GC–MS analysis of ethanolic extracts of *Cyathea nilgirensis, C. gigantea*, and *C. crinita*

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Aim
The present work aimed to study the chemical constituents present in selected *Cyathea* spp. using gas chromatography–mass spectrometry (GC–MS).

Materials and methods
GC–MS analysis was carried out using the Clarus 500 GC with a fused silica column packed with Elite-1 and the components were separated using helium as a carrier gas at a constant flow rate of 1 ml/min.

Results
The presence of potent chemical constituents in *Cyathea* spp. was confirmed by GC–MS analysis. The most main identified in *Cyathea nilgirensis* included methyloctadecyl dichlorosilane (29.19%), 2-methylbutane-1,4-diol, and 3-(1-ethoxyethoxy)- (42.37%) in *C. gigantea* and 2-hydroxy-5-methylbenzaldehyde (55.45%) in *C. crinita*. All the predicted compounds showed various biological properties.

Conclusion
The results of the present study may be useful in metabolomics research, nutraceuticals, and phytopharmaceuticals to evaluate their quality.

Keywords:
Cyathea spp., gas chromatography–mass spectrometry, metabolomics, pharmaceuticals

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Introduction

The selection criteria of medicinal plants are based on five principal approaches namely, random, taxonomic, phytochemical, ethnomedicinal, and information-managed approach. In the random approach, all the available species are collected, irrespective of previous knowledge and experience. In the taxonomic approach, plants of a specific genus or family are deemed to be of interest and collected from diverse locations. The phytochemical (chemotaxonomic) approach is based on a particular compound type, which is of biological interest. Taxonomic and phytochemical approaches are closely related and cannot be clearly differentiated from each other. In the ethnomedicinal approach, the focus is on information on the medicinal use of the plant. On the basis of this information, the plant is collected and evaluated [1]. Information-managed plant selection collates taxonomic, biological, ethnomedicinal, and phytochemical information to yield a list of plants for specific collection. The information is compiled through computerized databases such as NAPRALERT, a specialized relational database on natural products, on the basis of systematic literature searches [2].

The quantitative determination of phytoconstituents has been made very easy by developments in analytical instrumentation. Recent advancements in the isolation, purification, and structure elucidation of naturally occurring metabolites have enabled the establishment of appropriate strategies for the process of standardization. Chromatographic and spectroscopic techniques can determine the homogeneity of a plant extract. Gas chromatography in combination with mass spectrometry (GC–MS) is a powerful tool often used for standardization and to control the quality of both the raw material and the finished product [3–6]. Information on these chemical constituents can not only aid in the discovery of new therapeutic drugs, but this information can also enable the discovery of new sources of economic materials that are precursors for the synthesis of complex chemical substances [7].

'Tree fern' is an arbitrary term that has been applied to fern with a large, erect rhizome, a portion that bears leaves. Christensen [8] divided the genus *Cyathea* into three genera: *Cyathea*, *Hemitelia*, and *Alsophila* spp., mainly on the basis of the presence or the absence of indusium. The first investigation of flavonoid constituents in the genus *Cyathea* spp. was carried out by Harada and Saiki [9]. They examined the leaves of *Cyathea fauriei* and *C. hancockii* during

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a complete survey of the distribution of flavones, flavonols, and flavanones in Japanese ferns. Hiraoka and Hasegawa [10] detected flavonoid glycosides in five *Cyathea* spp. from Tokyo. Hiraoka and Maeda [11] isolated new acylated flavonol glycoside from the fronds of *C. contaminans* and chemically characterized as kaempferol-7-(6'-succinyl)-glucoside. Yamane *et al.* [12] identified 10 gibberellins from *C. australis* using GC–MS analysis.

