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SELECTION OF OPTIMUM PROCESS, SOLVENT AND DRYING METHOD FOR EXTRACTION OF ANTIOXIDANTS

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Abstract. An experimental study was performed for optimum process, solvent and drying method for the extraction of antioxidants from *Morinda citrifolia* fruit. Extractions were performed by soxhlet, ultrasonic and modified version of high hydrostatic pressure extraction at 100 bar and 50ºC with 15 grams of powdered morinda in 200 ml of solvent. Water, ethanol and ethyl acetate were used as solvents. The filtrate was dried by vacuum and spray dryers. The antioxidant activity or inhibiton percentage of the extracts was determined by Di-Phenyl picryl hydrazyl radical scavenging method. The yield was also determined. Optimization was performed using response surface methodology. It was found that the highest antioxidant activity or inhibition percentage of DPPH free radicals was exhibited by the extract obtained from HHPE process with ethyl acetate as solvent and vacuum dried. It was also found that the highest yield was obtained from HHPE process at 100 bar and 50ºC with ethanol as solvent and vacuum dried. It was noted that the nature of the process and the solvent used significantly contributed to the extraction of antioxidants from *Morinda citrifolia* fruit when compared with the drying methods.

Keywords: Antioxidant activity, DPPH radical scavenging method, high hydrostatic pressure extraction, *Morinda citrifolia*, ultrasonic extraction, soxhlet extraction

Abstrak. Satu uji kaji telah dijalankan untuk pemilihan proses optimum, pelarut, dan teknik pengeringan bagi pengekstrakan antioksidan daripada buah *Morinda citrifolia*. Pengekstrakan telah dijalankan dengan *soxhlet*, ultrasonik dan tekanan tinggi hidrostatik yang diubahsuai pada 100 bar dan 50ºC dengan 15 gram serbuk *Morinda* dalam 200 ml pelarut. Pelarut yang telah digunakan ialah air, etanol, dan etil asetat. Hasil penurasan kemudiannya dikeringkan melalui vakum dan pengering semburan. Aktiviti antioksidan atau peratusan ekstrak perencatan ditentukan dengan radikal Di-Phenyl picryl hydrazyl. Hasil ekstrak juga telah ditentukan dalam kajian ini. Pengoptimuman telah dilakukan dengan menggunakan kaedah gerak balas permukaan. Hasil kajian ini menunjukkan bahawa, aktiviti antioksidan atau peratusan perencatan DPPH radikel bebas yang paling tinggi dipamerkan daripada ekstrak yang diperolehi dari proses HHPE dengan etil asetat sebagai pelarut yang dikeringkan melalui vakum. Daripada kajian ini juga, hasil ekstrak yang tertinggi diperolehi melalui proses HHPE pada 100 bar dan 50ºC, di mana etil asetat digunakan sebagai pelarut dengan teknik pengeringan vakum. Di samping itu, didapati juga bahawa keadaan pengekstrakan dan pelarut secara langsungnya memberi kesan kepada

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pengekstrakan antioksidan daripada buah *Morinda citrifolia* jika dibandingkan dengan teknik pengeringan.

Kata kunci: Aktiviti antioksidan, radikel DPPH, pengekstrakan tekanan tinggi, *Morinda citrifolia*, pengekstrakan ultrasonik, pengekstrakan *soxhlet*

1.0 INTRODUCTION

Medicines from herbal and natural products have been used for centuries in every culture throughout the world. The true health benefits of these remedies without any recorded side effects have captured the interest in this field from scientists and medical professionals. *Morinda citrifolia* or mengkudu is one of the traditional folk medicinal plants that has been used for over 2000 years in Polynesia [1]. It has been reported to have a broad range of health benefits and for treatment of cancer, infection, arthritis, diabetes, asthma, hypertension, and pain [2]. The Polynesians utilized the whole plant known as Noni in their medicinal remedies and as dye for some of their traditional clothes. The roots, stems, bark, leaves, flowers, and fruits of Noni plant are all involved in various combinations in almost 40 known and recorded herbal remedies [3].

A number of phytochemicals has been identified in the Noni plant. Scopoletin, octoanoic acid, vitamin C, terpenoids, alkaloids, anthraquinones (such as nordamnacanthal, morindone, rubiadin, and rubiadin -1-methyl ether, anthraquinone glycoside), β-sitosterol, carotene, vitamin A, flavone glycosides, linoleic acid, alizarin, rutin, and a putative proxeronine are some of the major and rare antioxidants present [4].

