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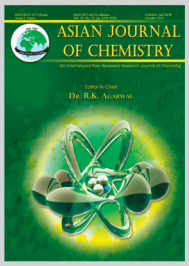

Extraction and Determination of Hormones in the Edible Bird's Nest

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Six kinds of hormones in two types of edible bird's nests, the white nest built by *Aerodramus fuciphagus* and the black nest built by *A. maximus*, were detected for the first time. These six kinds of hormones are testosterone, estradiol, progesterone, luteinizing hormone, follicle-stimulating hormone and prolactin. The ultrasonically assisted extraction methods, the optimal solvent used and the optimal ratio of of solvent to sample (v/w) were determined. The contents of each of these six kind of hormones were significantly different in the white and black nests as determined by full-automated microparticle chemiluminescent immunoassay. The determination of these hormones in the edible bird's nests may explain part of the effects of the edible bird's nest and may serve as an useful analytical technique for differentiating between white and black nest or the grades of the edible bird's nest products and it may be a useful method to determine adulteration.

Key Words: Edible bird's nest, Hormone, Solvent extraction, Ultrasonically assisted extraction, Full-automated microparticle chemiluminescent immunoassay.

INTRODUCTION

Edible bird's nests are the nests made from saliva of *Collocalia swiftlets* during the breeding and nesting season. They can be divided into two types: the white nest built by *Aerodramus fuciphagus* and the black nest built by *A. maximus*[1-3]. The white nest is made entirely of salivary "glue" (*A. fuciphagus* is the only species with a pure saliva nest) while the black nest is made of saliva and feathers[1,4].

The chemical constituents of the edible bird's nest have been reported for the investigations of the properties of the protein[5-7], the compositions and structures of carbohydrates[8-10], the compositions of lipids[7], in addition to the isolation and quantitative estimation of N-acetylneuraminic acids[11,12]. However, many bioactive components still remain undiscovered.

As edible bird's nest is the dried form of the nest made from saliva of the swiftlet during breeding season, we guess there may be hormones in the edible bird's nest. This thought was proved by a pre-experiment and six kinds of hormones [testosterone (T), estradiol (E2), progesterone (P), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin (PRL)] were detected.

Testosterone , E2 and P are three of the steroid hormones. Prolactin belongs to the family of protein hormones and LH and FSH are two kinds of gonadotropins, which are glycoproteins consisting of two subunits, designated as α and β. There are many reports on the extraction method of steroid and these protein hormones. Traditionally, steroids are extracted using solvent extraction. Different methods including vibration extraction, ultrasonically assisted extraction and reflux extraction and different kinds of solvents have been used[13-15]. Methods for extracting proteins are used to extract LH, FSH and PRL[16-19].

Ultrasonically assisted extraction is a relatively new technology. It has been employed to extract all kinds of components with steroids included[14]. It is reported to be used in extraction of sex hormones in Antler Velvet[15].

In the present paper we described relatively effective methods to extract and quantify the six kinds of hormones (T, E2, P, LH, FSH and PRL) in the white and black edible bird's nests.

EXPERIMENTAL

Unprocessed white and black edible bird's nests were obtained from CINRA Food Industries SDN. BHD.(Malaysia). The bird's nests were processed as described by Guo *et al.*[20]. The grounded nest samples were used in the following experiments.

Ultrasonically assisted extraction (1): Extractions were performed in an ultrasonic cleaner bath KQ-500 DB (Kunshan Ultrasonic Instrument Ltd., Suzhou, China) with useful volume of 6.0 L (internal dimensions: 30 cm × 15 cm × 15 cm), at the fixed frequency of 40 MHz. The temperature of the sonicated

water bath was 25 ºC. The edible bird's nest sample was mixed with 12 mL of solvent in the flask. Each nest sample was sonicated four times with each for 0.5 h. For each sonication, 12 mL of fresh solvent was added in the same flask. At the end of each sonication the extract was collected by centrifugation at 3000 rpm for 10 min (Eppendorf centrifuge 5804R, Eppendorf China Ltd., China). The whole extract was collected. The extractions were done for three kinds of solvents (acetone, methanol and 85 % ethanol) and four different ratios of solvent to sample (3:1, 6:1, 12:1 and 24:1, v/w).

Vibration extraction: Edible bird's nest sample (4 g) was mixed with 12 mL of acetone and the extraction was performed at room temperature four times for 0.5 h each. The rest procedures were the same as that in (1).

Reflux extraction: Four samples of the edible bird's nest (4, 2, 1 and 0.5 g) were extracted with 85 % ethanol under reflux at 90 ºC, respectively. The rest procedures were the same as that in (1).

Extraction of protein hormones: Protein hormones (LH, FSH and PRL) were extracted by the methods of ammonium sulfate precipitation[17] and ammonium acetate-ethanol extraction[18].

