NIR-Based Predictive Model Development for Antioxidant Activity and Quantification of Bioactive Compounds in *Gymnema inodorum*

Kanokporn Chareonpornpanich,[1] Thanarat Boonchalaem,[2] Phenphicha Fueangfukitjakarn,[2]
Kanchana Dumri[1*] and Kongsak Boonyapranai[2*]

1.Division of Biochemistry and Biochemical Innovation, Department of Chemistry, Faculty of Science, Chiang Mai University
2.Center for Non-Infectious Diseases and Environmental Health Sciences, Research Institute for Health Sciences, Chiang Mai University
*Corresponding authors email: kanchana.d@cmu.ac.th and kongsak.b@cmu.ac.th

ABSTRACT

Gymnema inodorum, a traditional medicinal plant from Northern Thailand, is known for its antioxidant and anti-inflammatory properties. This study aimed to evaluate the antioxidant activity and bioactive compound content of *G. inodorum* leaves collected from 30 different sources and to develop a predictive model using Near-Infrared Spectroscopy (NIR). Dried and powdered leaf samples were scanned by NIR prior to extraction with 50% ethanol. The extracts were freeze-dried, reconstituted in Type I water, and analyzed for antioxidant activity using DPPH and FRAP assays. Bioactive compounds were quantified using HPLC-DAD. Significant variation in antioxidant activity was observed among samples. In the DPPH assay (10 mg/mL), percentage inhibition ranged from 52.63% to 80.27%, with IC_{50} values between 3.931 and 8.101 mg/mL. In the FRAP assay (5 mg/mL), reducing power ranged from 0.349 to 0.620 mg/mL $FeSO_4$ equivalents. No significant correlation was found between bioactive compound content and antioxidant activity. These results indicate substantial variability among different sources of G. inodorum. The data will be used to develop chemometric models based on NIR spectral information for rapid, non-destructive quality assessment.

INTRODUCTION



Figure 1. *Gymnema inodorum*

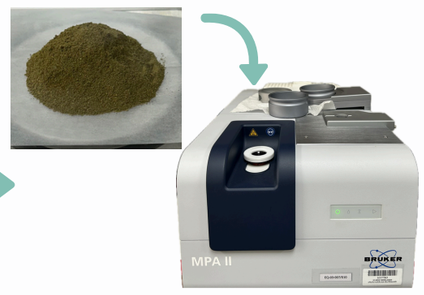


Figure 2. Near Infrared Spectroscopy (NIR)

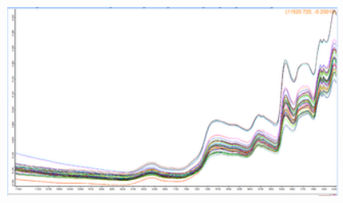


Figure 3. Spectra

- *Gymnema inodorum*, a traditional medicinal plant commonly found in Northern Thailand, is well known for its antioxidant, anti-inflammatory, and antidiabetic properties.
- Near Infrared Spectroscopy (NIR) is a rapid and non-destructive analytical technique that enables chemical analysis without chemical reagents and with minimal analysis time.
- Thus, NIR has potential as a rapid screening tool for evaluating the chemical composition and antioxidant-related properties of *G. inodorum* leaves.

OBJECTIVES

1. To evaluate the antioxidation activity of *Gymnema inodorum* leaves collected from 30 different sources.
2. To analyze the bioactive compound contents of the collected sample.
3. To develop predictive models for bioactive compounds and antioxidant activity using Near Infrared Spectroscopy (NIR).

METHODOLOGY



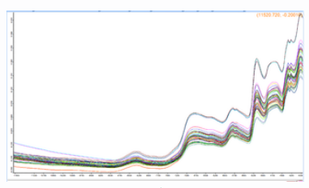
Sample powder → Extracted with 50% EtOH → Evaporation → Freeze drying → Dried extract powder



Sample powder



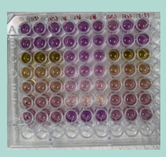
Collected spectra by NIR

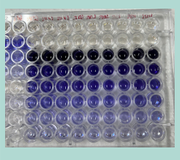


NIR spectroscopy was used to develop a calibration model, and the model performance was evaluated using cross-validation.