C. gigantea contains several active constituents namely, triterpenes, sterols, saponins, flavonoids, hentriacontane, β-sitostenone, β-sitostanone, diploterol, sitosterol, and hopan-29-ol, and the whole plant contains oleanolic acid [13]. Gopalakrishnan *et al.* [14] quantitatively estimated the presence of starch, total sugars, aminoacids, proteins, chlorophyll a, chlorophyll b, total chlorophylls, and carotenoids on the lamina of *C. crinita*, *C. gigantea*, and *C. nilgirensis*. They also studied the distribution of various aminoacids present in the chloroform and ethanolic extracts using the mobile phase n-butanol : acetic acid : water (12 : 3 : 5). Cyathea spp. has also been subjected to phytochemical studies related to the production of fernene, filicene, and hopane triterpenes [15,16], phenolic acids (coumaric and caffeic), protocatechuic acids, and flavonoids represented mainly by kaempferol glycosides [17]. Arai *et al.* [18] isolated dryocrassyl formate, sitostanyl formate, and 12 α-hydroxyfern-9 (11)-ene from fresh fronds of *C. podophylla*. On the basis of this information, the present study aimed to study the GC–MS profile of selected *Cyathea* spp.

Materials and methods

Collection of plant materials

Specimens for the present study were collected from various natural habitats of Tamil Nadu. *C. nilgirensis* were harvested in and around Kakkachi stream (1725 m), Kothayar, Tirunelveli hills (8°44′ N and 77°44′ E), *C. gigantea* from the road side near Nadugani (2637 m), Nilgiris hills (11°24′ N and 76°44′ E), and *C. crinita* from the Anglade Institute of Natural History, Shenbaganur, Kodaikanal (2195 m), Palni hills (10°13′ N and 77°32′ E), Western Ghats, South India. The plants were identified on the basis of the 'Pteridophyte Flora of the Western Ghats, South India' by Manickam and Irudayaraj [19]. Herbarium specimens were prepared and the voucher specimens were deposited in the St Xavier's College Herbarium (XCH), Palayamkottai, Tamil Nadu, India, for further reference (*C. nilgirensis* – XCH 25423; *C. gigantea* – XCH 25422 and *C. crinita* – XCH 25424).

Preparation of extracts

The collected species of *Cyathea* were washed thoroughly with tap water, followed by distilled water. They were blotted on a blotting paper and shade dried at room temperature in the dark. The shade-dried plant samples were ground to a fine powder using a mechanical grinder. Powdered samples of 30 g were extracted successively with 180 ml of ethanol using a Soxhlet extractor for 8–12 h at a temperature not exceeding the boiling point. The extracts were concentrated in a vacuum at 40°C using a rotary evaporator.

GC–MS analysis

The Clarus 500 GC (Perkin Elmer, Waltham, Massachusetts 02451, USA) used in the analysis included a fused silica column packed with Elite-1 and the components were separated using helium as a carrier gas at a constant flow rate of 1 ml/min. Ethanolic extracts of 2 μl of selected *Cyathea* spp. were used for GC–MS analysis [20]. The sample extracts were injected into the instrument and detected by the Turbo gold mass detector (Perkin Elmer) using Turbo mass, 5.1 software. During the 36th min of the GC extraction process, the oven was maintained at a temperature of 110°C with 2 min. The injector temperature was set at 250°C (mass analyzer). The different parameters of the operation of the Clarus 500 MS were also standardized (inlet line temperature: 200°C; source temperature: 200°C). Mass spectra were obtained at 70 eV, a scan interval of 0.5 s, and fragments from 45 to 450 Da. The MS detection was completed in 38 min.

Identification of components

The relative percentage amount of each component was calculated by comparing its average peak area with the total areas. The mass spectra of the respective peaks obtained in the GC–MS were compared with the mass fragmentation patterns of standards in the NIST (National Institute of Standards and Technology) version 2.0 – year 2005 library, which has more than 65 000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The compound name, molecular weight, molecular formula, and structure of the components present in selected *Cyathea* spp. were determined. The software used to handle mass spectra and chromatogram was GC–MS solution version 2.53. The biological activities of the components determined were predicted using PASS (Prediction of Activity Spectra for Substances) software and Dr. Duke's Phytochemical and Ethnobotanical Databases by D. Jim Duke of the Agricultural Research Service/

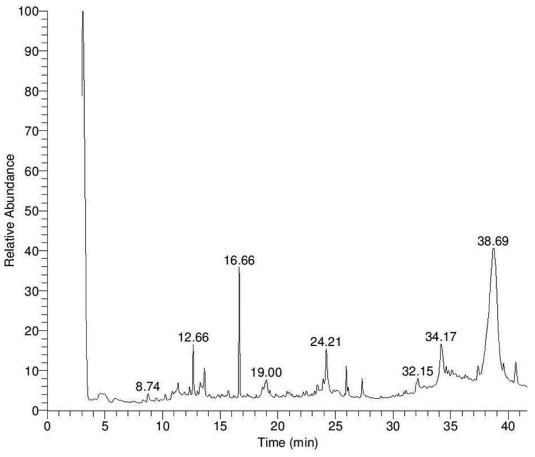
USDA. PASS online predicts over 4000 kinds of biological activity, including pharmacological effects, mechanisms of action, toxic and adverse effects, interaction with metabolic enzymes and transporters, influence on gene expression, etc.

