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Azadirachta indica A. Juss.내의 Azadirachtin과 Nimbin의 항산화 및 양적 추정

강원대학교 : Amal Kumar Ghimeray, 김성무, 김원우, 김현삼, Pankaja Sharma, 김슬기, 조동하*

Antioxidant activity and quantitative estimation of Azadirachtin and Nimbin in *Azadirachta indica* A. Juss.

Amal Kumar Ghimeray, Cheng Wu Jin, Wonwoo Kim, Hyan-sam Kim,
Pankaja Sharma, Seul Gi Kim, Dong Ha Cho*
Plant Biotechnology, Bioscience and Biotechnology, Kangwon National University,
Chuncheon 200-701, Korea

실험목적 (Objectives)

The main objective of research is to investigate the total polyphenol, total flavonoid and antioxidant activities of leaf and bark extracts of Neem in a comparative way. And also, quantitative estimation of Azadirachtin and Nimbin content in the Neem (seed, bark and leaf) using different solvents which can alter the pesticidal /insecticidal property of Neem.

재료 및 방법 (Materials and Methods)

The fractionated (hexane, ethylacetate, butanol and water) sample of leaf, bark and seed extracts of Neem were used for the estimation of total polyphenol, total flavonoids, and antioxidant activity (DPPH free radicals, Hydroxyl radical, DNA protection) in invitro assay. The quantitative estimation of Azadirachtin and Nimbin were also done by use of HPLC.

실험결과 (Results)

The results obtained in this study demonstrated that all the tested leaf and bark extracts/fractions of Neem grown in the foothills (subtropical region) have significant antioxidant property. Neem bark and leaf significantly scavenge the free radicals (DPPH) and hydroxyl radicals in invitro test and showed high DNA protection power by both the leaf and the bark extracts. Likewise, the azadirachtin and nimbin were distributed in leaf, bark and seed. However, high concentration of azadirachtin was observed in the seeds of the Neem. Although, in comparative study, bark was found to be more potent than the leaf in the entire assay with higher phenolic content, but, both the leaf and the bark can be used in the pharmacological applications as a valuable antioxidant natural source and medicine. Likewise, the presence of significant amount of Azadirachtin and Nimbin in all the parts of Neem grown in this region showed its potential use as natural insecticide

Corresponding author : Dong Ha Cho E-mail : chodh@kangwon.ac.kr Tel : 033-250-6475

* 시험성적

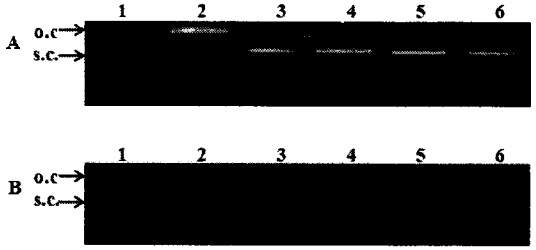

Figure 1. Effect of Neem Bark (A) and leaf (B) extracts on the protection of supercoiled DNA against ~OH generated by the H_2O_2. Lane 1: plasmid DNA (positive control); Lane 2: DNA+Fenton's reagent (DNA damage control); Lanes 3 – 6 of Fig 1 A and B: Hexane, Ethyl acetate, Butanol and water fraction sample respectively of Neem at the concentration of 1 mg/ml in presence of Fenton's reagent; Where, S.C. and O.C. represents supercoiled DNA and open circular DNA.

