TCDJPPS

Trinity College Dublin Journal of Pharmacy and Pharmaceutical Sciences

Official on-line publication of the School of Pharmacy and Pharmaceutical Sciences – Trinity College Dublin

TCDJPPS, Volume 2, Number 1, May 2010.

TCDJPPS

The Trinity College Dublin Journal of Pharmacy and Pharmaceutical Sciences - TCDJPPS

The Trinity College Dublin Journal of Pharmacy and Pharmaceutical Sciences is a brand new on-line publication on all areas of Pharmacy and Pharmaceutical Sciences. The journal will publish only short communications, in the format given in the template, on all aspects of pharmacy and pharmaceutical sciences with strong emphasis on originality and scientific quality. The Editors welcome all articles in this multidisciplinary field. Manuscripts submitted to the Journal are only accepted on the understanding that they are subject to editorial review (generally by two independent referees). The recommendations of the Declarations of Helsinki and Tokyo, for humans, and the European Community guidelines as accepted principles for the use of experimental animals have been adhered to. The periodicity of this publication will be every 3 months with on-line numbers in February, May, August and November.
Submissions are only accepted via e-mail and following the template. Please send your contribution to the editor-in-chief. Authors will be notified of the receipt of the manuscripts immediately and in a due time of the final decision of the referees.

e-mail for submission of papers: fabiofarm_tcd@yahoo.co.uk

TCDJPPS

It is a great pleasure to be releasing now the volume 2 of the TCDJPPS. Our commitment is to try and publish relevant aspects of the pharmaceutical sciences to reach a great number of students in this broad area worldwide. Next volume will be electronically available by the end of August. So, keep sending your contribution for evaluation. Help us to establish this important forum for undergraduate and postgraduate students of Pharmacy and Pharmaceutical Sciences.

Sincerely Yours

Dr. Fabio de Sousa Menezes
Editor-in-Chief TCDJPPS

TCDJPPS Contents Vol. 2:

Template……...……..4

Forde, É., Dul, M., Kamionka, M. Optimisation of the polyacrylamide gel percentage to enhance electrophoresis resolution around 8kDa protein product ……..….6

Mannion, F. and Menezes, F.S. Antioxidant activity of *Urera baccifera* Gaud extracts……...……8

Moreira, D.L., Fonseca, V.M., Velozo, L.S.M., Kaplan, M.A.C. Volatile Constituents from Brazilian Piperaceae: *Piper cabralanum* C.DC……...…10

Cunningham, F. and Menezes, F.S. Ethnopharmacology in Dublin: Surveys on the medicinal plants use profile……...12

TCDJPPS

The Trinity College Dublin Journal of Pharmacy & Pharmaceutical Sciences

2009
Vol. 0

Main Title Here, Remove this text and enter your title here

Author A[a], Author B[a] and Author C[b,*]

[a]*School of Pharmacy and Pharmaceutical Scieces, Panoz Institute, Trinity College Dublin, Ireland.*

[b]*Department of Biochemistry, MS 015, Unknown University, City, Country.*

correspondingauthor@domain.com

Received: July XX, 2009; Accepted: July XX, 2009

Enter your abstract here (A concise abstract briefly stating the purpose of the research, major findings, and conclusions, should appear here. 100 words maximum).

Keywords: (3-6) keywords which identify most important subjects covered in the manuscript.

The manuscript should be written as clearly and concisely as possible. Common headings such as Introduction, Results and Discussion, and Conclusions should not be used as text within these sections should be self explanatory. Please avoid lengthy surveys of the literature. References should be cited in brackets [1-2]. Paragraphs should not be indented and there will be single line between paragraphs. Never write more than 2 pages and use a maximum of 1500 words.

The manuscript should be written as clearly and concisely as possible. Common headings such as Introduction, Results and Discussion, and Conclusions should not be used as text within these sections should be self explanatory. Please avoid lengthy surveys of the literature. References should be cited in brackets [1-2]. Paragraphs should not be indented and there will be single line between paragraphs. Never write more than 2 pages and use a maximum of 1500 words.

Table 1: Please use this paragraph to type the the heading for a table.

Compds	IC_{50}, nM	Compds	IC_{50}, nM[a b]
1	24.00	**6**	1.7
2	0.87	**7**	3.0
3	0.59	**8**	2.4
4	2.8	**9**	2.1
5	0.91	**10**	9.0 ± 1.9

Please use this paragraph to type the table footer(s).

The manuscript should be written as clearly and concisely as possible. Common headings such as Introduction, Results and Discussion, and Conclusions should not be used as text within these sections should be self explanatory. Please avoid lengthy surveys of the literature. References should be cited in brackets [1-2]. Paragraphs should not be indented and there will be single line between paragraphs. Never write more than 2 pages and use a maximum of 1500 words.

The manuscript should be written as clearly and concisely as possible. Common headings such as Introduction, Results and Discussion, and Conclusions should not be used as text within these sections should be self explanatory. Please avoid lengthy surveys of the literature. References should be cited in brackets [1-2]. Paragraphs should not be indented and there will be single line between paragraphs. Never write more than 2 pages and use a maximum of 1500 words.

The manuscript should be written as clearly and concisely as possible. Common headings such as Introduction, Results and Discussion, and Conclusions should not be used as text within these sections should be self explanatory. Please avoid lengthy surveys of the literature. References should be cited in brackets [1-2]. Paragraphs should not be indented and there will be single line between

paragraphs. Never write more than 2 pages and use a maximum of 1500 words.