Results

GC–MS analysis of *Cyathea* spp. confirmed the presence of various phytochemical compounds in the ethanolic extracts. The active principals with their retention time (RT), molecular formula, molecular weight, and peak area (%) in the ethanolic extracts of the *Cyathea* spp. studied are presented in Tables 1–3. In total, 30 compounds were detected in each *Cyathea* spp. during the 38.7 min measurement period. The compounds were predicted on the basis of the MS attached with GC.

The major components present in *C. nilgirensis* included methyloctadecyl dichlorosilane (29.19%) and 2-methylbutane-1,4-diol,3-(1-ethoxyethoxy)- (24.48%) separated at the RTs of 38.75 and 3.12 min, respectively (Fig. 1 and Table 1). The ethanolic extracts of *C. gigantea* showed the major presence of 2-methylbutane-1,4-diol, 3-(1-ethoxyethoxy)- (42.37%) and 2-hydroxy-5-methyl benzaldehyde (16.26%) (Fig. 2 and Table 2). *C. crinita* confirmed the presence of the prevailing compound 2-hydroxy-5-methyl benzaldehyde (55.45%) separated at the RT of 16.68 min (Fig. 3 and Table 3).

Figure 1



Gas chromatogram of the *Cyathea nilgirensis* ethanolic extract.

Table 1 GC–MS analysis of the *Cyathea nilgirensis* ethanolic extract

Name of the compound	Retention time (min)	Peak area (%)	Molecular formula	Molecular weight
2-Methylbutane-1,4-diol, 3-(1-ethoxyethoxy)-	3.12	24.48	$C_9H_{20}O_4$	192
2-Propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, exo-	13.64	2.08	$C_{13}H_{20}O_2$	208
î-N-Formyl-l-lysine	24.21	3.04	$C_7H_{14}N_2O_3$	174
2-Propenoic acid, 2-methyl-, 2-((2,3,3a,4,7,7a(or3a,4,5,6,7,7a)-hexahydro-4,7-methano-1H-indenyl)oxy)ethyl ester	25.95	2.02	$C_{16}H_{22}O_3$	262
9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	32.15	2.21	$C_{57}H_{104}O_6$	884
19-Norethandrolone tbdms	34.17	4.14	$C_{26}H_{44}O_2Si$	416
Methyloctadecyl dichlorosilane	38.75	29.19	$C_{19}H_{40}Cl_2Si$	366

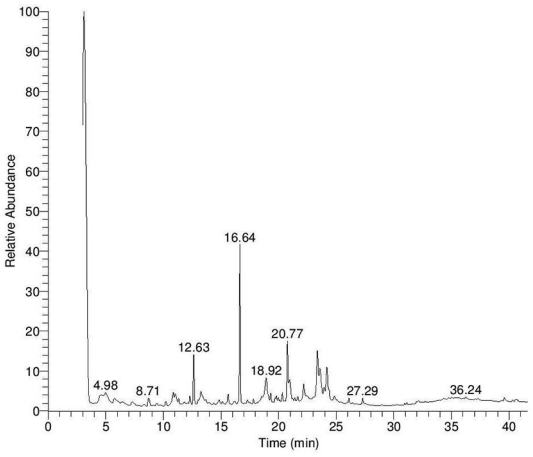
Table 2 GC–MS analysis of the *Cyathea gigantea* ethanolic extract