Preparation of samples for hormone determination: All of the extracts of ultrasonically assisted extraction, vibration extraction and reflux extraction were filtered to obtain clear filtrate. The filtrate was concentrated to a proper volume in a rotary evaporator (RE-2000, Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China) at less than 40 ºC under reduced pressure. The concentrated extract was stored at -26 ºC until analysis.

The samples obtained by the methods of ammonium sulfate precipitation and ammonium acetate-ethanol extraction were stored at -26 ºC and dissolved in 0.9 % sodium chloride aqueous solution before analysis.

Hormone determination: The contents of the six kinds of hormones (T, E2, P, LH, FSH and PRL) were detected by full-automated microparticle chemiluminescent immunoassay on the Beckman Coulter Access analyzer (Beckman Coulter Inc., USA). Tests were performed in triplicate.

Statistical analysis: Each of the above experiments was conducted on three individual samples of both types of nests. Statistical analysis were carried out with SPSS 13.0 for Windows (SPSS, Inc., Chicago, IL, USA). Analysis of variance was performed by ANOVA. Differences among samples obtained by different methods and solvents and ratios of solvent to sample were determined by Duncan's multiple range test. Comparisons of contents of each kind of hormone between the white and black nests were analyzed with independent-samples *t*-test. Differences reaching a confidence level of 95 % were considered as statistically significant.

RESULTS AND DISCUSSION

The hormones' contents are presented in Table-1 for different extracting methods and solvents and Table-2 for different ratios of solvent to sample on the white nest.

As there were almost non-detectable levels of hormones in the sample obtained by the ammonium acetate-ethanol extraction, the results were not shown here. The content of PRL obtained by ammonium sulfate precipitation was up to 0.405 ng/g white nest. However, this method was diminated from serious consideration because LH and FSH were not detected in the sample and it was time-consuming and inconvenient for the need of very strict conditions.

The ultrasonically assisted extraction is more effective than the other two methods. Ultrasounds produce cell disruption, particle size reduction and ultrasonic jet towards solid surfaces leading to a great contact area between solid and liquid phase and better access for solvent to valuable components. This is attributed to the phenomenon called acoustic cavitation[21,22].

The contents of hormones obtained by different solvents may be related to their polarities. For E2, 85 % ethanol is the most suitable extractant, for T and P is methanol and for PRL, FSH and LH is acetone. Comparisons of FSH contents in the black nest obtained by different solvents were not shown here. As is shown in Table-2, the extraction was the most effective when the ratio of solvent to sample (v/w) was 24:1 for T, E2 and P and 3:1 for PRL, FSH and LH.

The respective contents of hormones in white and black edible bird's nests are shown in Table-3. Follicle-stimulating hormone was not detected in all the white nest samples and

TABLE-1
CONTENTS OF HORMONES IN THE WHITE EDIBLE BIRD'S NEST EXTRACTED USING DIFFERENT METHODS AND SOLVENTS

	Vibration extraction	Ultrasonically assisted extraction			Reflux extraction
	Acetone	Acetone	Methanol	85 % ethanol	85 % ethanol
T (ng/g)	1.955 ± 0.006^{b}	2.860 ± 0.056^{c}	3.795 ± 0.013^{d}	N.D.	1.652 ± 0.004^{a}
E2 (pg/g)	132.834 ± 1.041^{a}	161.100 ± 1.146^{b}	298.212 ± 0.518^{d}	367.658 ± 0.195^{e}	256.618 ± 0.758^{c}
P (ng/g)	4.180 ± 0.096^{a}	6.587 ± 0.080^{b}	24.291 ± 0.547^{d}	19.875 ± 0.601^{c}	20.010 ± 0.370^{c}
LH (mIU/g)	0.182 ± 0.023^{a}	11.166 ± 0.281^{b}	N.D.	N.D.	0^{a}
PRL(ng/g)	0.065 ± 0.002^{c}	0.392 ± 0.002^{d}	0.030±0.003^{b}	N.D.	0.007 ± 0.003^{a}

Means ± standard deviation. Values in category row with different letters differ significantly ($p < 0.05$). N.D. = not detected.

TABLE-2
CONTENTS OF HORMONES IN THE WHITE EDIBLE BIRD'S NEST FOR DIFFERENT RATIOS OF SOLVENT TO SAMPLE (v/w)

	3:1	6:1	12:1	24:1
T (ng/g)	3.795 ± 0.013^{a}	7.318 ± 0.003^{b}	10.593 ± 0.006^{c}	12.148 ± 0.004^{d}
E2 (pg/g)	367.658 ± 0.195^{a}	419.791 ± 1.061^{b}	591.176 ± 1.057^{c}	802.333 ± 1.068^{d}
P (ng/g)	24.291 ± 0.547^{a}	28.728 ± 0.038^{c}	27.027 ± 0.638^{b}	37.724 ± 0.421^{d}
LH (mIU/g)	11.167 ± 0.028^{d}	6.588 ± 0.078^{c}	8.006 ± 0.024^{b}	5.875 ± 0.019^{a}
PRL (ng/g)	0.392 ± 0.002^{d}	0.210 ± 0.003^{c}	0.196 ± 0.003^{b}	0.168 ± 0.004^{a}

Means ± standard deviation. Values in category row with different letters differ significantly ($p < 0.05$).

