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COMPARISON OF *IN VITRO* NITRIC OXIDE ACTIVITY OF *ARECA CATECHU* LINN NUT BY SOXHLATION AND MICROWAVE EXTRACTION TECHNIQUE

Padmaa M Paarakh*

Department of Pharmacognosy, the Oxford College of Pharmacy, Bangalore 560 068, Karnataka, India.

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***Correspondence for Author**
Dr. Padmaa M Paarakh
Department of Pharmacognosy, the Oxford College of Pharmacy, Bangalore 560 068, Karnataka, India

ABSTRACT

The aim of the present study is to compare the *in vitro* nitric oxide activity of *Areca catechu* nut [unroasted, roasted and commercial sample from shop]by two different techniques of extraction viz., Soxhlation and Microwave extraction. *Areca catechu* nuts [unroasted, roasted and commercial sample from shop] were extracted separately in soxhlet and microwave extractor with methanol and distilled water. The study was carried out with 2 extracts prepared by both methods using *in vitro* nitric oxide scavenging activity. Ascorbic acid was used as standard. The study exhibited strong *in vitro* nitric oxide scavenging activity. The IC_{50} value were found to be 365.25,79.7;80.8,77.87 and 60.21,60.71 µg/ml by microwave assisted extraction and 84.06,191.9; 77.49,89.06 and 60.3,63.08 µg/ml by soxhlet method for unroasted methanol, unroasted water; roasted methanol, roasted water and commercial sample methanol and commercial sample water extracts respectively. The IC_{50} value for standard was found to be 38.9 µg/ml. This experiment has concluded the strong *in vitro* nitric oxide scavenging activity of nuts of *A.catechu*. Commercial sample showed the highest scavenging activity when compared all other extracts. There was difference in Microwave extraction technique and soxhlet technique only for unroasted nuts whereas both method yielded more or less same result in roasted and commercial sample.

KEYWORDS: *Areca catechu*, soxhlet extraction, microwave extraction, nitric oxide scavenging activity.

1. INTRODUCTION

Many diseases are caused due to oxidative stress and free radical generation. It has been implicated in the pathology of diseases for inflammatory conditions, cancer, atherosclerosis, Parkinsonism, diabetes and aging. Cardiovascular diseases, tumor growth, wrinkled skin, cancer, Alzheimer's disease are contributed by accelerated cell oxidation rendering a decline in energy and endurance.[1] Oxygen is normally used in regular body processes like respiration and some cell mediated immune functions which tend to produce free radicals like $O^{\cdot}$, $NO^{\cdot}$, and HOO etc. The oxidation of cellular oxidizable substrates can be prevented by substances called antioxidants. The antioxidants act by scavenging reactive oxygen species, activating a number of detoxifying proteins or by preventing the generation of reactive oxygen species.[2, 3] Nitric oxide [$NO^{\cdot}$], a short lived free radical generated endogenously exerts influence on a number of functions including vasodilation, neurotransmission, synaptic plasticity and memory in the central nervous system.[4] Besides mediating central nervous function, NO has been implicated in pathophysiological states. Overproduction of NO can mediate toxic effects viz., DNA fragmentation, cell damage, and neuronal cell death. [5] NO also shows neurotoxicity and acts as a pathological mediator in pathophysiological processes such as cerebral ischemia, epilepsy, Alzhemier's disease, Parkinson's disease, inflammation, reperfusion injury, atherosclerosis, acute hypertension, hemorrhagic shock, diabetes mellitus and cancer.[6]

Many a times the body is unprotected from deleterious effects of free radicals as the antioxidation activity normally carried out by the body is unable to sufficiently quench or scavenge the free radicals.

In order, to protect the human body from free radicals and retard the progress of many chronic diseases there has been a recent increase in interest in finding natural antioxidants.[7]
A number of studies have been carried out on various plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavonoids[8] which prevent free radical damage thereby reducing risk of chronic diseases. This beneficial role of plants to provide natural antioxidants has led to increase in the search for newer plant based sources.