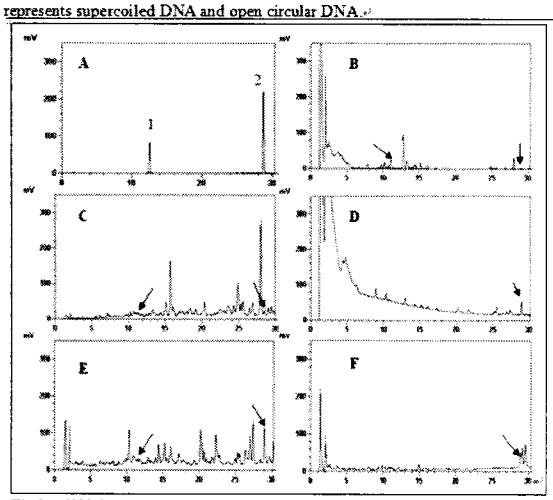

Fig 2. HPLC result of Neem (seed, bark and leaf extracts) showing Azadirachtin and Nimbin. The figure (A) represents the standard of Azadirachtin (1) and Nimbin (2) with their retention time 12.76 min and 28.93 min respectively. Arrow denotes the chromatogram of Azadirachtin and Nimbin in Neem seed methanolic (B); Seed hexane (C); Bark methanolic (D); Bark hexane (E); and Leaf hexane extracts (F). Fig B, C and E shows both the Azadirachtin and Nimbin content. Fig D & E showed only Nimbin content.

Table 1. DPPH free radical and Hydroxyl (OH) radical scavenging activity of bark and leaf fraction extracts of Neem expressed in percentage and shown in comparison.

Solvents used	DPPHscavenging percentage		Hydroxyl (OH) radical scavenging percentage	
	Bark	Leaf	Bark	Leaf
Methanol Crude	82.45 ± 1.06	12.56 ± 2.04	87.84 ± 0.09	36.47 ± 2.06
Hexane	8.89 ± 1.67	9.00 ± 1.15	84.89 ± 0.21	36.17 ± 2.32
Ethyl Acetate	83.73 ± 1.90	15.00 ± 1.19	86.38 ± 0.08	38.72 ± 0.60
n-Butanol	91.03 ± 1.03	33.25 ± 0.95	87.87 ± 0.12	35.72 ± 2.36
Water	93.11 ± 0.96	5.83 ± 3.45	92.12 ± 0.13	43.86 ± 1.24
Vitamin "C"(standard)	96.00 ± 2.82	-	-	-
BHA(standard)	-	-	88.89 ± 2.03	-

All the samples were used at the concentration of 0.1 mg/ml for DPPH free radical and 1mg/ml concentration was used for the OH radical scavenging activity. Vit. 'C' at 0.1mg/ml and BHA at 0.5mg/ml of concentration were used as standard. (-) Means not done.

Table 2. Total polyphenol (TPF) and Total Flavonoid (TF) contents in the bark and the leaf extracts of Neem in different solvents used. TPF and TF contents were expressed in Tannic acid (TAE) and Quercetin (QE) equivalent respectively.

Solvents used	Total Polyphenol contents (TAE µg/mg)		Total Flavonoid contents (QE µg/mg)	
	Bark	Leaf	Bark	Leaf
Methanol crude	651.07 ± 1.30	126.72 ± 3.84	14.21 ± 1.22	32.50 ± 1.95
Hexane	66.63 ± 1.67	23.85 ± 0.37	13.41 ± 1.28	20.67 ± 2.32
Ethyl Acetate	500.33 ± 8.70	149.59 ± 5.74	17.07 ± 2.38	71.71 ± 3.35
n-Butanol	468.48 ± 2.78	237.00 ± 7.96	12.87 ± 1.22	93.17 ± 3.23
Water	629.04 ± 2.96	96.44 ± 2.22	14.94 ± 1.59	13.72 ± 2.20

Each value is expressed as the mean ± SD (n = 3).

Table 3. Results of statistical analysis of estimation of Azadirachtin and Nimbin content in the Seed, Bark and Leaf extracts of Neem in different solvents.

sample	Azadirachtin (µg/g dw)	Nimbin (µg/g dw)
Neem seed (methanolic 80%)	3300	88
Neem seed Hexane	177	82
Neem leaf Hexane	-	112
Neem leaf water	17	-
Neem bark (methanolic 80%)	-	260
Neem bark Hexane	86	271
Neem bark butanol	-	60

The data are expressed in µg/g of dry weight. (-) Means not detected.