Name of the compound	Retention time (min)	Peak area (%)	Molecular formula	Molecular weight
2-Methylbutane-1,4-diol, 3-(1-ethoxyethoxy)-	3.13	42.37	$C_9H_{20}O_4$	192
2-Hydroxy-5-methylbenzaldehyde	16.64	16.26	$C_8H_8O_2$	136
l-Glucose	18.92	2.57	$C_6H_{12}O_6$	180
Mannitol, 1,3,4-tri-*O*-methyl-triacetate, d-	20.77	5.55	$C_{15}H_{26}O_9$	350
Desulphosinigrin	23.36	4.31	$C_{10}H_{17}NO_6S$	279
Gentamicin B	24.19	3.09	$C_{19}H_{38}N_4O_{10}$	482

Table 3 GC–MS analysis of the *Cyathea crinita* ethanolic extract

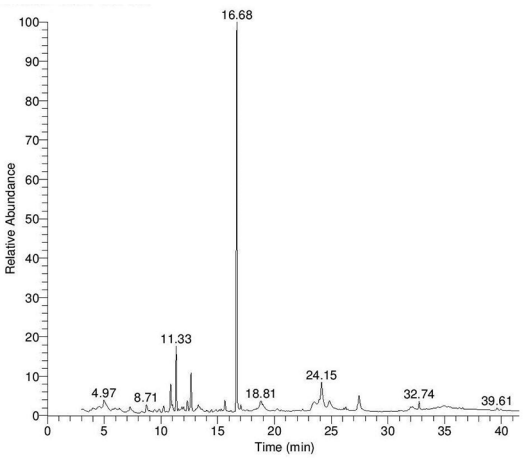
Name of the compound	Retention time (min)	Peak area (%)	Molecular formula	Molecular weight
Resorcinol	10.85	3.59	$C_6H_6O_2$	110
Benzofuran, 2,3-dihydro-	11.33	8.93	C_8H_8O	120
5-Acetoxymethyl-2-furaldehyde	12.65	5.35	$C_8H_8O_4$	168
2-Hydroxy-5-methylbenzaldehyde	16.68	55.45	$C_8H_8O_2$	136
α-d-Mannofuranoside, methyl	23.44	2.43	$C_7H_{14}O_6$	194
α-l-Rhamnopyranose	24.15	3.08	$C_6H_{12}O_5$	164
2-Propenoic acid, 3-(3-hydroxyphenyl)-	27.44	2.75	$C_9H_8O_3$	164

Figure 2



Gas chromatogram of the *Cyathea gigantea* ethanolic extract.

Figure 3



Gas chromatogram of the *Cyathea crinita* ethanolic extract.

Discussion

Metabolomics techniques are used to screen potential 'biomarkers' in plants [21]. These techniques play an important role in many aspects of biomedical and phytochemical research including biomarker screening, chemotaxonomy, quality control, bioactivity, and toxicity prediction [22]. The most widely used and powerful methods to identify the profile of low-molecular-weight chemicals are based on chromatographic separation, followed by detection and validation by MS [23]. GC in combination with MS can detect several chemicals including sugars, sugar alcohols, organic acids, amino acids, fatty acids, and a wide range of diverse secondary metabolites [24,25].

GC–MS analysis is an efficient way to analyze the metabolic fingerprinting of phytomedicine and to evaluate the overall chemical difference in medicinal plants that provides high separation efficiencies [26,27]. It is also a useful tool for the reliable identification of phytocompounds present in plant extracts. Chemical compounds found in plants, including secondary metabolites, have various functions ranging from defense against herbivores and microorganisms to ecological adaptations. Previous studies have reported a direct correlation between plant secondary metabolites and their biological activities [28,29].

In the present study, GC–MS analysis was carried out to identify some of the potent chemical constituents present in *Cyathea* spp. Biological properties of the *Cyathea* spp. studied were predicted using PASS software. The major components 2-methylbutane-1,4-diol, 3-(1-ethoxyethoxy)- showed various biological activities namely, sclerosant, hemostatic, antipruritic, allergic, cholesterol antagonist, cardioprotectant, antipyretic, antihypertensive, antithrombotic, apoptosis agonist, antiviral (influenza, herpes), etc.

