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Metabolomic quality control of claimed anti-malarial *Artemisia afra* herbal remedy and *A. afra* and *A. annua* plant extracts

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Abstract

Malaria remains a serious health problem world wide, especially in developing countries. Recent advances in the treatment of malaria have taken place and today combination therapies containing artemisinin (isolated in 1971 from *Artemisia annua*) and its derivatives have become the main weapon in the fight against this disease. Many herbal companies are now trying to make use of the success of artemisinin by selling *Artemisia* plant material in various formulations. We have therefore decided to test the product of one such company which claims that its capsules contain artemisinin. We have used a rapid NMR targeted metabolomics approach combined with principle component analysis (PCA) to verify that the capsules are indeed *A. afra* and not *A. annua*. In addition the concentration of artemisinin in the plant material was determined with a sensitive LC–MS method. This analysis indicated that even if the company has used *A. annua* in their capsules the dosage of artemisinin will be far to low to be effective. Our analysis shows that NMR with PCA can be a rapid and valuable tool in the quality control of herbal supplements.

Keywords: *Artemisia afra*; *Artemisia annua*; Artemisinin; Metabolomics; NMR

1. Introduction

Artemisia annua L. (Asteraceae) is an annual herb native to Asia and it has been used for centuries in traditional Chinese medicine for the treatment of malaria and fever. The active constituent, artemisinin, has been isolated in 1971 from the aerial parts of this species and is currently used to treat *Plasmodium falciparum* and *P. vivax* that has build up resistance against chloroquine treatment (Christen and Veuthey, 2001). Artemisinin is a sesquiterpene lactone with a rare endoperoxide bridge which is believed to confer activity for this molecule. According to Klayman et al. (1984) no other species of *Artemisia* contains artemisinin.

Artemisia afra Jacq. Ex Willd. (Asteraceae) is a common species in South Africa with a wide distribution from the Cederberg Mountains in the Cape, northwards to tropical East Africa and stretching as far north as Ethiopia. The list of uses covers a wide range of ailments e.g. coughs, colds, fever, loss of appetite, colic, headache, earache, intestinal worms and malaria. *A. afra* is used in many different ways and one of the most common practices is to insert fresh leaves into the nostrils to clear blocked nasal passages (Van Wyk et al., 1997). There are however some reports that extracts of *A. afra* shows some activity against *P. falcipirum* (Kraft et al., 2001; Gathirwa et al., 2007). There is however no reports that *A. afra* contains artemisinin or any of its derivatives.

Nowadays, plant materials are employed throughout the industrialized and developing world as home remedies, over-the-counter drugs, and ingredients for the pharmaceutical industry (Bandaranayake, 2006). As such, they represent a substantial proportion of the global drug market. The past decade has seen a tremendous increase in the production and sale of natural therapies, herbal supplements and medicinal plant remedies. We have decided to investigate one such product which is claimed to be an effective treatment against malaria. Nordman Superior Food supplements produces *Artemisia* anti-malaria capsules. The description of the product is that each capsule contains 400 mg of pure *A. afra* with no additives or fillers. The dosage of this product is to take two capsules daily from the day before you travel up to three days after the end of your journey in a malaria area. The dosage for children (15–45 kg) is one capsule daily and for children under 15 kg half a capsule daily. The website of this company (www.nordman.co.za) clearly states that artemisinin is

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responsible for the anti-malarial activity of these capsules. The aim of this research was therefore to find a rapid analysis for the quality control of this product. The possibility that the capsules contained *A. annua*, *A. afra* or a combination of the two herbs was tested with this method. In addition the concentration of artemisinin in all the samples was determined with the use of LC–MS.