Generation of free radicals or reactive oxygen species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress [5]. Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process [6]. This concept is supported by increasing evidence that oxidative damage plays a role in the development of chronic, age-related degenerative diseases, and that dietary antioxidants oppose this and lower risk of disease [7].

Apart from their role of health benefactors, antioxidants are added in foods to prevent or delay oxidation of food, initiated by free radicals formed during their exposure to environmental factors such as air, light and temperature [8]. At present, most of the antioxidants are manufactured synthetically. They belong to the class of synthetic antioxidants. The main disadvantage with the synthetic antioxidants is the side effects when taken *in vivo* [9]. Strict governmental rules regarding the safety of the food has necessitated the search for alternatives as food preservatives [10].

Wide range of uses of phytochemical antioxidants in nutraceutical, pharmaceutical and in food industries as preservatives has led to the development of technology for their extraction from plant matrices. Conventionally, the compounds are extracted with suitable solvent, followed by evaporation to separate solvent from the product. This method however yields less antioxidant [11]. There are many extraction methods, such as soxhlet, heat reflux, boiling, and distilling methods, which are traditional [12] and microwave, supercritical fluid technologies etc., that are sophisticated [13].

According to a recent work done by Mohd. Zin *et al.,* most of the antioxidants present in the *Morinda citrifolia* are of non-polar nature. The dried and powdered materials were extracted using methanol [14]. There are various extraction processes to extract desired phytochemicals from plants [15]. An experimental study has been performed to select the optimum process, solvent and drying method for the extraction of antioxidants from *Morinda citrifolia* fruit. Three extraction processes namely soxhlet extraction, ultrasonic extraction and our modified version of high hydrostatic pressure extraction (HHPE) process were used. Solvents chosen were water, ethanol and ethyl acetate in the order of polar, bipolar and non-polar respectively. Spray drying and vacuum oven were the drying methods used. The extraction time was 6 hours. Antioxidant activities of the extracts were determined by Diphenylpicrylhydrazyl (DPPH) radical scavenging method. The yield of the extracts after drying was also noted.

The data obtained was further analysed by the statistical and numerical optimization technique called Response Surface Methodology (RSM). RSM uses quantitative data from appropriate experiments to determine and simultaneously solve multivarient equations [16]. In general, RSM attempts to fit a polynomial of appropriate degree to the response of the system of interest. The goal of the system of interest is termed the response. This response is normally measured on a continuous scale and is a variable which likely represents the most important function of the system, though this does not rule out the possibility of investigating more than one system function, i.e., more than one response. Also contained in the system are input variables that affect the response and are subject to control [17].

2.0 MATERIALS AND METHODS

2.1 Chemicals and Equipment

Morinda citrifolia fruit was bought at fully grown unripe stage and washed with the tap water. The fruits at the right stage were cut and the seeds removed. The fruits were sun dried and made into powder using a micro-grinder with 1.5 mm sieve. The powder thus obtained was sieved using trays for different sizes of the particle. Ethanol and ethyl acetate (HPLC grade) were obtained from J. T. Baker (USA). 2,2-Diphenyl picryl hydrazyl (DPPH) was from Fluka Chemie, Germany.

The batch hydrostatic pressure extractor apparatus consists of a converted and altered parr 4842 series high pressure reactor. The inlet port on the lid of the reactor was removed and sealed. The volume of the extraction vessel is 300 ml and the equipment is connected to a nitrogen cylinder. The ultrasonic extractor apparatus is a sonics, vibra-cell disintegrator from Sonics, USA. The equipment has variable amplitude at 20 KHz along with the provision of fixing the duration of the ultrasound. 30% of the probe is dipped into the solution to provide ultrasound to the sample. The soxhlet extractor apparatus consists of a 24/29 neck size siphon and round bottom flask. The sample is taken in the cotton cuvette supplied by the manufacturer. The solvent was heated at 105ºC for water, 80ºC for ethanol and ethyl acetate.

2.2 Extraction Method

Extraction with soxhlet, ultrasonic and high hydrostatic pressure extraction at 100 bar and 50ºC were performed with water, ethanol and ethyl acetate as solvents. 15 grams of dried and powdered *Morinda citrifolia* fruit powder (50 μm – 1200 μm) was added to 200 ml of the solvent. The filtrate obtained using whatman no. 4 filter paper was divided into two parts. One portion of filtrate was dried in a vacuum oven maintained at 130 mm Hg and 40ºC and the other portion was spray dried. The 18 extracts obtained were immediately analysed for their antioxidant activity or inhibition percentage by Di-phenylpicrylhydrazyl (DPPH) radical scavenging method [18].