Methyloctadecyl dichlorosilane showed properties including anti-infective, skeletal muscle relaxant, antimyopathies, alopecia treatment, antifibrinolytic, antihematotoxic, astringent, antiviral (parainfluenza), antispirochetal, etc. 2-Hydroxy-5-methylbenzaldehyde showed antieczematic, immunosuppressant, hepatoprotectant, antihypotensive, antiviral (picornavirus), antiparasitic, antiprotozoal, antiperistaltic, antitreponemal, antiulcerative, and insecticidal properties. The NCBI pubchem biassays also confirmed the anticancer properties of 2-hydroxy-5-methylbenzaldehyde. Matsunaga *et al.* [30] isolated vanitaracin A (1) and B (2), together with three novel compounds 3, 4, and 5 from a culture broth of a fungus, *Talaromyces* spp. The ^{13}C NMR spectrum and HMBC correlations of *Talaromyces* spp. suggested that 3 had *m*-cresol and 3,5-dihydroxy-4-methylbenzaldehyde moieties. Matsunaga and colleagues identified a novel compound with anti-hepatitis B virus potential. Their results and our prediction also confirmed the antiviral properties of 2-hydroxy-5-methylbenzaldehyde moieties.

The presence of various bioactive compounds with biological properties confirmed the application of *Cyathea* spp. for various ailments. The computer program PASS [31,32] can predict biological activities for compounds from different chemical series on the basis of their 2D structural formulas very fast. The set of pharmacological effects, molecular mechanisms of action, and specific toxic compounds with side effects that might be exerted by a particular compound in its interaction with biological entities that is predicted by PASS is termed the 'biological activity spectrum' of the compound. It is the natural property of a compound

that depends only on its structure and physicochemical characteristics. Further hyphenated spectroscopic studies are required for structural elucidation and identification of compounds detected in the *Cyathea* spp. studied. However, isolation of individual phytochemical constituents may lead to a novel drug formulation.

Conclusion

The chemical profiles of *Cyathea* spp. using GC–MS analysis provide information on individual molecules that characterize the species or specific extract. The results of the present study may be useful in metabolomics research, nutraceuticals, and phytopharmaceuticals to evaluate their quality.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Cordell GA, Beecher CCW, Pezzuto JM. Can ethnopharmacology contribute to the development of new anticancer drugs? J Ethnopharmacol 1991; 32:117–133.

2 Farnsworth NR. Ethnopharmacology and future drug development: the North American experience. J Ethnopharmacol 1993; 38:145–152.

3 Bilia AR, Bergonzi MC, Lazari D, Vincieri FF. Characterization of commercial kava-kava herbal drug and herbal drug preparations by means of nuclear magnetic resonance spectroscopy. J Agric Food Chem 2002; 50:5016–5025.

4 Janakiraman N, Johnson M, Sahaya Sathish S. GC–MS analysis of bioactive constituents of *Peristrophe bicalyculata* (Retz.) Nees. (*Acanthaceae*). Asian Pac J Trop Biomed 2012; 2:S46–S49.

5 Rozylo JK, Zabinska A, Matysiak J, Niewiadomy A. OPLC and HPTLC methods in physicochemical studies of a new group of antimycotic compounds. J Chromatogr Sci 2002; 40:581–584.

6 WHO. *General guidelines for methodologies on research and evaluation of traditional medicine*. Geneva, Switzerland: World Health Organization; 2002.

7 Farnsworth NR. Biological and phytochemical screening of plants. J Pharmacol Sci 1996; 55:225–276.

8 Christensen C. Index Filicum. Hagerup, Hafniae 1905

9 Harada T, Saiki Y. Pharmaceutical studies on ferns: distribution of flavonoids in ferns. Pharm Bull 1955; 3:469–472.

10 Hiraoka A, Hasegawa M. Flavonoid glycosides from five *Cyathea* species. Bot Mag (Tokyo) 1975; 88:127–130.

11 Hiraoka A, Maeda M. A new acylated flavonol glycoside from *Cyathea contaminans* Copel. and its distribution in the Pterophyta. Chem Pharm Bull 1979; 27:3130–3136.

12 Yamane H, Yamaguchi I, Kobayashi M, Takahashi M, Sato Y, Takahashi N, *et al*. Identification of ten gibberellins from sporophytes of the tree fern, *Cyathea australis*. Plant Physiol 1985; 78:899–903.

13 Juneja RK, Sharma SC, Tandon JS. Studies on a fern, *Cyathea gigantean*. Pharm Biol 1990; 28:161–162.

14 Gopalakrishnan S, Rama V, Angelin S, Manickam VS. Phytochemical studies on tree ferns of Western Ghats. Indian Fern J 1993; 10:206–213.