The manuscript should be written as clearly and concisely as possible. Common headings such as Introduction, Results and Discussion, and Conclusions should not be used as text within these sections should be self explanatory. Please avoid lengthy surveys of the literature. References should be cited in brackets [1-2]. Paragraphs should not be indented and there will be single line between paragraphs. Never write more than 2 pages and use a maximum of 1500 words.

The manuscript should be written as clearly and concisely as possible. Common headings such as Introduction, Results and Discussion, and Conclusions should not be used as text within these sections should be self explanatory. Please avoid lengthy surveys of the literature. References should be cited in brackets [1-2]. Paragraphs should not be indented and there will be single line between paragraphs. Never write more than 2 pages and use a maximum of 1500 words.

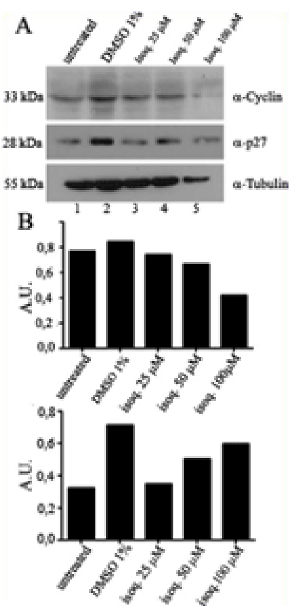

Figure 1: Please use this paragraph to type the footnote(s) for a figure.

The manuscript should be written as clearly and concisely as possible. Common headings such as Introduction, Results and Discussion, and Conclusions should not be used as text within these sections should be self explanatory. Please avoid lengthy surveys of the literature. References should be cited in brackets [1-2]. Paragraphs should not be indented and there will be single line between paragraphs. Never write more than 2 pages and use a maximum of 1500 words.

The manuscript should be written as clearly and concisely as possible. Common headings such as Introduction, Results and Discussion, and Conclusions should not be used as text within these sections should be self explanatory. Please avoid lengthy surveys of the literature. References should be cited in brackets [1-2]. Paragraphs should not be indented and there will be single line between paragraphs. Never write more than 2 pages and use a maximum of 1500 words.

The manuscript should be written as clearly and concisely as possible. Common headings such as Introduction, Results and Discussion, and Conclusions should not be used as text within these sections should be self explanatory. Please avoid lengthy surveys of the literature. References should be cited in brackets [1-2]. Paragraphs should not be indented and there will be single line between paragraphs. Never write more than 2 pages and use a maximum of 1500 words.

Acknowledgments - The inclusion of an acknowledgements section is optional. It may include credit to technical assistance, financial support and other appropriate recognition, and should be brief and placed at the end of your scientific paper before the references.

References

Please insert your references here. Some examples of references are given below.

[1] O'Neill PM, Posner GH. (**2004**) A medicinal chemistry perspective on artemisinin and related endoperoxides. *Journal of Medicinal Chemistry*, ***47***, 2945-2964.

[2] Paquette LA. (**2004**) Total synthesis of jatrophatrione, an unprecedented [5.9.5] tricyclic antileukemic diterpene. In *Strategies and tactics in organic synthesis.* Vol. ***4***, Harmata, M. (Ed). Elsevier, London UK. 97-131.

TCDJPPS

The Trinity College Dublin Journal of Pharmacy & Pharmaceutical Sciences

2010
Vol. 2

Optimisation of the polyacrylamide gel percentage to enhance electrophoresis resolution around 8kDa protein product.

Éanna Forde, Maria Dul, Mariusz Kamionka

School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Ireland.

kamionkm@tcd.ie

Received: May 10, 2010; Accepted: June 01, 2010

SDS-Polyacrylamide Gel Electrophoresis (PAGE) is run at a polyacrylamide percentage between 5% and 15%. It was used for the analysis of a recombinant human insulin construct. The resolution of the low molecular weight product, 8.2kDa, at typical polyacrylamide percentages was unsatisfactory. Therefore higher concentration gels were run (17.5% and 20%) and their resolution was compared to a 15% gel. These were expected to improve the resolution. They were more fragile and prone to damage. The results showed that resolution increased with increasing polyacrylamide percentage. However, fragility also increased. It is concluded that Tricine gels or another gel composition are needed.

Keywords: PAGE, polyacrylamide, electrophoresis,

Almost all analytical electrophoreses of proteins are carried out in polyacrylamide gels. The electrophoresis is carried out with a discontinuous buffer system i.e. the pH and ionic strength of buffer used in casting the gel and that in the reservoir are different. Before the proteins are loaded into the gel, the strongly anionic detergent, SDS, and a reducing agent combined with elevated temperature are used to denature them. After binding to SDS the denatured proteins become negatively charged. The amount of SDS is proportional to the molecular weight of the polypeptide and not its sequence. The SDS-polypeptide complexes are swept along by a moving boundary created when an electric current passes through the electrodes. Migration in the gel is therefore related to molecular weight. However, it should be noted that this is not entirely linear [1].