In order to establish a simple and rapid test procedure to differentiate these extracts a metabolomics approach with principle component analysis was used. Metabolomics is a growing field of interest and tries to quantify and identify all metabolites in a specific tissue or organism. In order to achieve this goal it is necessary to use a wide spectrum of analytical tools. Mass spectroscopy and nuclear magnetic resonance spectroscopy are mainly used in metabolomic analyses. To establish a rapid and easy to perform analysis for the plant samples NMR was used and the ^{1}H NMR data were subjected to a PCA analysis. This multivariate data analysis is mathematically defined as an orthogonal linear transformation that transforms the data to a new coordinate system such that the greatest variance by any projection of the data comes to lie on the first coordinate (called the first principal component), the second greatest variance on the second coordinate, and so on. PCA can be used for dimensionality reduction in a data set by retaining those characteristics of the data set that contribute most to its variance. Recently NMR and PCA have successfully been used in metabolomic profiling of several foodstuffs, phytomedicines and plant extracts (Choi et al., 2004, 2005; Gil and Duarte, 2006; Widarto et al., 2006; Rasmussen et al., 2006; Cho et al., 2007).

2. Materials and methods

2.1. Plant material

Two containers containing 60 capsules each were bought from Nordman Natural Therapies (Magaliessig, South Africa). Two batches of *A. annua* leaves were bought from Anamed (Winnenden, Germany). *A. afra* leaves were collected in March 2007 in the botanical garden of the University of Pretoria and at the Rietvlei Nature Reserve (Pretoria, South Africa) from four different plants.

2.2. Extraction of material

The plant material was removed from 20 capsules (10 from each container) after which it was allowed to air dry for 1 week. *A. annua* and the *A. afra* leaves were separately grounded into a fine powder and also allowed to air dry for 1 week. The three samples were divided into 10 *A. afra*, 7 *A. annua* and 7 capsule samples (100 mg each) yielding 24 samples in total. For the NMR analysis 100 mg of dried and grounded *A. annua*, *A. afra* and the material from the capsules were directly extracted with 1.5 ml of deuterated chloroform. The samples were sonicated for 30 min after which it was filtered and 0.8 ml transferred to NMR tubes.

For the LC–MS analysis 500 mg of plant material (two separate bathes of *A .annua*, four plants of *A. afra* and capsules from the two different containers) were sequentially extracted with hexane and chloroform (twice with each solvent). The samples were sonicated for 30 min after which it was filtered and dried on a rotary evaporator. A 1 mg/ml solution was prepared for each sample. The analysis was preformed in triplicate.

2.3. NMR analysis

The NMR analysis was performed according to Kim et al. (2005), on a 500 MHz Bruker spectrometer operating at a proton frequency of 500.13 MHz. For each sample 128 scans were performed after which the spectra were Fourier transformed with LB 0.3 Hz. The spectra were referenced to the