Dried extract powder

- Antioxidant activity
  - DPPH assay
    - sample 10 µL + DPPH 195 µL
    - Incubated in the dark for 30 minutes and Measured absorbance at 517 nm.
  - FRAP assay
    - sample 5 µL + FRAP reagent 195 µL
    - Incubated at 37°C for 5 minutes and measured absorbance at 593 nm.
- HPLC-DAD
  - Rutin
  - Quercetin
  - Gallic acid







The obtained results were correlated with the spectral data.

RESULTS AND DISCUSSION

NIR Spectra

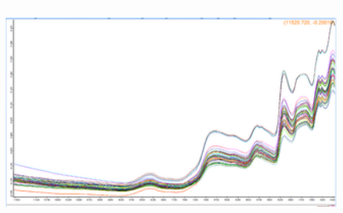


Figure 4. NIR spectra of *Gymnema inodorum.*

The NIR spectra showed absorption peaks around 5200 and 6900 cm^{-1}, corresponding to water (O-H vibrations). A peak near 4000 cm^{-1} was associated with lipid, while the intermediate region is related to carbohydrates.

DPPH assay

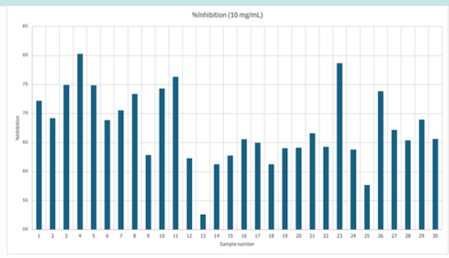


Figure 5. %Inhibition determined by DPPH assay (68.01 ± 5.679).

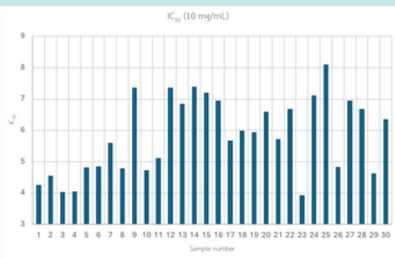


Figure 6. IC_{50} values determined by DPPH assay (5.838 ± 1.217).

The highest %inhibition was observed in sample 4 (80.27%). The IC_{50} value indicates the concentration required to inhibit 50% of the radical activity. Sample 23 showed the strongest IC_{50} values (3.931 mg/mL).

FRAP assay

Figure 7. FRAP values determined by FRAP assay.

Sample 3 exhibited the highest antioxidant capacity in the FRAP assay, with a standard equivalent concentration of 0.620 mg/mL.

Development of a predictive model

Figure 12. Prediction vs. Reference plot of unknown C.

Figure 13. Regression coefficient plot for unknown C.

HPLC-DAD

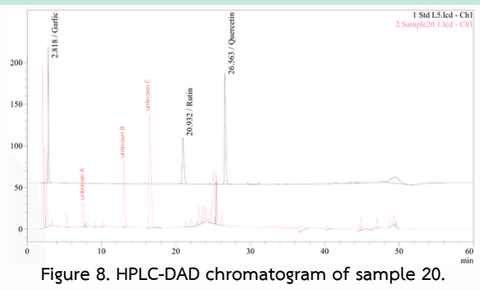


Figure 8. HPLC-DAD chromatogram of sample 20.

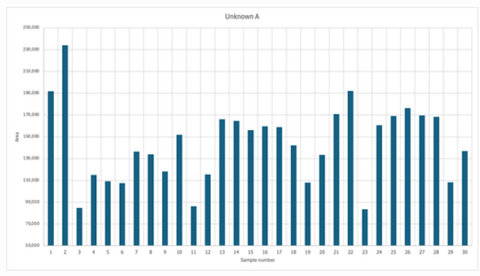


Figure 9. Peak area of unknown A determined by HPLC-DAD.

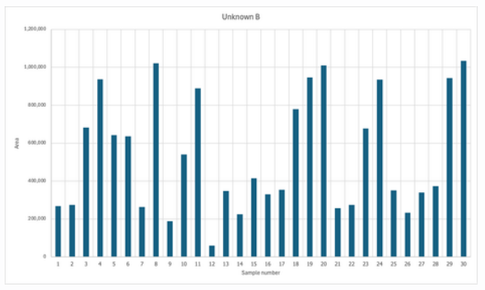


Figure 10. Peak area of unknown B determined by HPLC-DAD.

