Exploring the Chemistry of *Ocimum* Species under Specific Extractions and Chromatographic Methods: A Systematic Review

Andrea Beltrán-Noboa, Alejandro Jordan-Álvarez, Mabel Guevara-Terán, Blanca Gallo, Luis A. Berrueta, Francesca Giampieri, Maurizio Battino, José M. Álvarez-Suárez,* and Eduardo Tejera

Cite This: https://doi.org/10.1021/acsomega.3c00043

1. INTRODUCTION

Within the *Ocimum* genus, more than 65 native species have been described. It is considered the largest genus in the Lamiaceae family. The word *Ocimum* is derived from the Greek term “ozo”, which means “smell or fragrance”. Due to *Ocimum* species’ use in traditional cuisine, medicine, perfumes, and the pharmaceutical industry, *Ocimum* is considered the “king of herbs”. The diversity of the genus *Ocimum* depends mainly on geographical distribution, which has been defined as (1) tropical Asia, (2) tropical and subtropical regions of Africa, and (3) tropical parts of North and South America. According to Grayer et al., some of the principal species are *O. basilicum*, *O. gratissimum*, *O. kilimandscharicum*, *O. lamiifolium*, *O. minimum*, *O. citriodorum*, *O. gratissimum*, and *O. americanum*, which are classified by matching their chemical composition and closer morphology. However, as we will see later, the species’ chemical composition is difficult to analyze and is the central topic in this review. In this sense, the chemical variability according to Pandey et al. could derive from different changes in “edaphic factors” as well as different climatic zones. Other factors that may affect the chemical composition are cross-hybridization, polyploidization, morphogenesis, harvesting, drying, and storage. Previous studies have been carried out in order to explore chemical diversity across several *Ocimum* species. Chowdhury et al. reported a significant variation in chemical constituents from ethanol extracts, while Kruger et al. found similar results by studying the chemical composition of essential oils (EOs). In EOs, previous studies have found a large consistency in the presence of linalool, citral, and methyl cinnamate across different *Ocimum* species. However, studies related to the extensive chemical description of nonessential oils are scarce (as we will discuss later) in this genus. Besides uses in the culinary and food industries, *Ocimum’s* potential effects, such as antibacterial, anti-inflammatory, antioxidant, geno-protective, and antitumoral activities, have been reported recently using different extraction solvents. Even when the chemical composition is generally not fully reported in those studies focused on biological impact, the known presence of...
phenolic compounds, including eugenol, rosmarinic acid, apigenin, rutin, and caffeic acid, could be associated with some of those effects. Considering the importance of Ocimum species, given their medicinal and culinary uses, it is important to study the chemical composition and experimental methods used for extractions and characterization. In this sense, the aims of this review were (1) to consolidate the information regarding chemical composition (non-EOs) across different Ocimum species as reported by other authors and (2) to compare the chemical space across different studies/species. However, chemical composition will depend on the extraction and instrumental methods used as well as the geographical location; therefore, those variables will also be explored.

2. METHODOLOGY

2.1. Literature Search. A search on PubMed database (https://pubmed.ncbi.nlm.nih.gov/) was performed using the terms: “Ocimum” [Title/Abstract] OR “leaf” [Title/Abstract] OR “plant” [Title/Abstract] combined with “extract” [Title/Abstract] AND “alcoholic” [Title/Abstract] OR “hydroalcoholic” [Title/Abstract] OR “ethanolic” [Title/Abstract]. The search was delimited to articles published in English in the last 10 years, from 2012 to (June) 2022. We examined the titles, abstracts, methodology, and reports of the chemical composition across all retrieved articles and determined the eligibility of the articles. As a result of this PubMed search, a total of 365 results were further processed.

