Acacia rigidula versus other *Acacia* taxa: An alarming issue in the European novel food regulation and food supplement industry

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Abstract: Based on the signals recorded in the RASFF (Rapid Alert System for Food and Feed), *Acacia rigidula* is a repeatedly emerging unauthorized ingredient in weight loss dietary supplements in the European Union. Although the fruit, bark and gum of *Acacia nilotica* can be marketed as food supplement, and the gum of *Acacia senegal* as food ingredient, *A. rigidula* is an unauthorized novel food in the European Union. Here we present the first systematic overview of the phytochemical and pharmacological data reported on safety and efficacy of *A. rigidula*.

Keywords: *Acacia rigidula, counterfeit, RASFF, food supplements, safety*

1. Introduction

Acacia rigidula Benth. (blackbrush acacia) is a common constituent of illegal food supplements marketed as slimming agents. According to the records of the RASFF (Rapid Alert System for Food and Feed) portal, the presence of this plant was reported in several dietetic foods, food supplements, and fortified foods used to promote weight loss (1,2). However, there is little or no published clinical data about the potential biological effects of the plant or its products, and *A. rigidula* leaves have no documented history of use as food or as traditional herbal medicine (3). Acacia gum (the product of *Acacia senegal*) was on the market as a food or food ingredient and consumed to a significant degree before 15 May 1997, thus its access to the market is not subject to the Novel Food Regulation (EU) 2015/2283. Acacia gum may be the product of *Acacia nilotica* (syn.: *Acacia arabica*) as well, and this material can be marketed only as food supplement (4). However, certain species, such as *A. rigidula* are still not authorized as novel food ingredient (5,6).

Except acacia gum, relatively little is known about the chemical composition of the *Acacia* species (7); however, the presence of amines and alkaloids in *A. rigidula* is a warning sign regarding its safety. Our aim was to perform a comprehensive literature search in scientific databases and on the basis of the available data to assess the safety and potential efficacy of this plant and to present the scientifically valid information on this taxon.

Identification of *Acacia* species is difficult and their taxonomic relationships and nomenclature need clarification (8). It is now apparent that the name *Acacia amentacea* and *Vachellia rigidula* has been incorrectly applied for *A. rigidula* (9). Bentham (10,11) and Standley (12) used the name *A. amentacea* and listed *A. rigidula* as a synonym. Turner (13) considered both, *A. amentacea* and *A. rigidula* as distinct species. Hence, *A. amentacea* is an accepted name (14) syn. for *Vachellia rigidula*, and also synonym of *Acaciopsis amentacea* (14,15) in subgenus *Acacia.* As the genus *Acacia* is relatively large, and there are some inconsistencies in the appellation of the examined taxa, it is important to acknowledge these appellation parameters and separate *Acacia* taxons properly in the future.

2. Materials and methods

A comprehensive literature search was performed in several databases on *A. rigidula* and its synonyms, on PubMed/Medline, the Cochrane Library, ClinicalKey and Google Scholar, and Clingov. Data yielded from Scifinder and Web of Science were also reviewed. Literature search was carried out using the following search key: *Acacia rigidula*

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Table I *Unauthorized Acacia rigidula products.*

Year	Number of reports	Countries
1988–2015	0	-
2016	5	Netherlands, Poland, United Kingdom, Germany
2017	23	Poland, Lithuania, France, Malta, Spain, Belgium, Austria, Switzerland, Ireland, Sweden
2018–2019	0	-

In 2016 there were 5 reports concerning the Netherlands, Poland, United Kingdom and Germany. In 2017 the number of the reports quadrupled to 23 records, concerning Poland, Lithuania, France, Malta, Spain, Belgium, Austria, Switzerland, Ireland and Sweden.

OR *Acaciopsis rigidula* OR *Vachellia rigidula*. We systemically overviewed the available literature on the traditional use, novel food status, weight loss effects, phytochemistry and clinical investigations of *A. rigidula*. We also summed up and overviewed the reported signals on counterfeited food supplements containing *A. rigidula*, based on our previous research of the RASFF portal.

3. Results

In contrast to other *Acacia* taxons, the potential biological effects of *A. rigidula* have not been elucidated in detail, and the history of its use as food or traditional medicinal plant has not been documented (3). Furthermore, there were no clinically relevant results on the safe use of the extracts of *A. rigidula* and its use in weight loss products. The data presented below are important pieces of the puzzle of this plant, however, provide insufficient evidence for its safe use as food.

3.1. *Acacia* species and the novel food regulation

A. senegal is a small deciduous tree native to the semi-desert and north-western regions of Africa. *A. senegal* is the primary source of acacia gum (gum arabic), known locally as hashab gum (4,16).

