, which is more suitable for comparing models with different numbers of independent variables, is 99,98%.

Wetting time.

The wetting time for all 4 batches of tablets produced showed highest wetting time for the F13 formulation., as shown in figure 2. F13 (low crospovidone and high binder) showed highest wetting time 66.0s and F12 (High crospovidone and low binder) the lowest with 19.43s. The wetting time of all the tablet formulations was within the range of 19.5-73.0 sec.

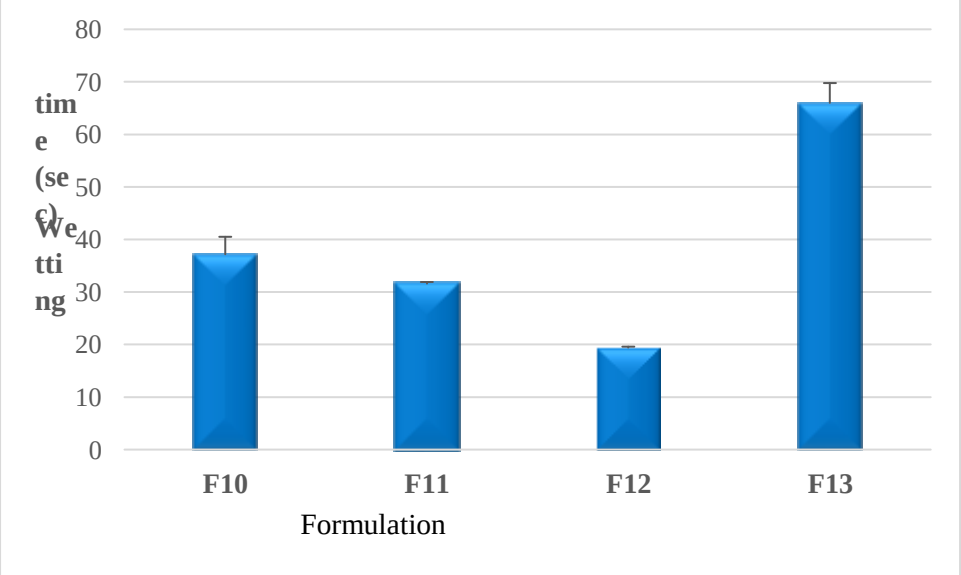

Figure 2: Wetting time values for wet granulation formulations

Optimisation of wetting time.

With above equation the lowest wetting time that can obtain is 19.34 sec under the following conditions: superdisintegrant level of 5% and Binder level of 5%. The relationship between the dependent and independent variable was further elucidated by constructing counter plots. The effects of X1 and X2 with their interaction on hardness at different levels (low and high level) are displayed in Figure 3; the interaction effect between X1 and X2 are shown in response surface plot figure 3.