2.2. Data Processing and Filtering. Manual processing was carried out to organize the information from the 356 retrieved articles. The process of inclusion and exclusion of studies are shown in Figure 1. The exclusion criteria were

- No full evaluation of the chemical composition of the plants (n = 189). This means that in those studies, no specific separation/identification experiments were implemented to identify the main chemical constituents (HPLC, GC, mass spectrometry, UV, etc.).
- Studies performed on essential oils (n = 12). In these studies, the extraction was made with water distillation.
- Extractions made with seeds, calluses, or a mixture of different plants (n = 6).
- Characterization of fermentation products or fungi, or articles using iontophoretic extraction (n = 5).
- Ocimum extracts used for nanoparticles synthesis (n = 21).
- Reviews and books (n = 8), articles with no access to the full text (n = 24), and extractions with the following solvents: butanol, chloroform, dichloromethane, and/or hexane (n = 12).

Finally, a total of 277 articles were rejected and 79 accepted for further analysis. The group of 79 articles comprises extractions using water, alcohol (with no differences between methanol and ethanol), hydroalcoholic mixture (with no differences between methanol and ethanol, or different water/alcohol proportions), acidic alcohol, and ethyl acetate (Table 1) (see Figure 1 for schematic representation). In Figure 1, the total sum is higher than 79 because some articles combine identification across more than one extraction procedure.

2.3. Molecule Analysis and Classification. Some challenges emerge in the comparison of the identified molecules across different publications. One of these is the ambiguity of chemical naming between different authors. In some cases, the same molecule could have a different name, e.g., apigenin 8-C-glucoside is the same as vitexin according to PubChem (https://pubchem.ncbi.nlm.nih.gov/compound/5280441). In the classification of the identified compounds...
in our search, manual curation was performed using the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) as our reference guide for searching for names and synonyms. Mass spectrometry is one of the most commonly applied methods for molecular identification in natural products, but in some cases, there are ambiguities during identification. For example, several articles mention "quercetin 3-O-hexoside" or "coumaroyl-glucoside derivate", which means that the specific determination of the aglycone or its connection with the rest of the molecule is unknown. These ambiguities and alternative nomenclature are difficult to curate; therefore, manual procedure and annotation were performed as follows:

- If the name of the molecule presented in the manuscript can be directly located in the PubChem database, the isomeric smiles code was retrieved, and the molecule was labeled as “0”. All possible synonyms were retrieved and renamed across all variations using a unique name.
- If the name of the molecule presented in the manuscript did not appear within the PubChem database, we carried out some additional inner codification and processing. For example, in Khaled et al., the following identifications were reported: apigenin-7-hexoside, quercetin-O-hexoside, and rosmarinic acid derivate. All these names were kept in our final database and labeled as “1” with no Smiles code associated. However, a Smiles code is necessary for further molecule classification and analysis. In these cases, a “closer” chemical representation was used and added to the data set (labeled as “2”). In this case, we classified the molecules that were similar in terms of chemical skeleton and had already a Smiles code as having “closer” chemical representation.

Following the same example, rosmarinic acid, quercetin-3-O-glucoside, and apigenin 7-glucoside were added to the database if they were not already identified in the manuscript.

Therefore, in each manuscript, the code “0” represents an unambiguously identified molecule, “1” represents a molecule that was not clearly identified, and “2” indicates an added molecule closer to those with code “1”. The main reason for adding a closer molecule is to represent as much as possible the chemical space of the molecules detected in a specific plant and using a specific extraction solvent. Another example is in Fernandes et al., where 6 glycosylated cyanidins were reported as “isomers”. All of them are labeled as “1” and a Cyanidin 3-glucoside was added only once in the database and labeled as “2”. All molecules with the corresponding Smiles and labeled as “0” are presented in Supporting Information 1.

All molecules were classified using their SMILES representation and the NPClassifier. The NPClassifier is a deep-learning algorithm used to predict super classes and classes of molecules (i.e., flavonoids, terpenes, phenolic acids, etc.) using the SMILES code of the molecule. This classification is useful to us to compare molecular types across different articles and/or plant species.