In the European Union gum arabic derived from the trunk and branches of *A. senegal* and closely related species like *Acacia seyal* are defined in the additives legislation (17) as food ingredient. Any other food uses of products derived from *A. senegal* require authorisation under the Novel Food Regulation. However, *A. seyal* is not listed in the novel food category list. According to information available to the Member States' Competent Authorities, fruit, bark, gum yielded from *A. nilotica* were used only as or in food supplements before 15 May 1997, hence only these three plant parts of *A. nilotica* are authorized. Any other food uses of this product have to be authorised pursuant to the Novel Food Regulation.

A. rigidula has no documented history of use as food or traditional herbal medicine. There was a request whether *A. rigidula* products require authorisation under the Novel Food Regulation or not. According to the information available to European Union Member States' Competent Authorities, this product was not used as a food or food ingredient before 15 May 1997. Therefore, before it may be placed on the market in the EU as a food or food ingredient, a safety assessment under the Novel Food Regulation is required (4). Since the safety of this plant has not been confirmed, at the moment it is an unapproved novel food ingredient in the European Union.

3.2. *Acacia rigidula* in illegal products

In the period between 1988–2019 *A. rigidula* was reported 28 times in the RASFF, and it was one of the most frequently used unapproved natural agent in weight loss products (2). The presence of *A. rigidula* was first reported in 2016 in the Netherlands, but later also in Belgium, Austria, France, Malta, Spain and other European countries. Overall 28 records can be found in the RASFF on the "unauthorized novel food ingredient Acacia rigidula", with no specification of the plant part used (gum, leaf etc.) (Table I and Figure 1). Unauthorized *A. rigidula* emerged overall 6 times in combination with other unauthorized. By the risk decision process conducted 2016, *A. rigidula* was considered to be a serious risk only in 2 cases, where other compounds like synephrine and oxilofrine were also present in the products. The rest of the notifications with *A. rigidula* were undecided or not serious. One of the serious reports resulted in market withdrawal. There were no available data referring to a mislabelling problem in the RASFF.

Zhao et al. (18) shed light to the problem of mislabelling. In their study, thirty-two dietary supplements were investigated and purchased from online retailers in September 2015. The selection of the commercial supplements for investigation was based on

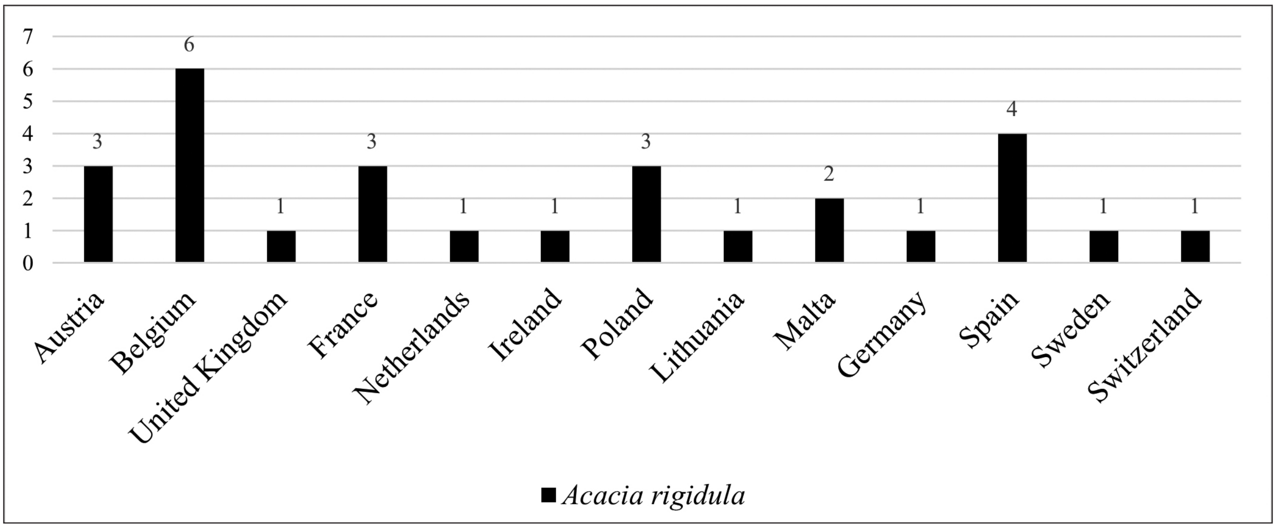

Figure 1 *Distribution of notifications of food supplements containing Acacia rigidula in the RASFF*

their label information that contain at least one of the following concepts or claims: 'weight loss', 'metabolic rate booster', 'myotrophic agent', 'appetite control/regulation', 'lipogenic', 'lipotrophic', 'thermogenic', 'fat burn', 'burn calories', 'gain strength/intensity', 'stimulant', 'energy aid/booster', 'mental focus', 'positive/uplifting mood', 'ephedra free', or 'Acacia'. The products were capsules, powders or tablets. Out of the 32 investigated products, 9 products listed *A. rigidula* plant materials in their ingredient lists. In case of 12 of the 16 products in which phenethylamine was detected, *A. rigidula* was not listed as an ingredient on their label information, which questions the real ingredients of the examined products.