The gels themselves are composed of a polymer of acrylamide cross linked with bisacrylamide. Ammonium persulfate (APS) is added to provide the free radicals to drive polymerisation. Tetramethylethylenediamine (TEMED) is also added to the mixture to catalyse the polymerisation. The amount of cross linking and the concentration of acrylamide determine the effective separation range. The pore size is inversely related to concentration of acrylamide and the sieving properties are related to pore size [1]. Table 1 illustrates this relationship:

Table 1: Relation: concentration of acrylamide and range of separation for proteins (molecular weight). *Adapted from Sambrook and Russell.*

Acrylamide Conc. (%)	Linear Range of separation (kDa)
15	10-43
12	12-60
10	20-80
7.5	36-94
5.0	57-212

Our product for analysis, an 8.2kDa recombinant human insulin construct is not adequately resolved by typical acrylamide concentrations. Analysis under acrylamide concentrations above this range was carried out to determine if any improvements could be seen before using an alternate gel system such as Tricine gels (which can resolve down to 1kDa [2]).

The concentrations that were investigated were 17.5% and 20.0% (along with a 15% gel for comparison).

The gels were all prepared according to the analogous protocol and run under identical conditions to ensure the higher standards of comparison. The lanes were loaded with a protein molecular weight reference ladder. The distance on the ladder between the bands for Lysozyme and Aprotinin (between the centres of each band) was measured on each gel to determine resolution. The distance from the bottom of the first band and the bottom of the last band in the gel was also measured. The former measurement was divided by the latter to standardize the results (Table 2).

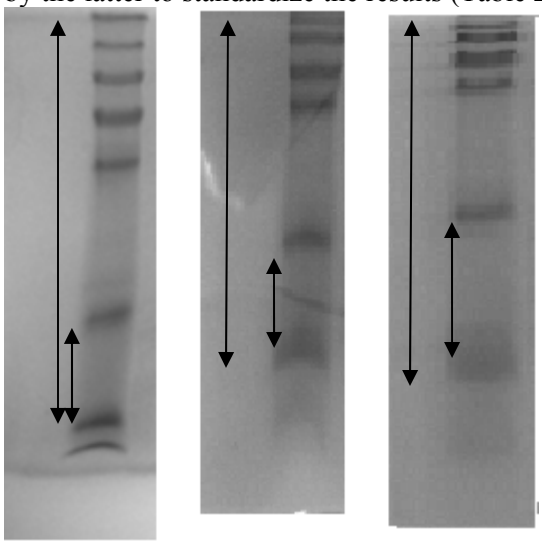

Figure 1: Captured images of gels using camera with EPI light source. 1 = 15%, 2 = 17.5%, 3 = 20%. The distances between bands measured are indicated.

The reasoning behind choosing these two markers is twofold: Firstly, they represent one of the most prominent gaps in markers on the ladder thus reducing experimental error. Secondly, the areas between these bands represent the area where the insulin sample would normally reside (6 – 16 kDa). Also, by determining the size of this gap relative to the distance between the first band and the Aprotinin band resolving properties of each gel were easily comparable.

Table 2 – Resolution according to the concentration of acrylamide.

Acrylamide %	Resolution
15	0.2504
17.5	0.3197
20	0.4194

After measurement of the distance in the three gels it was found that resolution in the investigated region improved with increasing acrylamide concentration.

However, a phenomenon observed was the fragility of the gels. With increasing concentration of acrylamide the gels became more fragile and harder to handle. They were particularly vulnerable to damage when being removed from the two plates. It was at this stage that two gels were badly damaged with one having to be repeated.

If more data points were available, i.e. enough to plot a statistically significant graph, it would have been possible to determine if a relationship exists between acrylamide concentration beyond 15% and resolution of >10kDa proteins.

From this study it can be seen that higher concentration gels are not optimal for an 8.2kDa polypeptide. Any advantage in the increase in resolution may be rendered merely academic by this increase in weakness and instability of the gels.

Tricine gels are the obvious alternative. It is the preferred electrophoretic system for resolution of proteins below 10kDa. They can also be stained by Coomassie Brilliant Blue although not all silver stains are compatible with them [2].

References

[1] Sambrook, J. & Russell, D. (**2001**). *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbour Laboratory Press.

[2] Schagger, H. & von Jagow, G. (**1987**). Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Analytical Biochemistry* , 368-79.

TCDJPPS

The Trinity College Dublin Journal of Pharmacy & Pharmaceutical Sciences

2010
Vol. 2

Antioxidant activity of *Urera baccifera* Gaud extracts.

Frances Mannion and Fabio de Sousa Menezes[a*]

[a]*School of Pharmacy and Pharmaceutical Scieces, Panoz Institute, Trinity College Dublin, Ireland.*

desouzaf@tcd.ie
Received: May 17, 2010; Accepted: July 01, 2010.

Samples of *Urera baccifera* Gaudi were collected in Brazil. Extracts were prepared (crude ethanol partition, hexane partition, dichloromethane partition, ethyl acetate partition, butanol partition and aqueous residue) and they were then tested for antioxidant activity using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), by analysing their total phenolic content and by estimating their flavonoid content. It was found that the ethyl acetate partition showed considerable antioxidant activity while the other five samples had poor antioxidant activity. The plant has previously been tested for anti-inflammatory activity and antiviral activity and is currently being tested by other researchers to isolate and identify its active constituents.

Keywords: *Urera baccifera*, Antioxidant activity, DPPH, Flavonoids, Total Phenols.

Urera baccifera Gaud is a member of the Urticaceae family and is indigenous to certain areas of the Greater and Lesser Antilles, Trinidad, and Tobago and on the mainland of South America from Mexico to Brazil, Argentine, Bolivia, and Peru [1]. It is known by several different names depending of which region is being considered. For example, it is known in Costa Rica as 'Chichicaste' or 'Ortiga brava'. Other names by which it is known include stinging nettle, ortiga, niguam, guaritoto and pica-pica. The plant grows in areas of rainforest on calcareous, acidic rocks and is generally shade intolerant. *U. baccifera*'s distribution is an important aspect of the maintenance of a balanced Brazilian rainforest [2].