Areca catechu Linn. an annual of the Palmaceae family is one of valuable medicinal plant. In many Asian cultures (such as India, Taiwan, and Southeast Asia), betel nut, Areca catechu L., is traditionally masticated either alone or as a quid along with a large variety of ingredients,

such as betel leaf (*Piper betel*; family Piperaceae), slaked lime, catechu, different types of tobacco, and various additives, perfumes, and stimulants. [9] It is estimated that over 600 million individuals all over the world consume betel nut (also called areca nut) in one form or another.[10] In herbal medicine, the areca nut has been used medicinally as a drug against parasitic worms.[11] In old Indian scripts, such as Vagbhata (4th century), and Bhavamista (13th century), betel nut has been described as a therapeutic agent. Its use was recommended in many diseases, such as leucoderma, leprosy, anaemia, and obesity. *Areca catechu* L plant contains a diverse group of phenolic compounds with antioxidant activity, including flavonoids, lignans and stilbenes, and simple phenolic acids, such as hydroxybenzoic acids and hydroxycinnamic acids.[12,13]

Most of the methods used in these studies are based on classical Soxhlet extraction. Nevertheless, this traditional sample extraction technique often uses large quantities of organic solvents and is usually time-consuming. In the last few years, established methods, such as supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) and microwave-assisted extraction (MAE), were used to reduce the volume of solvents required, to improve the precision of analyte recoveries and to reduce extraction time. Of these techniques, SFE is the most selective extraction method, but its use has been limited by the strong matrix dependence of the extraction process. Most of the time, extraction conditions need to be optimized for each new matrix MAE technique offer advantages over the SFE and PLE methods as the extraction can be completed within minutes with less solvent.[14]

Hence, the present study has been undertaken to investigate the two different technique of extraction viz., traditional and microwave assisted extraction technique and compare *in vitro* nitric oxide scavenging activity of methanol and water extracts of the nuts of *A.catechu*.

2. MATERIALS AND METHODS

2.1 Plant material

The *Areca catechu* nuts and commercial samples were collected from local market in Bangalore, Karnataka, India and it was identified and authenticated by Botanist, Natural Remedies Pvt Ltd., Bangalore. A voucher specimen was deposited in The Oxford College of Pharmacy, Bangalore. The nuts as such, roasted at 50^0C for 30 minutes and commercial samples were powdered coarsely respectively, passed through sieve no. 40 and stored in air tight container for further use.

2.2.1 Preparation of extract by soxhlet technique

Coarsely powdered nuts [unroasted, roasted and commercial sample] of *A.catechu* *20* g, each were subjected to extraction in soxhlet extractor with methanol and boiled with distilled water [150 ml] respectively. The two extracts of three types of nuts were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield was found to be 7.35, 9.4; 8.7, 9.8 and 34.75,29.2 % w/w respectively with reference to the air dried unroasted, roasted and commercial samples.

2.2.2 Preparation of extract by Microwave assisted extraction technique

Coarsely powdered nuts [unroasted, roasted and commercial sample] of *A.catechu* 20 g, each were subjected to extraction in Microwave extractor [Ragatech Company Microwave extractor with Power 3, set at temperature 50^0C for 30 minutes with continuous stirring] with methanol and water [150 ml] respectively. The two extracts of three types of nuts were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield was found to be 4.65,4.8;8,4.8 and 11.95,4.7 % w/w respectively with reference to the air dried unroasted, roasted and commercial samples methanol and water extracts respectively.

2.3 Drugs and Chemicals

The compounds, ascorbic acid, sodium nitroprusside, sodium nitrite, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, orthophosphoric acid, Sulfanilamide and Naphthyl ethylene diamine dihydrochloride were purchased from SD Fine Chemicals Ltd, Mumbai. Other reagents were procured from Loba Chemicals, Mumbai. All chemicals and reagents used in this study were at least of analytical grade.