3. RESULTS AND DISCUSSION

A total of 356 articles were analyzed, and the initial result indicated that 189 articles do not report a specific chemical composition. These articles are mainly focused on the in vitro and in vivo effects of aqueous and ethanolic extracts. The majority of these articles reported total quantities, like total amounts of polyphenols, tannins, and anthocyanins as well as...
total antioxidant capacities. In a few cases, computational analysis was performed but using oldest reports of chemical composition. The importance of these studies relies on the findings regarding antibiotic, anti-inflammatory, and anti-diabetic effects, as well as potential protection against oxidative stress. However, we consider that chemical composition, or at least the main chemical component analysis, could improve those manuscripts and contribute to the exploration of chemical diversity (or consistent component effects) across different species.

3.1. Distribution of Studies across Ocimum Species and Countries. The 79 articles under study comprise 12 Ocimum species, but O. basilicum, O. tenuiflorum, and O. gratissimum appear in the highest number of studies (Figure 2A) and in more studies across different countries (Figure 2B). In some articles, the cultivar of the species is named, but in others this information is missing. A total of 10 cultivars were identified: Purpurascens (Portugal), Maria Bonita (Brazil), Aroma2, Eleonora, Italiano Clasico, Basilico Rosso, and Dark Opal (Italy), Mitikas (Germany), and Red Basil (Greece), and all of them bred O. basilicum.

It is also noticed in Figure 2C that countries such as India, Nigeria, Brazil, Egypt, Pakistan, Portugal, Saudi Arabia, and South Africa report the highest number of articles. However, it

Figure 3. (A) Selection of molecules (n = 17) representing the most frequently reported ones across the articles. (B) The area represents the number of species in which the molecule was reported (from a maximum of 8 to a minimum of 3 species). (C) Distribution of molecules found in all Ocimum species according to superclasses defined by NPclassifier.
should be mentioned that the number of published studies may be related only to a single basil species, as in the case of South Africa, where a high number of articles have been published, but, according to our findings, only focused on *O. basilicum* and *O. gratissimum*.

As previously stated, the genus *Ocimum* includes about 65 species and is considered the largest genus in the Lamiaceae family. Moreover, there are huge numbers of reports regarding *Ocimum* species in the Tropicos database (https://tropicos.org), in which we can see that the greatest concentration is found in South America, Africa, and Asia. Nonetheless, our findings clearly indicate that chemical characterization (not including essential oils) was performed in very few cases. Indeed, chemical characterization is only available for 12 species of *Ocimum*. Additionally, even across those 12 species, the articles are mainly focused on 4 of them (*O. basilicum*, *O. tenuiflorum*, *O. gratissimum*, and *O. citriodorum*).

A possible explanation for the relationship between the countries and the number of studies (and species) we found in the literature (with the extraction methods we considered) is *Ocimum*’s uses in those countries. For instance, India has the highest number of articles related to *Ocimum* species (n = 12) (*O. tenuiflorum* (n = 9), *O. basilicum* (n = 2), and *O. gratissimum* (n = 1)) and *Ocimum* (especially *O. tenuiflorum*) plays an important role in Ayurvedic medicine. The plants are used as probiotics for their immunomodulator and anti-inflammatory effects in the treatment of convulsions or anxiety because of their anti-aminergic properties, in central nervous systems disorders, and as antibacterial agents in toothpaste, mouthwash, and gels. However, in other countries, like Italy, basil plants are traditionally used as the main ingredient of Mediterranean cuisine (particularly *O. basilicum*).

In this country, we found that all studies were performed on *O. basilicum*. Interestingly, the species *O. kilimandscharicum* has been only studied (with the methodology of our present work) in Egypt.

### 3.2. Extraction and Compound Identification Methods

Across the 79 articles accepted and processed, several strategies for molecular identification were used. In some cases, only ultraviolet (UV) was used (with liquid chromatography) and in others, nuclear magnetic resonance (NMR) and thin layer chromatography (TLC) were also used. In most cases, gas chromatography—mass spectrometry (GC-MS) or liquid chromatography—mass spectrometry (LC-MS) was used for separation and identification (Table 1). Most of the studies employed LC-UV (liquid chromatography with ultraviolet), which focuses on the quantification of specific compounds and consequently uses the retention time and reference standards for identification confirmation. Other reports mention the use of NMR, usually combined with LC or even LC-MS; additionally, some studies combine more than one identification strategy. Our results found that the most widely used identification methods for alcoholic and hydro-alcoholic extractions (which are the most common) are LC-UV and LC-MS. The section defined as “others” mentions less frequently used techniques: NMR, UV, TLC, and HPLC, which do not report the use of mass spectrometry.