It has been reported in local communities in Costa Rica that the locals chastise themselves with the stems of the nettle to ward off the cold when crossing high mountains. Locals also use *U. baccifera* as herbal treatment in various forms including infusion, oral and topical. The rubefacient effect is used to self treat rheumatic pains [3]. *U. baccifera* is also non-medicinally used to create effective barriers when planted in hedges and fences [1].

U. baccifera is of the family Urticaceae and the genus urera. The chemical constituents of *U. baccifera* have not yet been fully analysed but members of the same family such as *Urtica dioica* are reported as containing acids, amines, flavonoids, volatile oil constituents and lignans [4].

DPPH free radical method

DPPH is a free radical which gives a purple colour in ethanol. It has a maximum absorption at 515nm. This absorption decreases as antioxidants donate protons to DPPH causing it to become reduced. As can be seen in the reduction of DPPH below, the nitrogen atom is reduced by receiving a hydrogen atom from antioxidants. This becomes an uncoloured/yellow solution in the presence of antioxidants when the DPPH molecule becomes reduced. The extent of the decrease is absorption is taken as a measure of the extent of radical scavenging. The reducing ability of a compound is a good indicator of its antioxidant activity. DPPH is known to be sensitive to light, oxygen, solvent used and pH [5], which is why the reaction was carried out in the dark.

Based on the known uses of *U. baccifera* in folk medicine, the measured antioxidant activity of the plant was expected to be high. Also, other members of the same family, Urticaceae, have shown to exhibit antioxidant activity. *Urtica diocia* L., for example has been shown to have powerful antioxidant activity even when compared to standard antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), quercetin and tocopherol. Based on the results obtained, the ethyl acetate partition of *U. baccifera* can be determined as being the most active showing a lower EC_{50} value of 120.16 ± 0.32, when compared to the other partitions. The ethyl acetate partition was also the only one to exhibit a visual reduction, in colour from purple to faint yellow/colourless, in the DPPH molecule. When compared to other known antioxidants, the ethyl acetate can be assumed to show a high level of activity.

Rutin, a known antioxidant has been shown to exhibit EC_{50} value of 14.16 ± 0.20 when measured using the same method of analysis (Mensor et al, 2001). For the other partitions, no relevant antioxidant activity could be detected. This may be because the molecules present in these partitions may not contain available hydroxyl groups to undergo oxidation.

The DPPH method of screening for antioxidant activity proved effective and showed that the ethyl acetate partition may be rich in antioxidant activity due to the presence of free radical scavengers such as flavonoids and tannins.

Coumarins antioxidant activity has been shown to be dependent on the polar influence of a substituent [6]. This could explain why it is that the ethyl acetate partition, highly polar, showed the highest antioxidant activity. The antioxidant activity of the ethyl acetate partition was also seen to increase with increasing concentration.

Total phenolic content

Polyphenols work as antioxidants by neutralising the free radicals which contribute to disease. They can quench singlet and triplet oxygen and decompose peroxides. These phytochemicals have a quality, which can lower the mortality rate of various diseases [7]. The amount of total phenolics varied in the different partitions and varied from 0 to 77.75 mg GAE/g of dry material. The highest amount of total phenolics was detected in the ethyl acetate partition. This corresponds to the results already obtained from the DPPH analysis where ethyl acetate was shown to be the partition with the highest antioxidant activity. The ethyl acetate value of 77.75mg GAE/g dry material is very high when compared with value from other families. Therefore, we can deduce that *U. baccifera* must be high in plant polyphenols such as flavonoids. The ethyl acetate partition was found to be the partition showing the greatest activity. The butanol was again found to be the partition with the second greatest activity which also corresponds to the previous results.

Estimation of flavonoids content

Flavonoids are diphenylpropanes that are found in many plants. Flavonoids include flavones, isoflavones and flavanones. It has been reported that flavonoids inhibit the activity of many enzymes which include lipoxygenase, cyclooxygenase, monooxygenase, mitochondrial succinoxidase and NADH-oxidase and protein kinases. These effects are believed to be due to the antioxidant activity of flavonoids including their protecting reactions induced by iron [8].

From this analysis, the amount of flavonoids for each partition was determined. The values were seen to range from 0 to 27.14 mg/g Rutin equivalent. The ethyl acetate partition, as expected, showed the highest value of flavonoids present.

More research has been carried out on *Urera baccifera*. At this point it has been established that *U. baccifera* displays anti-inflammatory and analgesic activity in Sprague-Dawley rats [9]. More recently has shown to be effective at concentrations which are non-cytotoxic, against herpes simplex virus infection [10]. They also suggest that more research needs to be carried out on the plant in order to isolate the active constituents present as well as suggesting that coumarins may be responsible for the plants activity.

Urera baccifera has shown significant antioxidant activity. While this study suggests that flavonoids and coumarins may be responsible for the activity of the plant, it is essential that the plant be investigated fully, in order to determine the active constituents present. It is known that the plant exhibits activity against Herpes simplex virus and shows anti-inflammatory and analgesic action. It can now be said that the ethyl acetate partition of the plant has antioxidant activity.