2.3 Extract Yield Determination

The yield of extracts after drying was accurately measured using four decimal Sartorius electronic balance and is represented in terms of mg extract/15 g of sample.

2.4 Data Analysis

The data was analysed, evaluated and optimized by response surface methodology using the software package, Design expert 6.0.10. The statistical experimental design suggested by D-optimal method of this software is shown in Table 1. The data was numerically optimized and represented as interaction graphs in terms of inhibition percentage.

Table 1 Statistical experimental design suggested by D-optimal method of Design-Expert software

Std	Run	Block	Factor 1 A:Time hr	Factor 2 B:process	Factor 3 C:solvent	Factor 4 D:drying
14	1	Block 1	6.00	Level 2 of B	Level 2 of C	Level 1 of D
13	2	Block 1	6.00	Level 3 of B	Level 1 of C	Level 1 of D
27	3	Block 1	6.00	Level 2 of B	Level 2 of C	Level 1 of D
20	4	Block 1	2.00	Level 2 of B	Level 2 of C	Level 1 of D
25	5	Block 1	2.00	Level 1 of B	Level 3 of C	Level 1 of D
21	6	Block 1	4.00	Level 2 of B	Level 2 of C	Level 2 of D
24	7	Block 1	2.00	Level 1 of B	Level 2 of C	Level 1 of D
23	8	Block 1	2.00	Level 3 of B	Level 1 of C	Level 1 of D
17	9	Block 1	6.00	Level 1 of B	Level 2 of C	Level 2 of D
6	10	Block 1	6.00	Level 2 of B	Level 3 of C	Level 2 of D
16	11	Block 1	6.00	Level 2 of B	Level 1 of C	Level 2 of D
22	12	Block 1	2.00	Level 2 of B	Level 1 of C	Level 1 of D
1	13	Block 1	3.00	Level 3 of B	Level 1 of C	Level 2 of D
7	14	Block 1	6.00	Level 1 of B	Level 3 of C	Level 1 of D
28	15	Block 1	6.00	Level 3 of B	Level 1 of C	Level 1 of D
10	16	Block 1	2.00	Level 2 of B	Level 3 of C	Level 1 of D
18	17	Block 1	5.00	Level 1 of B	Level 2 of C	Level 1 of D
15	18	Block 1	2.00	Level 2 of B	Level 1 of C	Level 2 of D

2.5 Experimental Design

In Table 1, Level 1 of B or B1 represents the soxhlet extraction process, Level 2 of B or B2 represents the ultrasonic process and Level 3 of B or B3 represents the high hydrostatic pressure extraction process. Level 1 of C represents water, Level 2 of C represents ethanol and Level 3 of C represents ethyl acetate as solvent. D represents the drying method while Level 1 and Level 2 represent spray drying the vacuum dried extracts respectively.

3.0 RESULTS AND DISCUSSION

3.1 Antioxidant Activity Analysis at Various Process Conditions

3.1.1 Water Extraction vs Drying Method

The RSM single response analysis results of the extraction processes with water as solvent and spray dried are shown in Figure 1. The highest inhibition percentage or antioxidant activity was exhibited by the extracts obtained from high hydrostatic pressure extraction (HHPE). This was followed by the extracts from ultrasonic and soxhlet extraction.

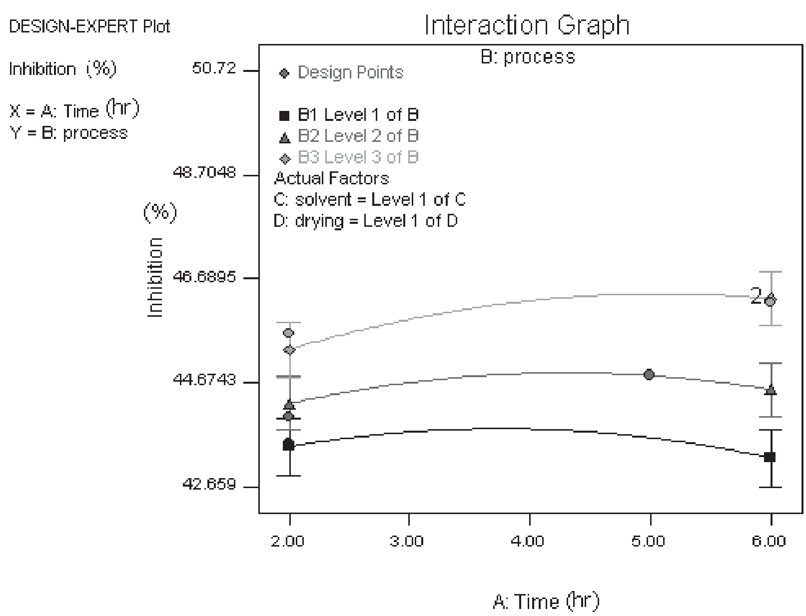

Figure 1 Extraction processes with inhibition percentage for water and spray dried extracts