Regarding molecular identification, a total of 325 molecules were labeled as “0” (the reported names were directly found in PubMed), 39 as “1” (the reported names are unspecific), and 22 as “2” (molecule inserted and similar to those labeled with “1” in order to maintain the chemical space) for a total of 335 unique smiles. The compounds with the highest number of identification as well as those most detected across different species are presented in Figure 3A and B.

Due to the high number of compounds, it is not possible to include all of them in a single image. We limited our graphs (Figure 3A and B) to those with the highest number of references (the full list can be found in Supporting Information 1). The most reported compound (Figure 3A) is caffeic acid, followed by rutin, rosamarinic acid, and chircoric acid. However, in terms of species, rutin and rosamarinic acid were found in 8 species (*O. americanum*, *O. basilicum*, *O. campechianum*, *O. cilantrolum*, *O. gratissimum*, *O. kilimandscharicum*, *O. selloi*, and *O. tenuiflorum*), followed by rosamarinic acid in the species: *O. basilicum*, *O. gratissimum*, *O. tenuiflorum*, *O. citriodorum*, *O. basilicum*, *O. gratissimum*...
Americanum, O. basilicum, O. campechianum, O. canum, O. citriodorum, O. gratissimum, and O. tenuiflorum. These are the most commonly found across Ocimum species. Moreover, we can notice that the majority of detected compounds are part of the flavonoid and phenylpropanoid (Figure 3C) chemical superclasses, which is consistent with the extraction methods considered in our methodology.

In Table 2, we can observe the top 10 most frequently detected molecules in different Ocimum species and countries (in some cases only a few numbers of molecules have been detected). In Figure 4A and B, we present the main metabolites in the two most abundant molecular superclasses.

We can examine how the chemical skeleton is very similar between molecules and here lies the difficulty of naming due to the spatial arrangement of the molecules. Moreover, we can notice (Table 2) that many compounds were not detected in all species. However, it is probably a result of a nonstandard methodology for identification. Many of these compounds (gallic acid, p-coumaric acid, ferulic acid, etc.) are related to the same metabolic route (phenylpropanoid pathway) with the p-coumaric acid as a core metabolite, as we can also see in the structural similarity (Figure 4A and B).

Moreover, for rosmarinic acid, besides 37 articles with direct references, 2 others refer to derivative compounds. A similar

**Figure 4.** (A) Principal molecules reported from the flavonoid superclass in all Ocimum species. (B) Principal molecules reported from the phenolic acid superclass in all Ocimum species.
situation was also found with quercetin glycosylated derivate and ferulic acid derivate. The most abundant type of molecules are the flavonoids, and, in this superclass, the following classes are ranked as follows: flavones, flavonols, flavanones, flavan-3-ols, dihydroflavonols, and chalcones with 35, 30, 9, 2, 2, and 1 molecule(s), respectively.

The terpenoid superclasses were not included, because in our study we excluded all articles with chemical characterizations of essential oils. In these studies, we should expect a wide range of terpenoids, so the chemical diversity and even the number of articles with identification for this family of compounds could be underestimated. This was why we did not focus our discussion on this family of molecules, where a
further review is needed. Moreover, several molecules were not classified \((n = 36)\) using the NPClassifier algorithm (Figure 5). A closer look at these molecules reveals several anthocyanins (cyanidins, pelargonidins, and peonidin), some phenolic compounds (resorcinol, pyrogallol, and catechol), quinone derivative (hydroquinone and glycosylated hydroquinone), and other molecules like ascorbic acid and glycerol.

3.3. Chemical Composition Across Different Analytical Methods. Mass spectrometry in combination with liquid or gas chromatography is the most common strategy for compound identification in natural products. In Table 3, we can clearly notice that the number of compounds identified using mass spectrometry combined with LC or GC is actually high.