Plant Samples

Aerial parts of *Urera baccifera* were collected in Rio de Janeiro, Brazil and were identified by Dr. Ana Claudia Vieira, Department of Pharmacognosy, Faculty of Pharmacy, Federal University of Rio de Janeiro, Brazil.

Extract Preparations

The plant material was allowed to dry and was extracted at room temperature with ethanol. The extract obtained was then concentrated under reduced pressure. To form different partitions, the residue was added to water and a liquid-liquid extraction with different solvents such as butanol, hexane, dichloromethane, ethyl acetate, ethanol etc. was carried out.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) photometric assay

To test the antioxidant activity of the sample, the Mensor *et al.*, 2001 method was followed [11].

Total Phenolic Content

To determine the amount of total phenolics, Folin-Ciocalteu reagent was used following the method of Lister and Wilson [12].

Estimation of flavonoids content

The amount of flavonoids in the samples was determined by using a UV spectrophotometer following the method of Lamaison and Carnat [13].

References

[1] Little, E.L., Jr., R.O. Woodbury, Wadsworth F.H. (**1974**) Trees of Puerto Rico and the Virgin Islands. Vol 2. Agriculture Handbook 449. U.S. Department of Agriculture, Washington, DC. 1,024 p. [2] Bertoni, J.E. de A., F.R. Martins, J.L. de Moraes, and G.J. Shephard. (**1988**) Composicao floristica e estrutura fitossocoiologica do parquet de Vacununga, santa Rita do Passa Quatro, Sao Paulo, gleba Praxides. Boletim Tecnico do Instituto Florestal, Sao Paulo 42. [3] Morton J. (1981). Atlas of Medicinal plants of Middle America. Charles Thomas, New York. P 154. [4] British Herbal Compendium, Vol 2. Peter Bradley, British Herbal Medicine Association. [5] Ozcelik, O., Lee, J. H., & Min, D. B. (**2003**) Effects of light, oxygen and pH on the Absorbance of 2,2-diphenyl-1-picrylhydrazyl. *Journal of Food Science*, 68, 487-490. [6] Orlov, Yu. E., (**1985**) Chemical structure and antioxidant activity among natural compounds. *Chemistry of natural compounds*, 22, 340. [7] Anderson, K. J., Teuber, S. S., Gobeille, A., Cremin, P., Waterhouse, A. L., & Steinberg, F. M. (**2001**) Walnut polyphenols inhibit in vitro human plasma and LDL oxidation. Biochemical and molecular action of nutrients. *Journal of Nutrition*, 131, 2837-2842. [8] Cao G., Sofic, E., & Prior, R.L. (**1996**) Antioxidant and prooxidant behaviour of flavonoids: structure-activity relationships. *Free radical biology and medicine*, 22, 749-760. [9] Badilla, B., Mora, G., lapa, A. J., & Emim, J. A. (**1999**) Anti-inflammatory activity of *Urera baccifera* (Urticaceae) in Sprague-Dawley rats. *Revista de Biologia Tropical.* [10] Martins, F. O., et al. (**2009**) In vitro inhibitory effect of Urera baccifera (L.) Gaudich extracts against herpes simplex. *African Journal of Pharmacy and Pharmacology*, 3(11), 581-584. [11] Mensor L., et al. (**2001**) Screening of Brazilian plant extracts for antioxidant activity by use of DPPH free radical method. *Phytotherapy Research,* 2001, 15, 127-130. [12] Lister, E., Wilson, P. (**2001**) Measurement of total phenolics and ABTS assay for antioxidant activity. Lincoln, New Zealand: Crop Research Institute. [13] Quettier-Deleu, C. et al. (**2000**) Phenolic compounds and antioxidant activities of buckwheat hulls and flour. *Journal of Ethnopharmacology,* 72, 35-42.

TCDJPPS

The Trinity College Dublin Journal of Pharmacy & Pharmaceutical Sciences

2010
Vol. 2

Volatile Constituents from Brazilian Piperaceae: *Piper cabralanum* C.DC.

Davyson de L. Moreira[a]*, Vinícius M. da Fonseca[a], Leosvaldo S.M. Velozo[b], Maria Auxiliadora C. Kaplan[b]

[a]*Departamento de Produtos Naturais – Farmanguinhos - FIOCRUZ, Rio de Janeiro, Brazil.*

[b]*Nucleo de Pesquisas de Produtos Naturais, Universidade Federal do Rio de Janeiro, Brazil.*

dmoreira@far.fiocruz.br

Received: May 10, 2010; Accepted: July 01, 2010.

The chemical composition of the essential oils from leaves and stems of *Piper cabralanum* C.DC was obtained by hydrodistillation and HS-SPME; and analyzed by GC-FID and GC-MS. Only sesquiterpenes were identified in all analyzed samples. Monoterpenes and arylpropanoids usually found in Piperaceae oils were not detected in this species. The sesquiterpenes α-muurolene, *E*-caryophyllene and δ-cadinene were found in all samples. The non-oxygenated sesquiterpene *E*-caryophyllene was identified in great amounts and as the major compound in the leaves essential oil obtained by hydrodistillation (38.8%) and HS-SPME (29.9%). The main compound recorded in the essential oil from stems was identified as α-muurolene (up to 15%).

Keywords: *Piper cabralanum*, Piperaceae, essential oil, sesquiterpenes, *E*-caryophyllene, α-muurolene.