Significant difference in yield was found between high hydrostatic pressure extraction at 100 bar and 50°C and the other two processes.

Figure 2 shows the inhibition percentage obtained for the *Morinda citrifolia* fruit extracts with water as solvent and vacuum dried. Higher antioxidant activity or inhibition percentage was obtained for the extracts dried using vacuum oven than spray dried (as shown in Figure 1). This may be due to the volatile nature of the antioxidants present in the filtrate. *Morinda citrifolia* fruit contains a number of volatile components [4] which can get vented into the atmosphere along with the high temperature and pressurized air used in spray drying.

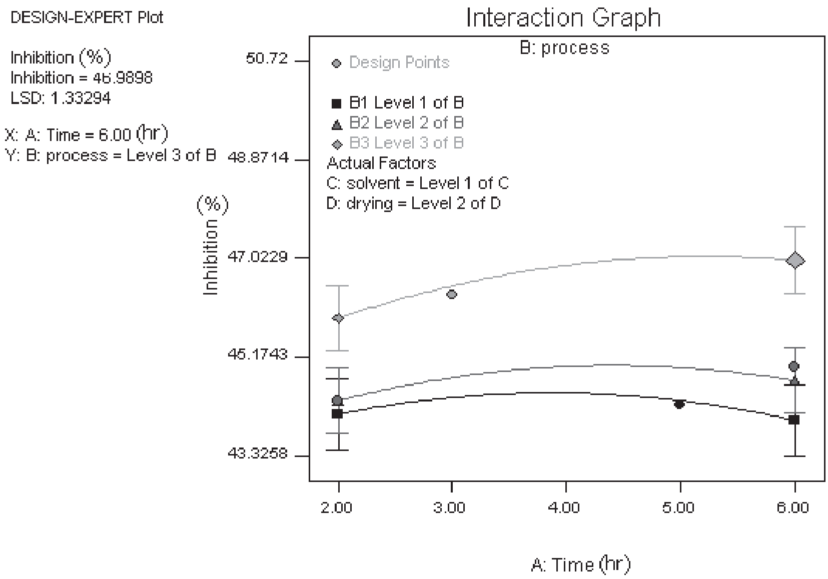

Figure 2 Extraction processes with inhibition percentage for water and vacuum dried extracts

3.1.2 Ethanol Extraction vs Drying Method

Figure 3 shows the interaction graph for single response analysis for the extraction processes with ethanol as solvent and the extracts dried with spray drier. The highest inhibition percentage for the extracts was exhibited by the extract obtained by HHPE. This was followed by the ultrasonic process. There was found to be a significant difference in antioxidant activity between HHPE the two processes. This may be due to the nature of the solvent and the process involved. Ethanol is a bi-polar solvent which is highly volatile. Volatile solvents have a higher penetration capacity which can be increased with pressure and temperature. This is based on the phase behavior theory, that the solubility is greater as the pressure increases. Under the process of high hydrostatic pressure extraction, the differential pressure between the cell interior and the exterior of cell membranes is so large that it will lead to rapid permeation. Consequently, the concentration between the cell interior and the exterior of cell membranes can reach equilibrium in a short time. HHPE involves the application of pressure for extraction of antioxidants from the *Morinda citrifolia* fruit matrices.