Table 3. Compounds Identified with Different Extraction and Identification Methods

<table>
<thead>
<tr>
<th>Analysis methods</th>
<th>Extraction method</th>
<th>Number of compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC</td>
<td>Hydroalcoholic</td>
<td>2</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Alcoholic</td>
<td>131</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Hydroalcoholic</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>31</td>
</tr>
<tr>
<td>LC-UV</td>
<td>Alcoholic</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Alcoholic-acid</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Hydroalcoholic</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>37</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Alcoholic</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>Alcoholic-acid</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Hydroalcoholic</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>55</td>
</tr>
</tbody>
</table>

The use of only GC or LC in almost all articles is focused on the identification of specific compounds, requiring reference standards for retention time confirmation. However, a full metabolomic exploration was carried out in combination with mass spectrometry. Of course, several factors can affect the amount of identified compounds: mass instrument parameters, general annotation strategy \((i.e.,\) reference standards, literature reference, and library-based annotation), identification experience, derivatization, general sample treatment, and even general chromatographic conditions. On the same page, the compounds reported in Egypt, Brazil, Israel, and India were detected using mainly GC-MS, while Italy, Pakistan, Portugal, Romania, and South Africa reported compounds mainly identified using LC-MS. In alcoholic extracts, more compounds were identified using GC-MS than LC-MS \((131 vs 94)\). However, the degree of agreement is quite low. For example, in alcoholic extracts comparing GC-MS and LC-MS, only 17 molecules have been commonly detected: arachidic acid, caffeic acid, catechin, chlorogenic acid, ellagic acid, epicatechin, eugenol, ferulic acid, gallic acid, linalool, oleic acid, palmitic acid, \(\alpha\)-coumaric acid, protocatechuic acid, stearic acid, vanillic acid, and \(\alpha\)-linolenic acid.

In the hydro-alcoholic extract, as should be expected, the majority of identified compounds \((n = 60)\) were identified using LC-MS. Interestingly, between hydro-alcoholic and alcoholic LC-MS identifications, we found 28 molecules in common: apigenin, caffeic acid, caftaric acid, chioric acid \((2,3\)-dicafeoyl-1-tartaric acid\), chlorogenic acid, cirsineol, cirs-

maritin, epicatechin, eugenol, gallic acid, gentisic acid, kaempferol, linalool, linoleic acid, lithospermic acid A, luteolin, \(\alpha\)-coumaric acid, protocatechuic acid, quercetin, quercetin 3-O-glucoside, rosmarinic acid, rutin, sinapic acid, stearic acid, ursolic acid, vanillic acid, and vicenin-2.

4. CONCLUSIONS

This study presents a systematic review of the principal findings of chemical composition and different extraction methods in basil species, excluding the studies that were performed \textit{ex vivo} and \textit{in vivo} with animal models, EOs extracts, and nanoparticles. A total of 356 articles were retrieved from the PubMed database and further analysis.

The first interesting finding we obtained was that 189 articles do not mention chemical composition; most of these articles mention the total antioxidant capacity or total phenolic content but not the identification of molecules. These types of articles, in which no tentative identification is made of the main compounds, do not permit chemical analysis related to species or even possible bioactive relationships. Although both techniques (GC-MS and LC-MS) lead to major compound identification, the methodology used in these articles requires further reviewing because we found a very low agreement between the compounds identified using the same extraction procedure. Moreover, in all the articles with chemical composition analysis, we found only 12 \textit{Ocimum} species. Of these species, \textit{O. basilicum} and \textit{O. tenuiflorum} comprise the majority of the published articles, indicating that a wide spectrum of basil species is mostly unknown.