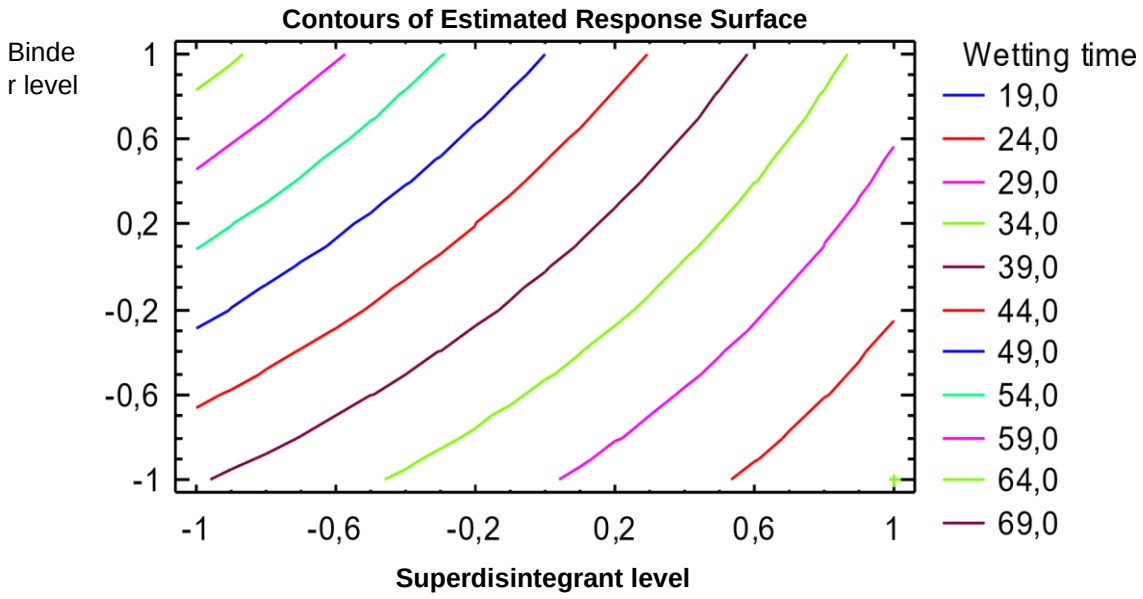

Figure 3: Contour plot for wetting time

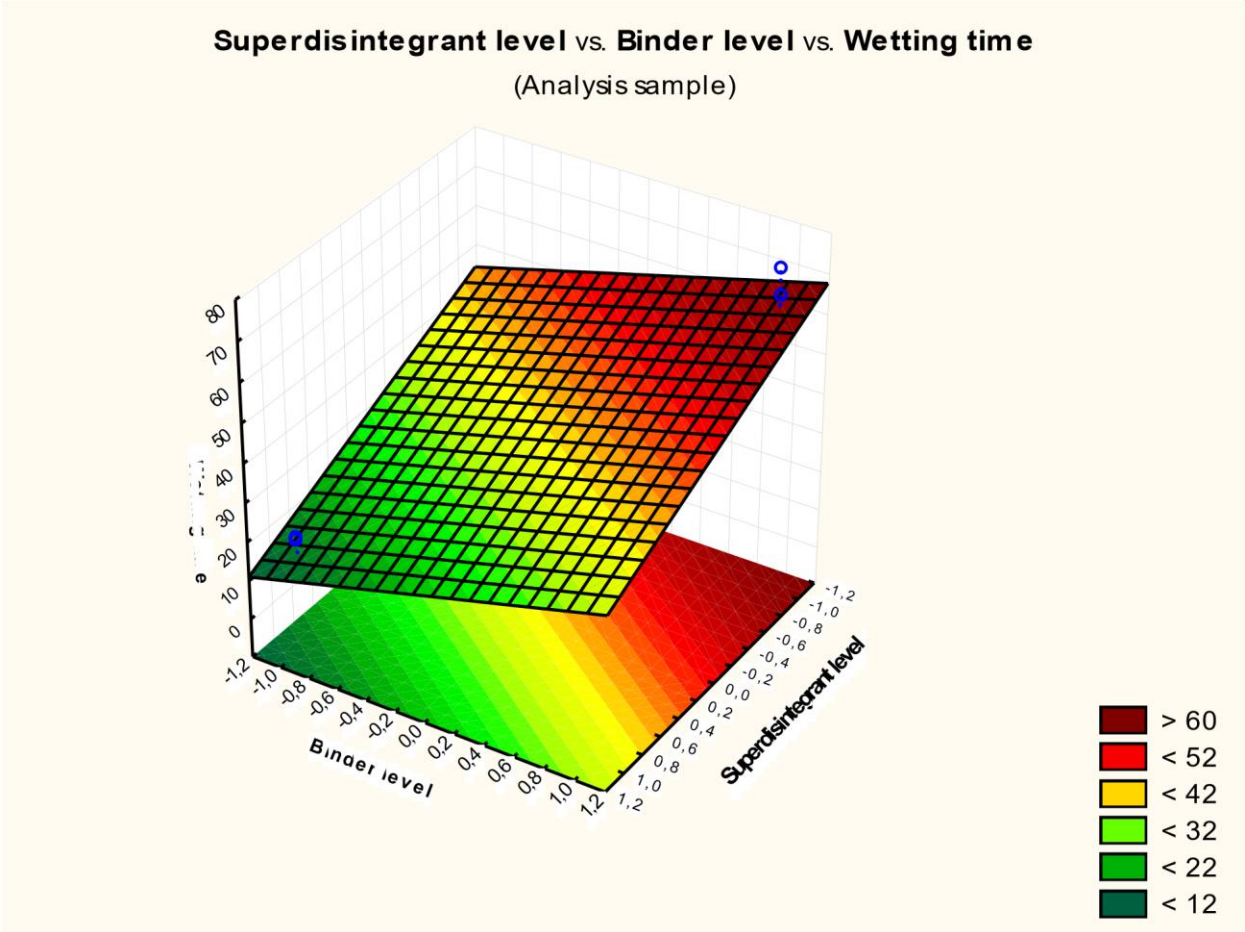

Figure 12: Response surface plot for wetting time Water absorption ratio

DISCUSSION

The percentage yield 42.96% proved to be relatively higher as compared to other researchers who used maceration and obtained a yield of 12.3% [6, 24] and 27.3% when rotated to increase yield [13, 19]. The basic principle is to grind the plant material (dry or wet) finer, which increases the surface area for extraction thereby increasing the rate of extraction. Earlier studies reported that solvent to sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal [2, 14, 25].

In our study water was chosen; which is a universal solvent and is frequently used to extract plant products; components easily extracted with water include anthocyanins, starches, tannins, saponins, terpenoids, polypetides and lectins [15], most of which were of interest in our study. However, other solvents could further reveal the presence of more phytochemicals. In conclusion, decoction though a laborious and time-consuming process is a method of choice in the case of heat sensitive material and to increase yield. Distilled water is an acceptable solvent in the extraction process for MIABE in the case of human consumption.

Phytochemical screening

The presence of active pharmaceutical components such as saponins, phenols, tannins, flavonoids and coumarins in our MIABE concurs with findings by other researchers and further support the use of *M. indica* stem bark in herbal medicine to fight diseases [26]. In contrast, similar studies done by Mada S *et al* in Nigeria [18] showed the presence of alkaloids and terpenoids in addition to the phytochemicals mentioned above; they also showed positive for cardiac glycosides, xanthoproteins and anthroquinones. Alkaloids were absent but resins were detected in addition to flavonoids [27, 28].