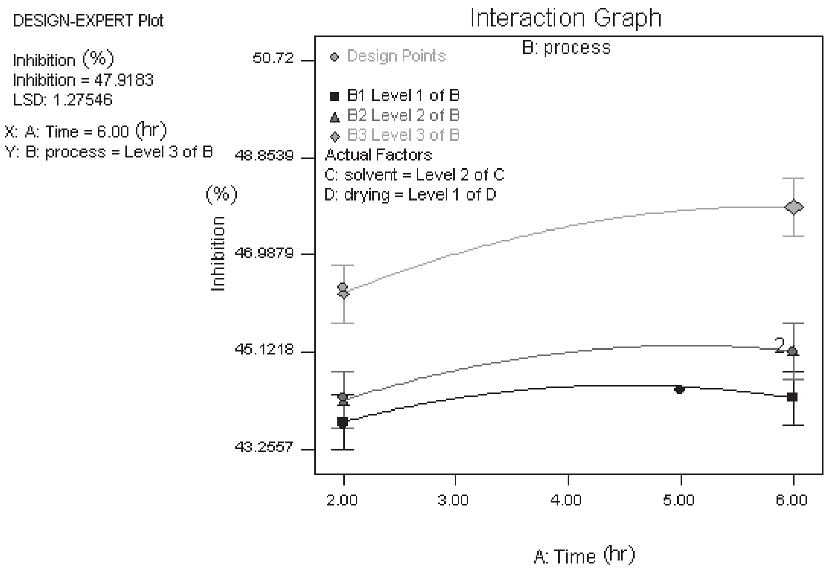

Figure 3 Extraction processes with inhibition percentage for ethanol and spray dried extracts

Figure 4 shows a countable increase in antioxidant activity can be noted for the extracts dried using vacuum oven.

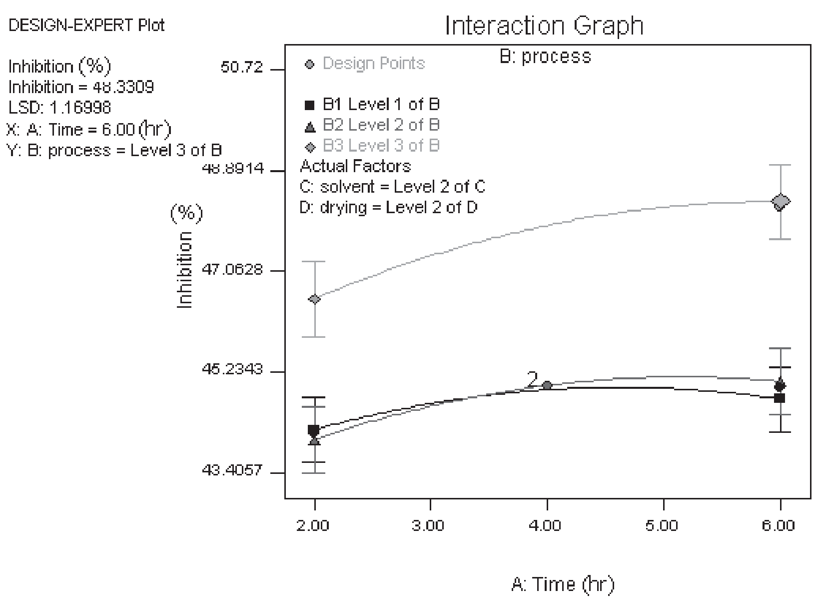

Figure 4 Extraction processes with inhibition percentage for ethanol and vacuum dried extracts

3.1.3 Ethyl Acetate Extraction vs Drying Method

From Figure 5, it can be noted that the extraction processes with ethyl acetate as solvent exhibits the highest antioxidant activity or inhibition percentage for spray dried extracts. Comparatively more difference was obtained between the HHPE and other processes. The inhibition percentage obtained was also higher for HHPE when compared with the HHPE with ethanol as solvent, as shown in Figure 3. This may be due to the polarity of the solvent used. Ethyl acetate is a non-polar solvent. The antioxidants in the *Morinda citrifolia* fruit are mostly non-polar in nature [14].

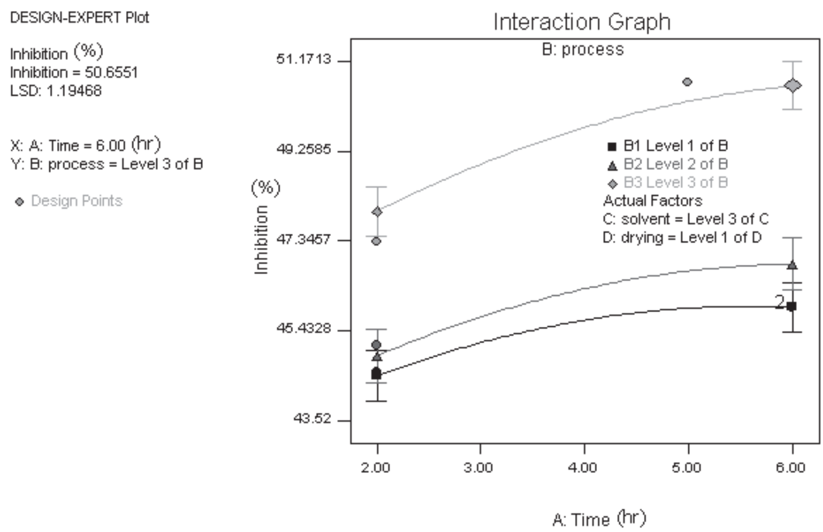

Figure 5 Extraction processes with inhibition percentage for ethyl acetate and spray dried extracts