The family Piperaceae belongs to the superorder Nymphaeiflorae, order Piperales (*sensu* Dahlgren, 1980), and it is composed by 5 genera (*Piper, Peperomia, Pothomorphe, Ottonia* and *Sarcorhachis*) and approximately 2000 species with a cosmopolitan distribution, and great occurrence in Rio de Janeiro State, Brazil [1,2] The chemical investigation of Piperaceae essential oils has shown the presence of monoterpenes, sesquiterpenes and arylpropanoids that, non rare, are the main constituents of the mixture [3-5]. *Piper cabralanum* C.DC. is a shrub measuring 1 to 3 meters high, commonly found in the Atlantic Forest (Southeast Brazil). Continuing the phytochemical and pharmacological studies with species of Piperaceae from the Rio de Janeiro State, *P. cabralanum* was collected near the town of Teresópolis. Since it was observed a pleasant fragrance of the leaves and stems of this species, the chemical composition evaluation was proceeded.

The chromatographic profile of the essential oils obtained from leaves and stems of *P. cabralanum* were very similar with a rich fraction composed by non-oxygenated sesquiterpenes (Table 1). Monoterpenes and arylpropanoids that are very common in the oils of Piperaceae were not detected in these samples. It was possible to identify more than 70% of the compounds in the volatile fraction obtained by hydrodistillation from leaves and stems. Considering the volatile fraction analyzed by HS-SPME it was possible to identify more than 90% in the sample from leaves and stems (Table 1). Oxygenated sesquiterpenes were identified only in the samples obtained by hydrodistillation due to the low volatility of these compounds (exception for *E*-nerolidol identified in low concentration in leaves).

The sesquiterpene *E*-caryophyllene was identified in all analyzed samples and in high amounts in the essential oil from leaves obtained by hydrodistillation (38.8%) and HS-SPME (29.9%). This sesquiterpene was also recorded in great amount in the oil from stems obtained by HS-SPME (14.3%). The compounds identified in higher amounts in the oils obtained by hydrodistillation and HS-SPME from the stems were α-muurolene (15.7%; 18.1%) and δ-cadineno (9.3%; 11.9%). Also, the sesquiterpene *Z*-caryophyllene was registered in high amount in the volatile fraction from stems obtained by hydrodistillation (12.1 %). It is interesting to note that α-bergamotene was recorded in high percentage in the volatile fraction from stems (16.3%).

The non-oxygenated sesquiterpene *E*-caryophyllene has many biological activities, including anti-inflammatory, local anaesthetic, antimicrobial, and antioxidant [6]. This is the first result on the chemistry of *P. cabralanum*, an endemic species from Brazilian Atlantic Forest.

Table 1: Identified compounds in the essential oil from leaves of *Piper cabralanum.*.

Compounds	RI_{cal}	RI_{lit}	HD %		HS-SPME %	
			leaves	stems	leaves	stems
α-cubebene	1347	1351	-	-	0.3	0.4
cyclosativene	1363	1368	0.4	2.5	0.8	0.9
α-copaene	1377	1377	-	-	6.5	9.7
longifolene	1408	1402	-	-	4.9	4.1
Z-caryophyllene	1409	1404	2.1	12.1	1.8	1.5
E-caryophyllene	1411	1418	38.8	8.2	29.9	14.3
α-berbamotene	1428	1438	0.5	1.8	5.4	16.3
α-humulene	1445	1454	1.4	-	-	-
alloaromadendrene	1451	1461	0.3	-	-	-
γ-muurolene	1476	1477	-	-	0.9	1.5
germacrene D	1484	1480	0.4	1.6	-	-
viridiflorene	1495	1493	-	-	12.5	12.5
bicyclogermacrene	1496	1494	1.7	-	-	-
α-muurolene	1499	1499	18.7	15.7	19.1	18.1
δ-amorphene	1513	1512	-	-	0.5	-
δ-cadinene	1516	1524	10.8	9.3	11.1	11.9
Elemol	1544	1549	0.5	-	-	-
E-nerolidol	1558	1564	3.4	4.8	0.9	-
caryophyllene oxide	1572	1581	1.2	3.7	-	-
globulol	1575	1583	0.4	0.9	-	-
α-muurolol	1645	1645	1.4	4.3	-	-
cubenol	1646	1647	2.6	6.1	-	-
% of Identified Compounds			**84.6**	**71.0**	**94.6**	**91.2**

RI_{cal} = Retention Index values calculated; RI_{lit} = Retention index values from literature data; HD = hydrodistillation; HS-SPME = Head Space-Solid Phase Microextraction.

Experimental

Plant material **-** Leaves and stems of *P. cabralanum* were collected in August 2009, in the city of Teresópolis, Rio de Janeiro State, Brazil. The plant material was identified by Profa. Dra. Elsie Franklin Guimarães comparing the collected samples with those deposited at Herbarium of Jardim Botânico do Rio de Janeiro.

Extraction of the Essential Oil: Hydodistillation **-** Fresh leaves and stems of *P. cabralanum* (150 g) have undergone hydrodistillation for two hour in a modified Clevenger extractor, yielding colourless oil (0.2 %) with a pleasant odour. A sample of this oil was dissolved in dichloromethane for GC-FID and GC-MS analysis [4].

Head Space-Solid Phase Microextraction (HS-SPME) **–** Fresh leaves and stems of *P. cabralanum* (400mg, each) were reduced to small fragments and placed in separated vials. The vial headspace materials were separately extracted by HS-SPME technique using a CAR-DVB fiber (75μm) at 80°C, and sample/headspace equilibration time was left for 15min. The extracted materials were immediately desorbed directly in the injector of the GC-MS apparatus.