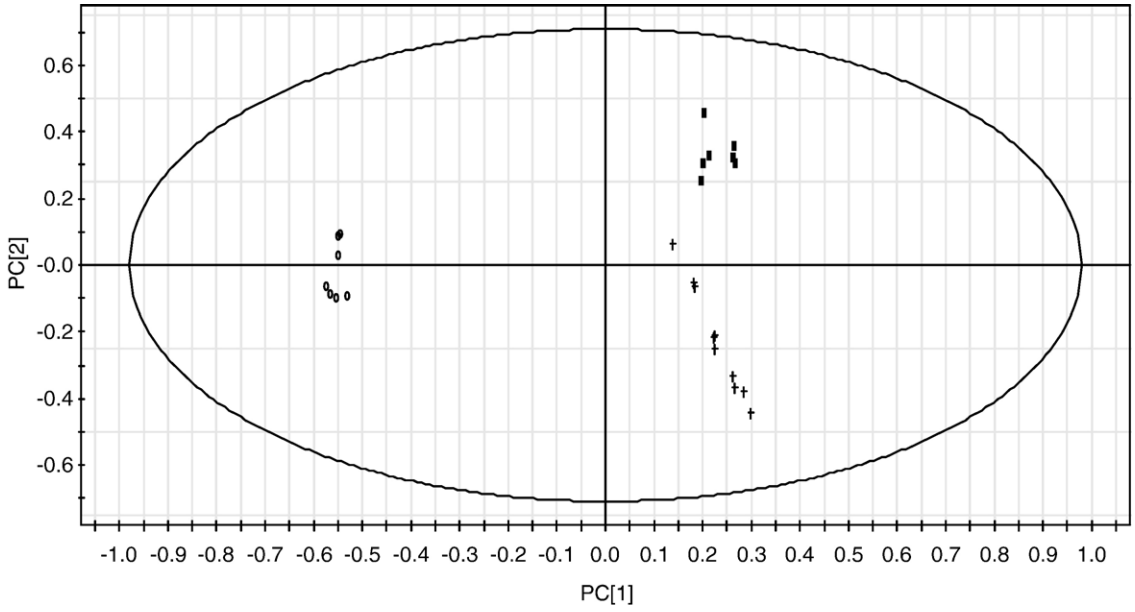

Fig. 1. Scoring plot of the Artemisia samples showing the clear differentiation in PC 1 between the *A. annua* (circles) samples and the *A. afra* (crosses) and herbal remedy material (boxes).

residual chloroform peak at 7.26 ppm. The total analysis time was 6 min per sample.

2.4. LC–MS analysis

The method of Huang et al. (2005) was adapted to quantify artemisinin in the plant samples. The LC–MS analyses were performed on an Agilent 1100 LC–MSD. An Agilent XDB-C18 Eclipse column (5 μm, 4.6×150 mm) was used to separate the mixtures. The mobile phase consisted of 0.1% formic acid (A) and methanol containing 0.1% formic acid (B). The following gradient system was employed to separate the extracts. Time=0 min: 60% B, time=8 min: 90% B, time 10 min: 90% B, time 12 min: 60% B and time 15 min: 60% B. The total run time per sample was 15 min. The MS conditions were as follows: APCI positive ionization in the Scan and SIM mode (SIM ion 283). The fragmentor was set to 0, drying gas flow 5 l/min, drying gas temperature 350 °C, vaporisor temp. 350 °C, capillary voltage 4000 V and the corona current 4.0 μA. UV detection was also preformed at 228, 254 and 280 nm to observe possible overlapping peaks. A standard curve for artemisinin (obtained from Sigma-Aldrich) was prepared with a concentration range of 1.56, 3.13, 6.25, 12.50, 25.0 μg/ml. The concentration vs. the area of the SIM ion peak was plotted with Excel software. The regression coefficient was 0.9915.

2.5. PCA analysis

The NMR data were automatically reduced to ASCII files using AMIX (v. 3.8, Bruker Biospin). The spectral intensities were reduced to integrated regions, also referred to as buckets, of equal width (0.04 ppm) corresponding to the region of 10.0 to −0.1 ppm. The residual chloroform region 7.28 to 7.24 was excluded. The files were imported into Excel files and transferred to SIMCA-P (10.0 Umetrics, Umea, Sweden) for PCA analysis.

3. Results and discussion

3.1. NMR and PCA analysis

The PCA analysis revealed that the capsules contained only *A. afra* and not *A. annua* or a mixture of the two herbs. The scoring plot (Fig. 1) shows a clear difference based on principle component 1 between the *A. annua* and the *A. afra* and capsule material samples. Based on PC 1 the samples could be differentiated as *A. annua* or *A. afra*. The loading plot revealed that artemisinin is an important chemical marker to differentiate between the two species. PC 1 was mainly affected by the three methyl signals of artemisinin at 0.99, 1.21 and 1.44 ppm.

3.2. Concentration of artemisinin in the plant material

The LC–MS analysis indicated the presence of artemisinin in only the *A. annua* samples as expected. The concentration being 0.840%±0.016% and 0.780%±0.009% for the two samples tested. The claimed concentration of artemisinin in this material is 0.570%–0.830% (Anamed –– oral communication). No trace of artemisinin could be detected in the *A. afra* sample as well as in the plant material in the capsules.

The NMR analysis clearly shows a difference between the samples. Based on PC 1 which explains 54.1% of the difference the two species can be differentiated into *A. annua* and *A. afra*. The observable difference between the *A. afra* plant and capsule samples based on PC 2 can probably be explained by them being different chemotypes, harvested in different seasons or being of different ages.

The LC–MS analysis showed that *A. afra* does not contain any artemisinin while the concentration in *A. annua* was calculated to be about 0.780–0.840% as expected. Our results have shown that the content in the capsules are indeed *A. afra* but that it, as well as the *A. afra* plant extract, does not contain any trace of the anti-malarial compound artemisinin.