We extracted more than 300 molecules across the 79 processed articles, but many of these molecules were not systematically found across the same species and similar experimental methods. Even so, one can notice a wide variety of chemicals: flavonoids, phenolic acids, terpenoids, anthocyanins, and others. These varieties of molecules, some of them with well-known biological activity, should be a driving force to continue the study of chemical constituent in this genus. These plants can be a source of useful bioactive molecules, as proven by the high number of articles \((189 in our review) related almost exclusively to the biological effect of their extracts.\cite{29,30}

Recent works, also reviews but across different genera, have focused on relating the identified molecules with their possible biological functions.\cite{31–34} In our case, even when occasionally discussed, the focus was to explore the chemical space, species identification, and consistency across experimental methods. The presented list of molecules could be valuable for further computational as well as experimental assays focused on the identification of specific compound–activity response. In this sense, in order to improve the reproducibility and even the causes of the chemical variation of these plants, we need to include other variables like plant maturity \((or at least morphological information)\), combine different analytical methods \((i.e.,\) liquid and gas chromatography\), and simultaneously study a wide number of species under similar or identical sample preparation, chromatographic, and mass spectrometry conditions.

5. FUTURE PERSPECTIVES

Our study focused on specific extraction procedures. We rejected all articles related to essential oil characterization. However, several molecules detected in alcoholic extracts are probably present also in essential oils. A similar analysis needs
to be carried out for EO extraction and also across different species. We also recommend expanding further the review, exploring different sample preparation, chromatographic, and mass spectrometry conditions in order to better explain the low agreement in identification across different studies, even with similar extraction protocols.

Our findings also indicate that in the Ocimum genus, an extensive chemical composition study has been only explored in a few species, especially O. basilicum and O. tenoriflorum. Considering the wide variety of compounds with potential bioactive effects we found, it is important to expand our experimental studies into a wide variety of species, taking into consideration the geographical location and the plants’ developmental stage as well as the combination of different chromatographic approaches. Finally, the provided list of molecules identified in our review could be used for exploring (or validating) biological functions by using experimental protocols or computational modeling.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c00043.

Supplementary Table S1. Molecules reported in Ocimum species with the corresponding SMILES representation (XLSX)

**AUTHOR INFORMATION**

**Corresponding Authors**

José M. Álvarez-Suárez — Departamento de Ingeniería en Alimentos. Colegio de Ciencias e Ingenierías, Universidad San Francisco de Quito, Quito 170901, Ecuador; orcid.org/0000-0001-8509-1498; Email: jalvarez@usfq.edu.ec

Eduardo Tejera — Grupo de Bioquímioinformática, Universidad de Las Américas, Quito 170513, Ecuador; orcid.org/0000-0002-1377-0413; Email: eduardo.tejera@udla.edu.ec

**Authors**

Andrea Beltrán-Noboa — Grupo de Bioquímioinformática, Universidad de Las Américas, Quito 170513, Ecuador; orcid.org/0000-0002-3444-9088

Mabel Guevara-Terán — Grupo de Bioquímioinformática, Universidad de Las Américas, Quito 170513, Ecuador; orcid.org/0000-0002-4890-4899

Blanca Gallo — Departamento de Química Analítica, Facultad de Ciencia y Tecnología, Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV/EHU), 48940 Leioa, Biscay, Spain

Luis A. Berrueta — Departamento de Química Analítica, Facultad de Ciencia y Tecnología, Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV/EHU), 48940 Leioa, Biscay, Spain

Francesca Giampieri — Research Group on Food, Nutritional Biochemistry and Health, Universidad Europea del Atlántico, 39011 Santander, Cantabria, Spain

Maurizio Battino — Department of Clinical Sciences, Università Politecnica delle Marche, 60121 Ancona, Italy; International Research Center for Food Nutrition and Safety, Jiangsu University, 212013 Zhenjiang, China; orcid.org/0000-0002-7250-1782

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c00043

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We thank the Universidad de Las Américas, Quito, Ecuador, for the financial support of the PhD students A. Beltrán-Noboa and M. Guevara-Terán. We also want to thank Universidad de Las Américas for supporting A. Jordan-Alvarez and E. Tejera and Y. Castillo, and Universidad San Francisco de Quito, Ecuador, for supporting J.M. Alvarez-Suarez.

**REFERENCES**