PRL not in the black nest samples. The contents of each of the other four kinds of hormones (T, P, E2 and LH) are significantly different ($p < 0.05$) between the white and black nest.

TABLE-3
HORMONE CONTENTS IN WHITE AND BLACK EDIBLE BIRD'S NESTS

	White bird's nest	Black bird's nest
T (ng/g)	12.148 ± 0.004^b	4.293 ± 0.004^a
E2 (pg/g)	802.333 ± 1.068^a	906.086 ± 1.519^b
P (ng/g)	37.724 ± 0.421^b	24.966 ± 0.111^a
LH (mIU/g)	11.167 ± 0.281^b	1.420 ± 0.002^a
FSH (mIU/g)	0^a	0.149 ± 0.003^b
PRL (ng/g)	0.392 ± 0.003^b	0^a

Means ± standard deviation. Values in category row with different letters differ significantly ($p < 0.05$).

So far no literature has been published about the hormones in the edible bird's nest. Our present work detected six kinds of hormones (T, E2, P, LH, FSH and PRL) in the white and black nests for the first time and we determined the method for hormone extraction and quantification in the edible bird's nest and the contents of the six kinds of hormons in the white and black nests.

A. fuciphagus produces the best quality white nests. They are normally very clean, with few strands of black feathers. These nests even unprocessed could fetch up to three times the price of cleaned nests obtained from the black nests. It is known that the colour of the nest cement can be easily removed by chemical treatment to produce pure white nest materials[23]. As the hormone contents in white and black edible bird's nests are different, the determination of hormones may serve as a useful analytical technique for differentiating between white and black nest. As there was no FSH in the white nest and no PRL in the black nest, it may be an easier method to detect one of the two kinds of hormones.

Some adulterants for example karaya gum and tremella fungus have been found to be routinely incorporated into the edible bird's nests during commercial processing prior to final sale[7]. The determination of hormones in the nests may be also a method to determine whether the nests are adulterated or not.

Edible bird's nest is highly esteemed for their nutritional and medicinal value[23]. Edible bird's nest has been used for a long time in traditional Chinese medicine. It was used in consumption disease, stomach ulcers, haematemesis general debility and asthenia[24]. It has been shown that edible bird's nest possesses the first known avian epidermal growth factor (EGF)[25] and a glycoprotein which could potentiate mitogenic response of human peripheral blood monocytes to stimulation with concanavalin A or phytoemagglutinin A[26]. It has haemogglutination inhibiting action against the influenza virus and the ability to neutralize its infectivity[20]. It is claimed that consuming edible bird's nest regularly can give a person exuberant physical and mental strength as well as to restore the fine and fair complexion of one's youthfulness[23] and the bird's nest soup which is produced by cooking the nests in a double boiler with sugar is often administered to the old and young[7].

These six kinds of hormones all play important roles in biological systems. They are all important in reproduction. T, E2 and P perform hormone replacement therapy (HRT)[15,27] and play important roles in skeletal development and maintenance[28,29]. E2 and P have significant modulating effects circadian rhythms and sleep patterns[30,31]. E2 can regulate angiogensis[32] and it has numerous effects in the adult brain and facilitates functions from the display of sexual and aggressive behaviour to learning and memory[33]. LH and FSH play a central role in the mammalian reproductive process[34]. PRL play important roles in the water-electrolyte homeostasis, influence on brain and behaviour, regulation of growth and development[35,36] and immunoregulation[37,38]. The containing of these hormones may be one of the reasons as to why the edible bird's nests have great nutritional and medicinal values.

It has been reported that the frequency of consuming bird's nests should be carefully regulated. It is consumed regularly, twice a week in a small quantitives for optimum results. The recommendation have been used by many people who have claimed to have benifited positively from consuming the bird's nests[23]. This may to some extent also be related to the containing of hormones in the nests, as irregular intake of hormones may lead to endocrine dyscrasia in human body.

Conclusion

The contents of six kinds of hormones (T, E2, P, LH, FSH and PRL) were detected in the white nest built by *A. fuciphagus* and the black nest built by *A. maximus*. Ultrasonic assisted extraction was used to extract hormones in the nests. The determination of these hormones in the edible bird's nests may serve as a useful analytical technique for differentiating between white and black nest or the grades of the edible bird's nest products and it may be a useful method to determine adulteration. Further studies will be required in identification and purification of the variety of bioactive components which will be related to the overall effects of the edible bird's nest.

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