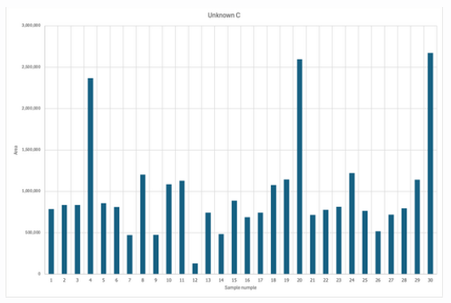


Figure 11. Peak area of unknown C determined by HPLC-DAD.

Sample 2 showed the highest amount of unknown A, whereas sample 30 showed the highest amounts of unknown B and unknown C.

Parameter	R^2	RMSECV	Bias	RPD
%Inhibition	-4.232	6.37	0.178	0.979
IC_{50}	6.756	1.16	0.0123	1.04
FRAP	80.22	0.0298	-0.000658	2.25
unknown A	64.49	20.8	-0.566	1.68
unknown B	90.97	90.7	-2.23	3.33
unknown C	94.17	139	-0.846	4.14

Table 1. Statistical parameters of the prediction models.

- %Inhibition and IC_{50}: Low model performance; not suitable for reliable prediction.
- FRAP: Moderate performance; suitable for rough screening and sample classification.
- unknown A: Suitable for preliminary classification and approximate estimation.
- unknown B: Good performance; applicable for screening and comparative studies.
- unknown C: Very good performance; suitable for broader applications including quality control.

Validation of the Prediction Model

Name	Predicted value	True value	Range	Add. Info.
FRAP	0.444 mg/mL	0.457 mg/mL	-inf.- +inf. mg/mL	MDI = 0.1
unknown A	121,060	133,475	-inf.- +inf.	MDI = 0.1
unknown B	1,084,090	1,010,982	-inf.- +inf.	MDI = 0.1
unknown C	2,467,557	2,467,557	-inf.- +inf.	MDI = 0.2

Table 2. Validation of the prediction equation using calibration samples.

Name	Value	Range	Add. Info.
FRAP	1.530 mg/mL	-inf.- +inf. mg/mL	MDI = 38.6
unknown A	506.949	-inf.- +inf.	MDI = 1801
unknown B	-12,785.318	-inf.- +inf.	MDI = 255.7
unknown C	18,348,525	-inf.- +inf.	MDI = 750.9

Table 3. Prediction results of fresh leaf sample using the NIR model.

Name	Value	Range	Add. Info.
FRAP	0.501 mg/mL	-inf.- +inf. mg/mL	MDI = 0.3
unknown A	210,491	-inf.- +inf.	MDI = 0.8
unknown B	264,844	-inf.- +inf.	MDI = 0.4
unknown C	1,384,727	-inf.- +inf.	MDI = 0.6

Table 4. Prediction results of powdered leaf sample using the NIR model.

Validation using calibration samples showed that the predicted values were close to the reference values. Fresh leaf samples could not be predicted by the model due to their high moisture content. However, after grinding into powder, the sample could be successfully analyzed. Moreover, these samples fell within the model prediction range.

CONCLUSIONS

The developed NIR model demonstrated the potential to predict FRAP values and bioactive compounds (unknown A, B, and C). However, increasing the sample size (approximately 150 samples) would further improve the reliability and predictive accuracy of the model.

ACKNOWLEDGEMENTS

- Division of Biochemistry and Biochemical innovation, Department of Chemistry, Faculty of Science, Chiang Mai University.
- Center for Non-Infectious Diseases and Environmental Health Sciences, Research Institute for Health Sciences, Chiang Mai University.

REFERENCES

Kebal, L., Pokajewicz, K., Djebli, N., Mostefa, N., Poliwoda, A., & Wieczorek, P. P. (2022). HPLC-DAD profile of phenolic compounds and *In vitro* antioxidant activity of *Ficus carica* L. fruits from two Algerian varieties. Biomedicine and Pharmacotherapy, 155. https://doi.org/10.1016/j.biopha.2022.113738