Figure 6 expresses the same trend as Figures 2 and 4 for HHPE and other processes. However, countable increase in antioxidant activity can be noted for the extracts dried using vaccum oven. The highest antioxidant activity among all the extracts was obtained for HHPE extract with ethyl acetate as solvent and vacuum dried as shown in Figure 6.

3.2 Yield of the Extracts at Various Processing Conditions

The following graphs show the multiple response analysis of RSM with yield as the second response considered for study. The pattern of the graph was found to be similar to that of the antioxidant activity. The highest yield was obtained from the HHPE process followed by ultrasonic and soxhlet. The trendline in the graphs were found to be more or less parallel to the *x*-axis. This may be due to the nearing of equilibrium by the yield after the first hour of extraction process in case of HHPE and ultrasonic and slow extraction in case of soxhlet extraction.

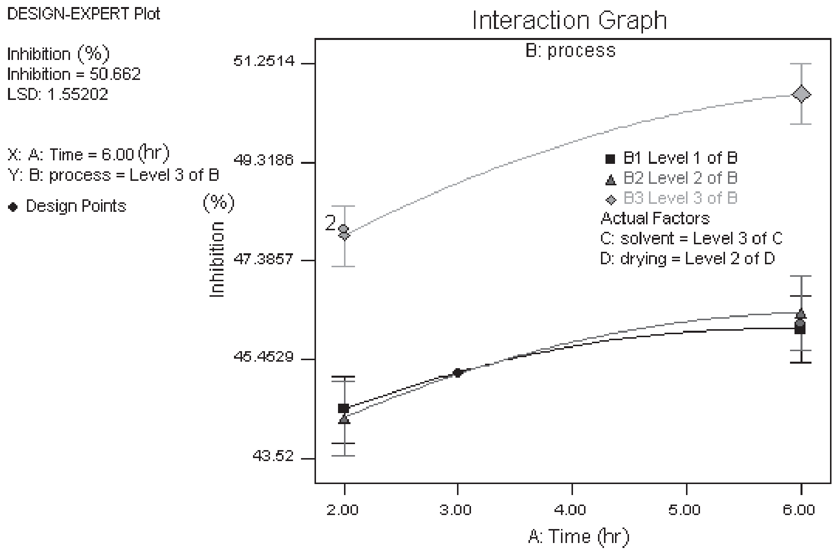

Figure 6 Extraction processes with inhibition percentage for ethyl acetate and vacuum dried extracts