2.4. Determination of in vitro nitric oxide scavenging activity

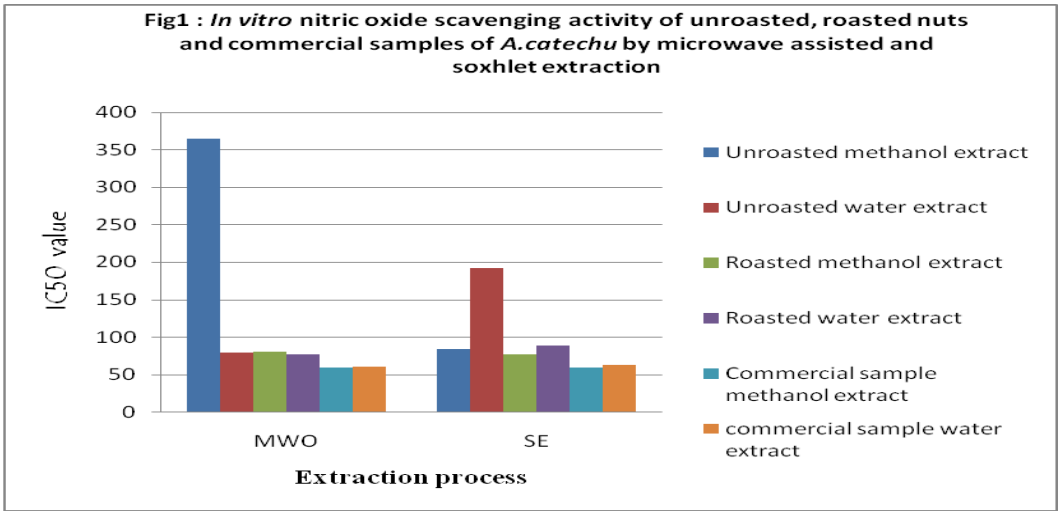
Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generated nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated by the use of griess reagent.[15, 16] Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside[5 mM] in phosphate buffered saline[PBS] was mixed with 3.0 ml of different concentration[100 to 500 µg/ml] of the different extracts dissolved in methanol and incubated at 25^0C for 150 min. the samples from above were reacted with griess reagent[1 % sulphanilamide, 2 % orthophosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride]. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthyl ethylene

diamine was read at 546 nm and referred to the absorbance of standard solution of sodium nitrite treated in the same way with griess reagent. Reference standard compound being used was ascorbic acid [20 to 100 µg/ml]. The IC_{50} value is the concentration of sample required to inhibit 50% of the nitric oxide free radical was determined.

3. RESULTS AND DISCUSSION

Table 1: *In vitro* nitric oxide scavenging activity of unroasted, roasted nuts and commercial samples of *A.catechu* by microwave assisted and soxhlet extraction

Sl no.	Sample/standard	IC_{50} value	
		Microwave assisted extraction [MWO; µg/ml]	Soxhlet extraction [SE; µg/ml]
1	**Unroasted methanol extract**	365.25 ± 0.31	84.06 ± 0.36
2	**Unroasted water extract**	79.70 ± 0.29	191.90 ± 0.19
3	**Roasted methanol extract**	80.80 ± 0.56	77.49 ± 0.85
4	**Roasted water extract**	77.87 ± 0.63	89.06 ± 0.92
5	**Commercial sample methanol extract**	60.21 ± 0.86	60.30 ± 0.75
6	**commercial sample water extract**	60.71 ± 0.55	63.08 ± 0.24
7	**Ascorbic acid**	38.90 ± 0.35	



Nitric oxide generated from SNP at physiological pH was found to be inhibited by the extracts. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) of *A.catechu* extracts and ascorbic acid are indicated in Table 1and Fig 1. The IC_{50} value were found to be 365.25,79.7;80.8,77.87 and 60.21,60.71 µg/ml by microwave assisted extraction and 84.06,191.9; 77.49,89.06 and 60.3,63.08 µg/ml by soxhlet method for unroasted methanol, unroasted water; roasted methanol, roasted water and commercial sample methanol

and commercial sample water extracts respectively. The IC_{50} value for standard ascorbic acid was found to be 38.9 µg/ml.

4. CONCLUSION

This experiment has concluded the strong *in vitro* nitric oxide scavenging activity of nuts of *A.catechu.* Commercial sample showed the highest scavenging activity when compared all other extracts. There was difference in Microwave extraction technique and soxhlet technique only for unroasted nuts whereas both method yielded more or less same result in roasted and commercial sample. Further, investigation on *in vivo* antiinflammatory activity has to be carried out to understand its mode of action and to discover the main constituent of *A.catechu* nut responsible for this activity.

5. ACKNOWLEDGEMENT

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6. ETHICAL ISSUES

There is none to be applied.

7. CONFLICT OF INTEREST

None to be declared.

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