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Extraction and Microencapsulation of Polyphenols from *Orthosiphon Stamineus* Leaves

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Motivation

Misai kucing (*Orthosiphon stamineus*) is traditionally used in Malaysia for treatment of bladder inflammation, gout, eruptive fever, edema, hepatitis, jaundice, hypertension, diabetes mellitus, rheumatism and diuretic (Ho *et al*., 2010). Previous scientific studies revealed that extract of *O. stamineus* contained various terpenoids, polyphenols and sterols (Tezuka *et al*., 2000) leading to various activities such as antibacterial, antifungal, antimicrobial and antitumor. Effectiveness of nutraceutical products derived from *O. stamineus* in preventing diseases depends on the bioavailability of the active ingredients. The first step to recover and purify bioactive compounds from plant materials involves an extraction process which depends on the solvent used, extraction method and condition. Conventional extractions such as soxhlet extraction and maceration (ME) are normally performed at high temperatures for several hours. In recent years, a better extraction method has been developed such as the ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE) and supercritical extraction. Supercritical extraction is less favorable owing to its energy consumption and higher capital cost. Extraction is a mass transfer process involving solvent transport to the solid phase (inner transport), dissolution of the solutes and release of solutes from the solid matrix to the bulk phase (external transport). Both the MAE and UAE reduce the inner and external mass transfer limitation and hence may increase the yield of extraction. Therefore, both MAE and UAE methods were employed in this work. Solvent type plays important role in essential oil extraction. A combined effect of different extraction methods (ME, and MAE, UAE) and varying solvent polarity to the polyphenol extraction from *O. stamineus* has never been studied previously, and hence this is the objective of this work. A solid pharmaceutical dosage in the form of tablets is desirable for convenience of administration besides having longer shelf life and ease of handling. A method to produce high quality solid powder products from *O. stamineus* extracts must be established as the product of conventional method via hot spray is prone to thermal degradation. Thermal degradation of other bioactive compounds such as Vitamin E, Vitamin A and antioxidants have been reported by Xie et al. (2010). Thermal degradation is undesirable because the degraded product is of low nutritional value and consequently, hampers the intention to produce a nutraceutical product. Microencapsulation technique can minimize the thermal degradation during spray drying of *O. stamineus* extract. No literature concerning microencapsulation of flavonoids from *O. stamineus* extract is presently available in the literature.

Methodology, Results and Discussion

The polyphenol content in the plant extracts were analysed by using Singleton's method, aluminium chloride assay and ultra-performance liquid chromatography (UPLC). The UPLC method developed for the first time in this work is capable of a rapid and accurate qualitative and quantitative analysis of *O. stamineus* extract with about three times faster than other known methods. The results suggest that the polyphenol extraction from *O. stamineus* is affected by the solvent type. The highest phenolic content of 96.41 mg GAE/g DW was obtained using 50% aqueous methanol, whereas the highest yield of rosmarinic acid (38.70 mg RA/g DW) was obtained using 70% aqueous methanol (Table 1). The highest yield of sinensetin (261.21 µg Sin/g DW) and eupatorin (2.71 mg Eup/g DW) was obtained using isopropanol. Aqueous solvent provides a wider range of polarity than the pure solvent, and hence enhances simultaneous extraction for both methoxylated and hydroxylated compounds. It was found that the extraction time of 2 minutes and power setting at 300W gave the highest yield of polyphenol using microwave assisted extraction. However, extraction beyond 90 minutes or at a temperature higher than 60 °C induces degradation and hence reducing polyphenol yield. The microwave assisted extraction provides rapid extraction of polyphenol without significantly compromising the extraction yield. Microencapsulation of polyphenols from *O. stamineus* by spray

drying using encapsulant consisting of WPI or mixture of WPI and maltodextrin resulted in a high retention of polyphenols content (Fig. 1). Higher solid concentration leads to bigger particle size and lower moisture content. Microencapsulation using a least amount of protein (0.05 wt.%) yielded better retention of romarinic acid (82.08%), sinensetin (79.57%) and eupatorin (81.08%). The results suggest that eupatorin is more susceptible to thermal degradation than both sinensetin and rosmarinic acid during the microencapsulation process. The most effective formulation consisting of 1:9 protein to maltodextrin ratio produced high retention of rosmarinic acid (89.41%), sinensetin (89.14%) and eupatorin (86.66%) compared to the other formulation, i.e., 1:1 and 9:1. Results obtained from this work demonstrated that whey proteins and maltodextrin formulation can be effective microencapsulating agents for polyphenols derived from *O. stamineus*.