The biological functions of flavonoids include protection against allergies, inflammation, free radical scavenging, platelets aggregation, microbes, ulcers, hepatoxins, viruses and tumors. Mangiferin, catechin and epicatechin are the major phyto-constituents of *M. indica*. These flavonoids are responsible for the antioxidant effects of the mango stem bark and leaves [24, 29]. The mango stem barks contain polyphenols, which have the ability not only to protect the human organism from the attack of oxidative chemical species (OCS) but also are able to reach the target organs and tissues. The antioxidant activity of all those polyphenols is governed by the number and location of these aromatic hydroxyl groups. Phenols protect plants from oxidative damage and perform the same functions for humans. The outstanding phytonutrients feature of phenols is their ability to block specific enzymes that causes inflammations. They also modify the prostaglandin pathways, thereby protecting platelet from clumping [13, 30].

Saponins' natural tendency to ward off microbes makes them good candidates for treating fungal and yeast infections. These compounds served as natural antibiotics, helping the body to fight infections and microbial invasion. These compounds also appear to greatly enhance the effectiveness of certain vaccines. Plant saponins help humans to fight fungal infections, combat microbes and viruses, boost the effectiveness of certain vaccine and knock out some kinds of tumour cells particularly lung and blood cancers. They also lower blood cholesterol thereby reducing heart disease. The most outstanding and exciting prospects for saponins are how they inhibit or kill cancer cells [31]. They may also be able to do it without killing normal cells in the process, as is the mode of some cancer fighting drugs. Cancer cells have more cholesterol type compounds on their membranes than normal cells. Saponins therefore bind cholesterol and thus, interfere with cell proliferation [26].