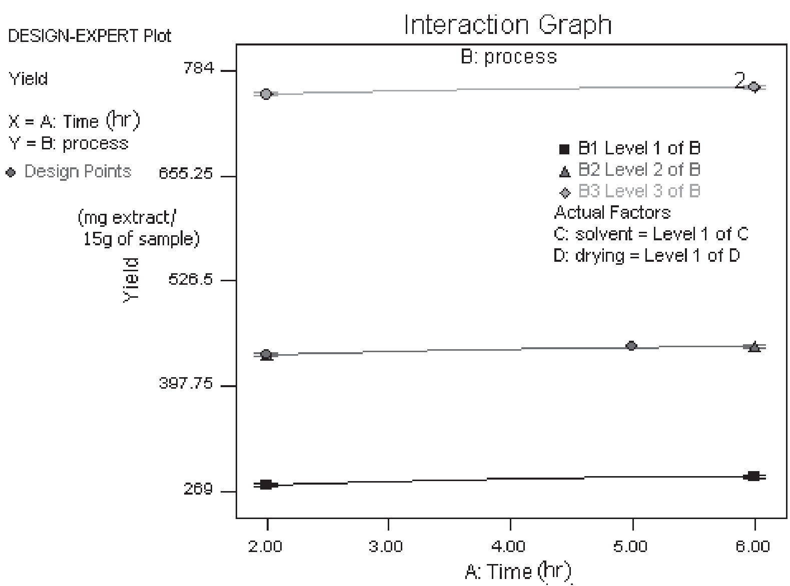

Figure 7 Extraction processes with yield for water and spray dried extracts

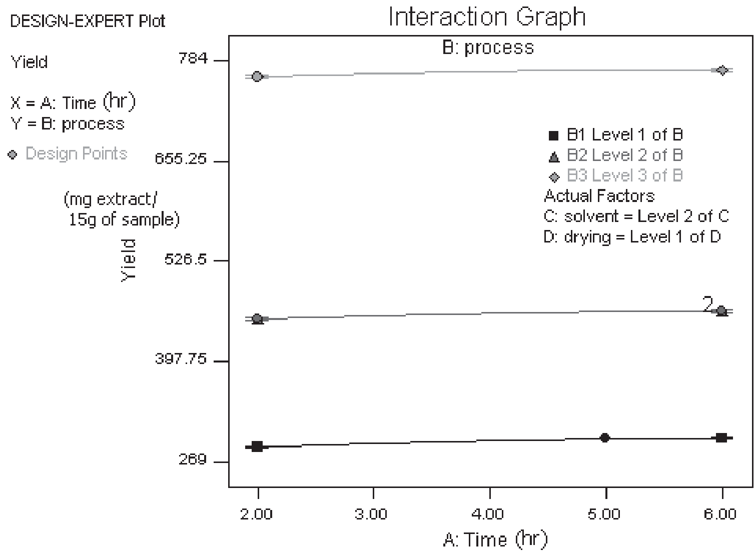

Figure 8 Extraction processes with yield for ethanol and spray dried extracts

From Figures 7 and 8, it was found that the yield was higher comparatively for the processes with ethanol as solvent and spray dried.

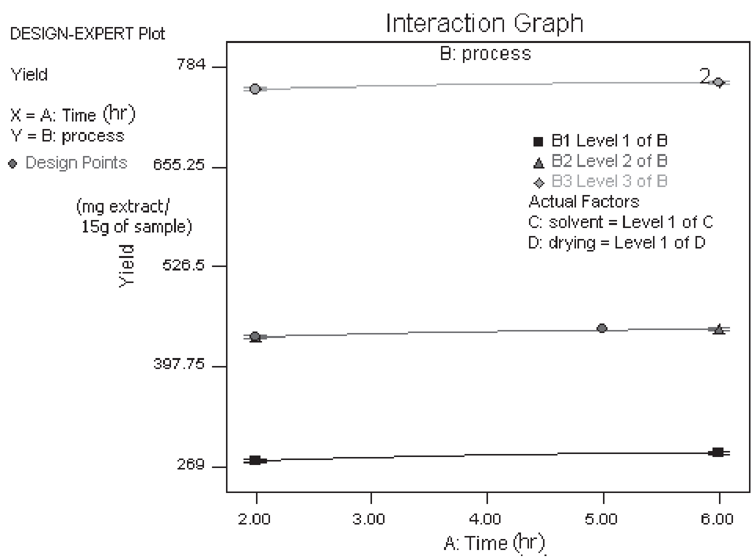

Figure 9 Extraction processes with yield for ethyl acetate and spray dried

The lowest yield was obtained for processes with ethyl acetate (Figure 9). This may be due to the factor that ethyl acetate is non-polar.

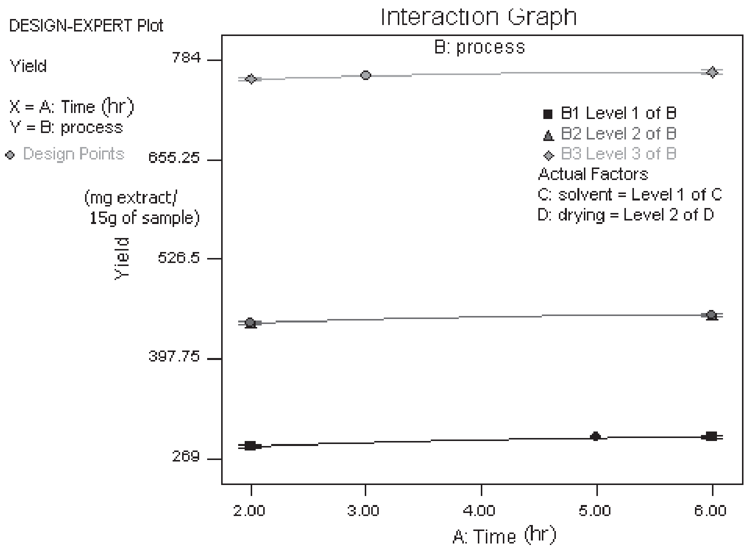

Figure 10 Extraction processes with yield for water and vacuum dried extracts

From Figures 10 and 11, it was noted that the highest yield was obtained for extraction processes with ethanol and vacuum dried. The yield was also found to be higher than the spray dried extracts. This was found due to the process involved in drying. In case of spray drying, some of the extracted phytochemicals may be vented into the atmosphere along with the pressurized air. Ethyl acetate extracted extracts exhibited lower yield comparatively as shown in Figure 12.