Table 1: Influence of extraction methods and solvent to polyphenol extraction from *O. stamineus*

Solvent type	**Polyphenol** (mg GAE/g DW)	**Flavonoid** (mg QE/g DW)	**Bioactive component**		
			Rosmarinic Acid (mg RA/g DW)	Sinensetin (µg Sin/g DW)	Eupatorin (mg Eup/g DW)
UAE					
Methanol	43.03 ± 1.15^{g}	103.57 ± 2.18	33.13 ± 0.19	254.99 ± 1.28	1.87 ± 0.01
Isopropanol	13.46 ± 0.67	18.83 ± 0.94	3.37 ± 0.09	261.21 ± 1.01	2.71 ± 0.02
Water	44.47 ± 1.23	38.09 ± 1.15	ND	ND	ND
50% Methanol	57.22 ± 1.86	163.05 ± 2.15	34.84 ± 0.002	150.15 ± 1.98	0.45 ± 0.05
70% Methanol	60.83 ± 1.04^{a}	154.06 ± 2.10	38.70 ± 0.06	164.12 ± 0.67	0.98 ± 0.01
50% Isopropanol	64.67 ± 1.23	171.18 ± 3.56	35.33 ± 0.05^{b}	202.69 ± 0.31^{f}	1.57 ± 0.01
70% Isopropanol	60.58 ± 2.03^{a}	169.04 ± 4.45	36.91 ± 0.12	248.16 ± 0.55	2.38 ± 0.03
MAE					
Methanol	42.43 ± 0.17^{g}	52.21 ± 1.51	23.63 ± 0.81	202.46 ± 9.92^{f}	1.26 ± 0.07
Isopropanol	7.81 ± 1.19	15.19 ± 4.24	2.93 ± 0.04	168.44 ± 1.06	1.77 ± 0.02
Water	74.77 ± 2.38	67.62 ± 2.38	13.47 ± 0.15	ND	ND
50% Methanol	96.41 ± 0.17^{c}	96.50 ± 0.30^{h}	36.47 ± 0.10	203.56 ± 1.85^{f}	0.62 ± 0.02
70% Methanol	95.08 ± 1.87^{c}	96.41 ± 0.40^{h}	35.78 ± 0.63^{b}	212.31 ± 2.00	1.08 ± 0.05
50% Isopropanol	95.99 ± 2.46^{c}	113.62 ± 1.21	34.78 ± 0.40^{e}	207.13 ± 2.47	1.28 ± 0.01
70% Isopropanol	80.12 ± 4.50^{i}	107.20 ± 1.82	34.42 ± 0.37^{e}	215.64 ± 2.07^{d}	1.71 ± 0.03
Maceration					
70% Isopropanol	81.70 ± 3.62^{i}	98.54 ± 1.44	35.61 ± 0.12	210.25 ± 3.48	2.17 ± 0.02

Note: Means (three replicates) followed by at least one same letter are not significantly different (P > 0.05).

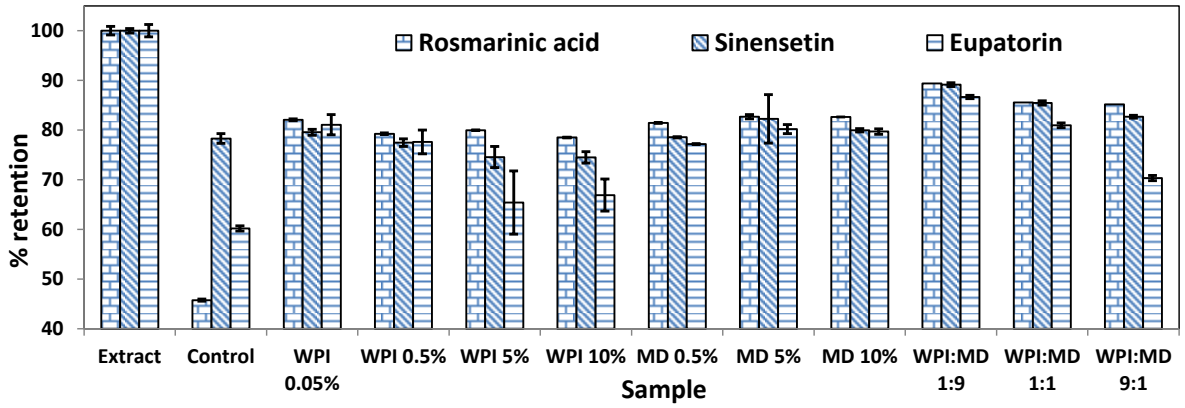

Fig. 1: Retention of polyphenol with different encapsulating agent and without encapsulation

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