Tannins are reported to exhibit antiviral, antifungal, antibacterial, anti-tumour activities. It was also reported that certain tannins are able to inhibit HIV replication selectivity and also used as diuretic. Plant tannins have been recognized for their pharmacological properties and are known to make trees and shrubs a difficult meal for many caterpillars [32, 33]. Tannins have important roles such as been stable and potent antioxidants. Herbs that have tannins as their main component are astringent in nature and are used for treating asthma, pneumonia, and dysentery, thus justifying the use of the plant in traditional medicine practice [13, 34]. In conclusion, the presence of these phytochemicals could explain the use of MIABE in traditional medicine. It should be noted that many factors account for the variability in results such as geographical location, extraction method, season and time of harvest [35].

The various factors that can influence flowability were the object of our pre-compression studies. Bulk density is basically how much a material will compact under various loads and it is an indicator of flow. Generally, a free-flowing powder will show very small change in bulk density from the initial value to its tapped value (consolidation stress). A cohesive or poor flowing powder will generally show a large increase in bulk density (30-50%) from bulk density as tapping increases [36]. Bulk density depends on a number of factors including particle size distribution, true density, particle shape and cohesiveness due to surface forces including moisture. It generally decreases with decreasing particle size and decreases as the particle shape becomes less spherical and more irregular. This explains the lesser values of bulk density obtained direct compression (mean value of 0.64g/cm^3) versus wet granulation (mean value of 0.67g/cm^3).

The smaller the particles, the greater the surface of the powder; this phenomenon increases the friction between particles and subsequently decreases bulk density (case of direct compression powders). Another explanation stems from the fact that lactose used in direct compression is denser and bigger (diameter) than microcrystalline cellulose (MCC) used in wet granulation [7, 37]. This is significant in that small differences in particle size have been observed to make a big difference in flowability of powder and powder mixtures. Increase in lactose from 1.93% in F7 to 6.68% in F8 resulted in

considerable decrease in bulk density from 0.66g/cm^3 to 0.56g/cm^3 respectively. High particle density has been observed to favour free flow of powders, therefore an increase in lactose, will consequently improve powder flow characteristics. Same observation is gotten when comparing F10 and F11 of wet granulation where MCC was used in 11.19% and 3.19% respectively; an increase in bulk density was observed as MCC (filler) was increased. These results are similar to that of other researchers where flow properties are improved as MCC is increased [27, 38]. This means that once the lubricant and the respective excipient were blended, a lubricant film was formed around the excipient particles, easing their rearrangement, sliding and packing in the powder bed. As a consequence, the powder bulk and tap densities increased and porosity decreased, as compared to the un-lubricated materials. Other authors have found similar results, especially when lubricants such as talc and stearic acid are employed. In terms of efficiency, highly hydrophobic lubricants such as magnesium stearate induced the largest volume reduction in most excipients [9, 39].

Research has shown that lactose gives higher volume reductions after tapping than microcrystalline cellulose [21, 40]. Finer particles are expected to give larger values of volume reduction (hence higher tapped density values). MCC is more porous and hence less compressible (consequently possess good flowability and low cohesiveness) than lactose. It is therefore expected that MCC will impart increase flowability to the mixture in which it is included.

Fillers greatly influence bulk properties of powders and so selection of fillers is very crucial when formulating. Filler such as MCC and lactose could cover up the effect of a poor flowing active ingredient if active ingredient is low dose. The technique also influences the flow properties; with wet granulation creating heavier particles with increased sizes (good flow) [41]. The better flow characteristics displayed by the powder mix for wet granulation as opposed to direct compression is in accord with findings by other teams [12, 42]. Some researchers have however shown some superiority of direct compression over wet granulation. For the purpose of our study, we decided to proceed to tableting of wet granulation powder blends which had better flowability.