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References

Bandaranayake, W.M., 2006. Quality control, screening, toxicity, and regulation of herbal drugs. Modern Phytomedicine 25–57.

Cho, I.H., Kim, Y.-S., Choi, H.-K., 2007. Metabolomic discrimination of different grades of pine-mushroom (*Tricholoma matsutake* Sing.) using ^{1}H NMR spectrometry and multivariate data analysis. Journal of Pharmaceutical and Biomedical Analysis 43, 900–904.

Choi, Y.H., Kim, H.K., Hazekamp, A., Erkelens, C., Lefeber, A.W.M., Verpoorte, R., 2004. Metabolomic differentiation of *Cannabis sativa* cultivars using ^{1}H NMR spectroscopy and principal component analysis. Journal of Natural Products 67, 953–957.

Choi, Y.H., Sertic, S., Kim, H.K., Wilson, E.G., Michopoulos, F., Lefeber, A.W. M., Erkelens, C., Kricun, S.D.P., Verpoorte, R., 2005. Classification of *Ilex* species based on metabolomic fingerprinting using nuclear magnetic resonance and multivariate data analysis. Journal of Agricultural and Food Chemistry 53, 1237–1245.

Christen, P., Veuthey, J.L., 2001. New trends in extraction, identification and quantification of artemisinin and its derivatives. Current Medicinal Chemistry 8, 1827–1839.

Gathirwa, J.W., Rukunga, G.M., Njagi, E.N.M., Omar, S.A., Guantai, A.N., Muthaura, C.N., Mwitari, P.G., Kimani, C.W., Kirira, P.G., Tolo, F.M., Ndunda, T.N., Ndiege, I.O., 2007. In vitro anti-plasmodial and in vivo anti-malarial activity of some plants traditionally used for the treatment of malaria by the Meru community in Kenya. Journal of Natural Medicine 61, 261–268.

Gil, A.M., Duarte, I.F., 2006. High-resolution nuclear magnetic resonance spectroscopy of fruit juices. Modern Magnetic Resonance 3, 1595–1599.

Huang, J., Yu, Y., Weng, W., Shen, T., Shi, L., 2005. Determination of artemether and its metabolite dihydroartemisinin in human plasma by HPLC with atmospheric pressure chemical ionization mass spectrometry. Zhongguo Linchuang Yaoxue Zazhi 14, 274–278.

Klayman, D.L., Lin, A.J., Acton, N., Scovill, J.P., Hoch, J.M., Milhous, W.K., Theoharides, A.D., Dobek, A.S., 1984. Isolation of artemisinin (qinghaosu) from *Artemisia annua* growing in the United States. Journal of Natural Products 47, 715–717.

Kraft, C., Jenett-Siems, K., Siems, K., Jakupovic, Mavi, J.S., Bienzle, U., Eich, E., 2001. In vitro antiplasmodial evaluation of medicinal plants from Zimbabwe. Phytotherapy Research 17, 123–128.

Kim, H.K., Choi, Y.H., Erkelens, C., Lefeber, A.W.M., Verpoorte, R., 2005. Metabolic fingerprinting of *Ephedra* species using H-NMR spectroscopy

and principle component analysis. Chemical Pharmaceutical Bulletin 53, 105–109.

Rasmussen, B., Cloarec, O., Tang, H., Staerk, D., Jaroszewski, J.W., 2006. Multivariate analysis of integrated and full-resolution ^{1}H NMR spectral data from complex pharmaceutical preparations: St. John's Wort. Planta Medica 72, 556–563.

Van Wyk, B., Van Oudtshoorn, B., Gericke, N., 1997. Medicinal plants of South Africa. Briza Publications, Pretoria.

Widarto, H.T., Meijden, E., Lefeber, A.W.M., Erkelens, C., Kim, H.K., Choi, Y.H., Verpoorte, R., 2006. Metabolomic differentiation of *Brassica rapa* following herbivory by different insect instars using two-dimensional nuclear magnetic resonance spectroscopy. Journal of Chemical Ecology 32, 2417–2428.

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