15 Arai Y, Koide N, Ohki F, Ageta H, Yang LL, Yen KY. Ferns constituents: triterpenoids isolated from leaflets of *Cyathea spinulosa*. Chem Pharm Bull (Tokyo) 1994; 42:228–232.

16 Arai Y, Hirohara M, Mtsuhira M, Tyoasaki K, Ageta H. Ferns constituents: triterpenoids isolated from leaflets of *Cyathea lepifera*. Chem Pharm Bull (Tokyo) 1995; 43:1849–1852.

17 Bringmann G, Gunther C, Jumbam DN. Isolation of 4-O-bD-glucopyranosylcaffeic acid and gallic acid from *Cyathea dregei* Kunze (Cyatheaceae). Pharm Pharmacol Lett 1999; 9:41–43.

18 Arai Y, Hattori T, Hamaguchi N, Masuda K, Takano A, Shiojima K. Fern constituents: dryocrassy formate, sitostanyl formate and 12 alpha-hydroxyfern-9(11)-ene from *Cyathea podophylla*. Chem Pharm Bull 2003; 51:1311–1313.

19 Manickam VS, Irudayaraj V. *Pteridophyte flora of the western ghats, South India*. New Delhi, India: BI Publications Private Limited; 1992.

20 Merlin NJ, Parthasarathy V, Manavalan R, Kumaravel S. Chemical investigation of aerial parts of *Gmelina asiatica* Linn by GC–MS. Pharmacognosy Res 2009; 1:152–156.

21 Kooy FV, Maltese F, Choi YH, Kim HK, Verpoorte R. Quality control of herbal material and phytopharmaceuticals with MS and NMR based metabolic fingerprinting. Planta Med 2009; 75:763–775.

22 Liu NQ, Cao M, Frederich M, Choi YH, Verpoorte R, van der Kooy F. Metabolomic investigation of the ethnopharmacological use of *Artemisia afra* with NMR spectroscopy and multivariate data analysis. J Ethnopharmacol 2010; 128:230–235.

23 Tolstikov RN, Fiehn O. Analysis of highly polar compounds of plant origin: combination of hydrophilic interaction chromatography and electrospray ion trap mass spectroscopy. Ann Biochem 2002; 301:298–307.

24 Fiehn O, Kopka J, Dormann P, Altmann T, Trethewey RN, Willmitzer L. Metabolic profiling for plant functional genomics. Nat Biotechnol 2000; 18:1157–1161.

25 Roessner U, Luedemann A, Burst D, Fiehn O, Linke T, Willmitzer L, Femie AR. Metabolic profiling allows comprehensive phenotyping of genetically or environmentally modified plant systems. Plant Cell 2001; 13:11–29.

26 Hall R, Beale M, Fiehn O, Hardy N, Sumner L, Bino R. Plant metabolomics: the missing link in functional genomics strategies. Plant Cell 2002; 14:1437–1440.

27 Sumner LW, Mendes P, Dixon RA. Plant metabolomics: large scale phytochemistry in the functional genomics era. Phytochemistry 2003; 62:817–836.

28 Fernie AR, Trethewey RN, Krotzky AJ, Willmitzer L. Metabolite profiling: from diagnostics to systems biology. Nat Rev 2004; 5:1–7.

29 Saito K, Dixon R, Willmitzer L. *Plant metabolomics*. Heidelberg, Germany: Springer Verlag; 2006.

30 Matsunaga H, Kamisuki S, Kaneko M, Yamaguchi Y, Takeuchi T, Watashi K, Sugawara F. Isolation and structure of vanitaracin A, a novel anti-hepatitis B virus compound from *Talaromyces* sp. Bioorg Med Chem Lett 2015; 25:4325–4328.

31 Gloriozova TA, Filimonov DA, Lagunin AA, Poroikov VV. Evaluation of computer system for prediction of biological activity PASS on the set of new chemical compounds. Chim Pharm J 1998; 32:32–39.

32 Poroikov V, Filimonov D. Computer- aided prediction of biological activity spectra: application for finding and optimization of new leads. In: Holtje HD, Sippl W, eds *Rational approaches to drug design*. Barcelona, Spain: Prous Science; 2001:403–407.