Analysis of the Essential Oil: GC-FID analysis **-** Quantitative and qualitative analysis were carried out on GC 2010 Shimadzu with a DB-1MS fused silica capillary column (30m x 0.25mm x 0.25μm film thikness). The operating temperatures used were: injector 260°C, detector 290°C and column oven 60°C up to 290°C (3°C/min). Hydrogen at 1.0mL x min^{-1} was used as carrier gas. The percentages of the compounds were obtained by GC-FID analysis.

GC-MS analysis **-** Quantitative and qualitative analysis were carried out on a GC-MS QP 5000 Shimadzu with a ZB-5MS fused silica capillary column (30m x 0.25mm x 0.25μm film thikness). The operating temperatures used were the same of GC-FID analysis. Helium at 1.0mL x min^{-1} was used as carrier gas.

Chemical elucidation of the essential oil **-** The compounds identification was performed by comparison of their retention indices and mass spectra with published data. The results were also confirmed by comparison of the compound elution orders (retention times) with their relative retention indices reported in the literature [7]. The retention indices were calculated for all volatile constituents using the retention data of linear n-alkanes (C_8–C_{26}).

References

[1] Yunker TG (**1972**) The Piperacee of Brazil. *Hoehnea*, ***2***, 19-366.

[2] Candolle C **(1896)** Piperaceae: In. Prodromus, Systematis naturalis regni vegetabilis (De Candolle, A.P., ed.). ***16***(1), 235-471.

[3] Parmar VS, Jain CS, Bisht KS, Jain R, Taneja P, Jha A, Tyagi OD, Prasad AK, Wengel J, Olsen CE, Boll PM (**1997**) Phytochemistry of the genus *Piper*. *Phytochemistry*, ***46***(4), 597-673.

[4] Santos PRD, Moreira DL, Guimarães EF, Kaplan MAC (**2001**) Essential Oil Analysis of 10 Piperaceae species from the Brazilian Atlantic Forest. *Phytochemistry*, ***58***(4), 547-551.

[5] Moreira DL, Cardoso G.L, Souza PO, Pereira NA, Guimarães EF, Kaplan (**2001**) Effect of Leaf Essential Oil from *P. solmsianum* C.DC. in Mice Behaviour. *Anais da Academia Brasileira de Ciências*, ***73***(1), 33-37.

[6] Fernandes ES, Passos GF, Medeiros R, Cunha FM, Ferreira J, Campos MM, Pianowski LF, Calixto JB (2007) Anti-inflammatory effects of compounds alpha-humulene and (−)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. *European Journal of Pharmacology*, ***569***, 228–236

[7] Adams RP. (**2001**) Identification of essential oil components by gas chromatography/quadrupole mass spectrometry. Allured Publishing Corp, Carol Stream, IL.

TCDJPPS

The Trinity College Dublin Journal of Pharmacy & Pharmaceutical Sciences

2010
Vol. 2

Ethnopharmacology in Dublin: Surveys on the medicinal plants use profile.

Francis Cunningham[a] and Fabio de Sousa Menezes[a*]

[a]*School of Pharmacy and Pharmaceutical Scieces, Panoz Institute, Trinity College Dublin, Ireland.*

desouzaf@tcd.ie

Received: May 17, 2010; Accepted: July 01, 2010.

A field study was carried out on 18 natural health shops in Dublin area to assess the use of medicinal plants and other natural medicines. Store assistants were interviewed and asked questions from a previously prepared questionnaire. Results were evaluated and statistically analysed. Questions asked included best sellers, new products on the market, common prescribers, ailments for which plants are required, among others. Results indicated that Echinacea is the best seller, nutritionists accounted for approximately one third of the medical profession that prescribed plants, and cold and flu were the main pathologies for what natural approach was searched for. It could be notice that people in the greater Dublin area use medicinal plants either as a complement or the only source of treatment.

Keywords: Medicinal plants, ethnopharmacology, Echinaceae, Nutritionists, Dublin.

Ethnopharmacology concerns the intersection of medical ethnography and biological studies of therapeutic action i.e., a transdisciplinary exploration that spans the biological and social sciences [1]. The main goal of ethnopharmacology has been to discover novel compounds, derived from plants and animals used in indigenous medical systems, which can be employed in the development of new pharmaceuticals [2]. Ethnopharmacologic exploration, involving both field visits, as well as experimental research has lead in past to highly valuable information about medicinal plants used in different cultures and many were developed into drugs [3,4]. In years past enormous ethnopharmacological research was carried out by in the early days of medicinal plant research 250 years ago**.**

The collection of information on commonly used natural products in the greater Dublin area was the main focus of this research. A group of selected shops in the Dublin area were visited and one or more members of staff were questioned using a previously assembled questionnaire. The questionnaire contained closed questions; however this was amended in certain shops to allow more quantity and accuracy in information gathering. The main reasons questions were tailored were to due to the different functions practiced by the shops e.g. while certain shops focused on a more medicinal approach others were more dependent on a dietary approach. The information was gathered to get a detailed view on the natural medicines used in the area along with the customer's knowledge and approach to their use.

Fieldwork

A total of 18 health shops in the Dublin area were interviewed to gather information on the different types of natural medicinal products supplied. A survey with 12 questions was assembled, and asked in general to at least one person that worked in each health shop. To increase the number of answers, and therefore the accuracy of results two members of staff from each store were generally interviewed.