Disintegration time the most important parameter that needs to be optimized in the development of orally dispersible tablets is the disintegration time of tablets. In the present study tablets, three formulations out of four (F10, F11, F12) disintegrated under 3 minutes thereby fulfilling the official requirements (<3 min, i.e. 180s) for dispersible tablets [9, 43]. F12 (high superdisintegrant and low binder levels) had the least disintegration time of 22.2seconds and F13 (low superdisintegrant and high binder levels) had the highest disintegration time of 195.1seconds**.**

It was observed that among the various disintegrants used such as Corn starch, Crospovidone, Croscarmellose Sodium and Sodium Starch Glycolate, Crospovidone shows relatively faster

disintegration time at same concentration as compared to others Crospovidone is a synthetic homopolymer of cross-linked N-vinyl pyrrolidinone and it is a white, free flowing, compressible powder and hygroscopic in nature. It might be due to high water uptake capacity and low gelling capacity of Crospovidone [17, 44] when compared to others. Difference in swelling may also play a role in disintegrating agent efficiency it causes the tablet to disintegrate quickly. And because of its high crosslink density, it swells rapidly in water without gel formation. Therefore, crospovidone uses a combination of mechanisms to provide rapid disintegration (wicking and swelling).

The optimum concentration of superdisintegrant can be selected according to critical concentration of disintegrant. Below this concentration the tablet disintegration time is inversely proportional to the concentration of superdisintegrant. If the concentration of superdisintegrants incorporated in tablet is above the critical concentration, the disintegration time remains approximately constant or even increases [25]. However, in high dose tablets, research shows that increasing the strength of the super disintegrant Crospovidone did not alter the DT considerably, unless mixed with other disintegrants. Addition of CP alone to the dry extract reduced the DT of the tablet appreciably when compared to the tablets containing lower amount of the dry poly herbal extract [16].

The measurement of wetting time may be used as another confirmative test for the evaluation of fast dissolving tablets. The wetting volume is important to check minimum volume of water required for wetting of tablet. It is related to the contact angle and gives an insight into the disintegration properties of tablets; a lower wetting time implies a quicker disintegration of the tablet [8].

Superdisintegrant has a negative coefficient in the equation 14 and binder level a positive coefficient. This implies that the higher superdisintegrant, the lower the wetting time and the higher the binder, the higher the wetting time. We can note that the coefficient of the binder (7.75) is much higher than the superdisintegrant value (2.55) implying that the binder has an effect of approximately two times that of superdisintegrant. It also worth noting that the superdisintegrant effect is antagonistic to binder influence on the disintegration time; this is shown by the reduction of coefficient when combined (interaction effect, impossible to detect with the OFAT and best guess method).

CONCLUSION

Based on the results from this study, the following conclusions were reached that the aqueous extraction of *Mangifera indica* L. through digestion had more yield as compared to other extraction methods and the extract contains the following phytochemicals: tannins, saponins, phenols, tannins, flavonoids and coumarins compounds, known by literature to possess pharmacological activities. Direct compression and wet granulation techniques greatly influenced the flow properties of the powder blend with wet granulation showing superiority by producing free flowing granules of MIABE ODTs. Formulation

variables, lubricant and diluent amount were also found to play a role in powder flow. Their presence, if well selected could significantly improve powder flow. Superdisintegrant and binder levels were seen to influence significantly the tablet properties (disintegration time, wetting time and hardness) of MIABE ODT with a non-significant influence on the water absorption ratio. The statistical design has the advantage of performing a small number of experiments and the fitted model from the statistical analysis can be used to predict values of responses at any point inside the experimental space. The design can be successfully used to optimize the wet granulation formulation. In conclusion, in this project, ODTs of *Mangifera indica* L. stem bark extract was successfully prepared using a QbD (DoE) approach, using a wet granulation method. The design and evaluation of the formulations in this study resulted in successful formulation optimization of an Improved Traditional Medicine. DoE proved to be an excellent method to optimize formulations of ITMs, providing several tools that increase a much better understanding of the formulation and manufacturing process. Further studies on this formulation DoE are needed to evaluate the effect of more process variables (compression force and speed) and more formulation variables such as palatability.

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