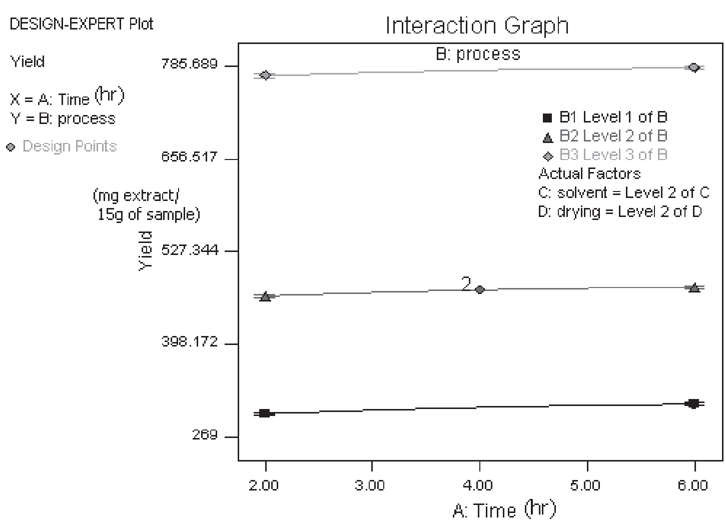

Figure 11 Extraction processes with yield for ethanol and vacuum dried extracts

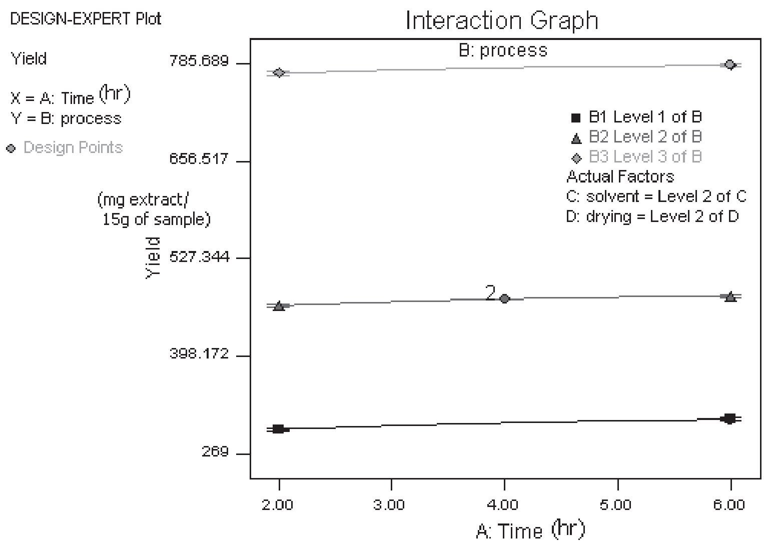

Figure 12 Extraction processes with yield for ethyl acetate and vacuum dried extracts

From the multiple response analysis of antioxidant activity or inhibition percentage and yield, it can be noted that the optimum antioxidant activity and yield was from HHPE process at 100 bar and 50°C with ethyl acetate as solvent at a time duration of 6 hours.

4.0 CONCLUSION

18 extracts of *Morinda citrifolia* were obtained from various combination of extraction process as mentioned in materials and methods section. The highest antioxidant activity or inhibition percentage of DPPH free radicals was exhibited by the extract obtained from HHPE process with ethyl acetate as solvent and vacuum dried. Process optimization performed through Response Surface Methodology also showed that the highest yield was obtained from HHPE process at 100 bar and 50°C with ethanol as solvent and vacuum dried. It was noted that the solvent used determined the yield and quality of the antioxidant. The yield which is based on the amount of extract was found to be higher for ethanol as solvent due to its bi-polar nature. However, the antioxidant activity which is based on the amount of active antioxidants was found to be higher in the extracts using ethyl acetate as solvent and so it can be concluded that the active antioxidants in the *Morinda citrifolia* extracts are of non-polar nature.

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