Research site

Dublin is the largest city and capital of Ireland. It is located near the midpoint of Ireland's east coast, at the mouth of the River Liffey. There are over 1.6 million residents in the county of Dublin. The use of Health shops in the Dublin area has been on the increase in recent years as residents are becoming tired with the, perceived lack healthcare provided by the state. Statistics provided in 2007 by the central statistics office showed the following about Ireland health system;

- 47.6% of Ireland's population were covered by private health insurance, and 31.9% of the population were covered by Medical Cards.
- There were 53 publicly-funded acute hospitals, with a total of 12,094 in-patient beds available and 1,253 day beds available.

Many of the shops, although natural health stores had very different functions to the community. While some focused purely on the medicinal side others focused more on a dietary approach thus giving a wider range of answers. To

tailor for this the questions were adapted, if required, to allow the maximum amount of information to be gathered. During the month of October, the selected health stores were visited on the list provided to me. The following stores were selected; 1-Country Cellar, 8 Patrick St. Dun Laoghaire; 2-General Health Food Store, GPO Arcade Dublin 1; 3-Health Matters, Ashleaf S.C. Crumlin Dublin 12; 4-Health Matters, 8 Grafton St. Dublin 2; 5-Hopsack Health Store, The Swan Centre Rathmines; 6-La Sante, Marine Mall Dun Laoghaire; 7-Nourish, GPO Arcade Dublin 1; 8-Nourish, Liffey St. Dublin 1; 9-Nourish, 16 Wicklow St. Dublin 1; 10-Organic Supermarket, 2c Main Street, Blackrock; 11-The Health Store, The Square Tallaght Dublin 24; 12-The Health Store, Nutgrove Office Park Dublin 24; 13-Vegeplanet, 19a Main Street Blackrock Market; 14-Down To Earth, Great Georges Street South Dublin; 15-Full life health food store, Dun Laoghaire shopping centre; 16-Nature Store, 324A NCR Phibsboro Dublin 7; 17-Nourish, Omni S.C Santry Dublin 9; 18-Nourish, Nutgrove S.C. Rathfarnham Dublin 16.

The main results of the field research are as follows:

Figure 1: Main natural products sold in health shops in Dublin area.

The use of plant derived remedies is still a vital area in medicinal product invention. Approximately 60% of the anti-tumoral and anti-infective agents, either commercially available or in late stages of clinical trials are of natural origin [5]. Echinacea and general multi-vitamin were the dominant products selling in the health stores visited. When the survey was carried out, in early October, the swine flu epidemic had just become publicized; along with the impending winter sales of medicines with the ability of immune boosting functions were among the higher sellers, hence its position as the number one seller. The second best seller, multi-vitamins, was number 1 for the rest of the year commonly, as stated by staff when the interview was conducted. Staff claiming this reason was due to customer request for a supplement that could re-energize and reduce fatigue. From the survey it becomes apparent that there is an almost equal amount of people that enter stores with a clear view on what they want, to those who enter and seek advice. There were also two clear products for which patients had researched privately and requested in these stores, which could not be legally sold. The two products were Gingko Biloba and St. John's Wort, the former available in the United Kingdom and the later being made prescription only in 2000.

Nutritionists were named by all health stores as the main prescribers of their medicines. Kinesiologists, which had been previously been excluded from the list was the second highest profession prescribing natural medicinal products. All others professions appeared with approximately the same ratio of prescriptions. All stores stated that prescribing was generally from a mixture of all the professions present on the list**.**

The main reason for potential patients visiting health stores at the time of visiting was for herbal products for the prophylaxis of cold and flu's. Mentioned previously personnel in the stores visited stressed the point that many people had become worried about contracting such diseases and the public outbreak of the H1N1 virus had caused people to become more fearful of their health. The general pattern of the oncoming winter season and outbreaks of new diseases annually ensures that preparations with immune boosting function become the largest seller at that period.

The second largest reason for the rise in popularity of Health Stores was a more natural approach to health. Many of the store assistants interviewed said that people purchasing medicines from their store were becoming weary of general practitioners and pharmacies and believed natural remedies would provide a safer option. Customers believed that products with a more "natural" origin would cause less potential problems and side effects. Health store staff believed patients are becoming less trusting in other members of the health profession such as pharmacists and general practitioners. Patients perceived a significant number of modern medicines as harmful to the body. An increase in the number of health articles and alternative treatments available in recent years has caused this trend.

References

[1] Etkin, N.L., Elisabetsky, E. (**2005**) Seeking a transdisciplinary and culturally germane science: the future of ethnopharmacology. *Journal of Ethnopharmacology*, 100, 23–26.

[2] Anna Waldstein (**2006**).Mexican migrant ethnopharmacology: Pharmacopoeia, classification of medicines and explanations of efficacy.

[3] Bruhn, J.G., Holmstedt, B. (**1981**) Ethnopharmacology: objectives, principles and perspectives. In: Beal, J.L., Reinhard, E. (Eds.), Natural Products as Medicinal Agents. Hippocrates-Verlag, Stuttgart, pp. 405–430.

[4] Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D., Guo, Z. (**1985**) Medicinal plants in therapy. Bulletin of the World Health Organisation, 63, 965–981.

[5] Silva,C.G; Herdero, R.S. *et al.* (**2005**) Evaluation of Antioxidant Activity of Brazilian Plants. *Pharmacological Research*, 52, 3, 229-233.