3.3 Phytochemistry of *Acacia* sensu lato

Although the genus *Acacia* is quite large and is widespread in the warm subarid and arid parts of the world, relatively little is known about the chemistry of most species except acacia gum (7).

According to preliminary chemical screening studies, members of the genus *Acacia* sensu lato contain amines, simple alkaloids, cyanogenic glycosides, cyclitols, essential oils, diterpenes, fatty acids from seed oils, polysaccharides, non-protein amino acids, triterpenes, phytosterols, saponins, flavonoids, and both hydrolysable and condensed tannins. In general, this genus (as well as other mimosoid legumes) appears to lack acetylenes, anthraquinones, coumarins, glucosinolates, lignans, naphthoquinones, phenylpropanoids, stilbenes and unusual fatty acids. However, few species have been examined specifically for these substances (7).

3.3.1 Gum

From a chemical point of view, the original acacia gum (mainly from *A. senegal*/*A. seyal*) contains polysaccharides based on a galactan main chain carrying heavily branched galactose/arabinose side-chains. Rhamnose and/or glucuronic acid may be present as side-chain terminations (6,19). The *A. seyal* complex includes *A. rigidula*, so it is suspected that *A. rigidula* may also be used to produce acacia gum (20). The available information on the gum of *A. rigidula* is limited.

Idris et al. (21) reported that common acacia gum comprises 39–42% galactose, 24–27% arabinose, 12–16% rhamnose, 15–16% glucuronic acid, 1.5–2.6% protein, 0.22–0.39% nitrogen, and 12.5–16.0% moisture. Small concentrations of tannins, around 0.4% (22), can be found in the gum resulting in slightly coloured products. Variability in tannins content was reported both for *A. senegal* (0.3-0.6%) or *A. seyal* (0.6-1.2%) gums (22). Others emphasized that tannins can be found in *Acacia* gums except that of *A. senegal* var. *senegal* (23). *Acacia* gums also contain enzymes such as oxidases and peroxidases, diastases and pectinases (24–26).

3.3.2. Amines and alkaloids

In a study focusing on the azotoids of *A. rigidula*, leaves and stems, in total, 44 amines and alkaloids, including 29 phenethylamine derivatives were identified (27). Four previously encountered amines in *A. berlandieri* (*N*-methyl-β-phenethylamine, tyramine, *N*-methyltyramine, and hordenine were found also in *A. rigidula rigidula* (28,29). The majority of the alkaloids detected in *A. rigidula* were related to the parent compound β-phenethylamine (27). These compounds generally varied in the degree of *N*-methylation (amphetamine family), and in oxygenation of the aromatic ring (tyramine, dopamine, and mescaline families). The 2-cyclohexylethylamine and the *N*-cyclohexylethyl-*N*-methylamine are the saturated analogues of the phenethylamine and *N*-methyl phenylethylamine, respectively. Tryptamine, *N*-methyltryptamine, and *N,N*-dimethyltryptamine were also reported from blackbrush (27). Tryptamine and *N,N*-dimethyltryptamine were also detected in the related species *A. berlandieri* (guajillo) (29). Overall, four amphetamine derivatives were also detected in *A. rigidula* (27).

Other noteworthy alkaloids found in blackbrush including mescaline, nicotine, nornicotine, and four tetrahydroisoquinoline alkaloids, anhalamine, anhalidine, anhalonidine, and peyophorine. The amides of the amino acids pipecolic acid and p-hydroxypipecolic acid were also detected from the plant (27). All of the above-mentioned amines and alkaloids were detected in the leaves and attached stems of *A. rigidula,* however, it should be noted that these compounds were detected by GC-MS and the presence of the majority of these compounds has not been confirmed by subsequent studies by others.

Amines and relatively simple alkaloids are found in most of the taxa of genus *Acacia* sensu lato (30). The seeds of neotropical species of subgenus *Aculeiferum* section *Monacanthea* is especially abundant in these compounds, as well as the African species *A. brevispica*, *A. caesia*, *A. kraussiana*, *A. schweinfurthii*, and *A. pentagona*, which lack most of the non-protein amino acids found in other members of the subgenus. These six mentioned *Acacia* species often contain *N*-methyltyramine in their seeds, which is a biologically active amine (31).

3.3.3. Cyanogenic glycosides

Many species of *Acacia* contain cyanogenic glycosides, substances that can release hydrogen cyanide if the plants are damaged (30). The cyanogenic glycosides of subgenus *Acacia* are a series of related aliphatic compounds (linamarin, lotaustralin, proacacipetalin, epiproacacipetalin, heterodendrin, proacaciberin, and 3-hydroxyheterodendrin) (7,32).

Linamarin and lotaustralin are the major cyanogenic glycosides of *A. farnesiana*. The amount of cyanide produced by plants within one population of the taxon varies from below the level of detection with picrate paper to approximately 5 μmol per g (1.4%) dried leaves (33). Maslin et al. (34) identified proacacipetalin by ^{1}H nuclear magnetic resonance spectroscopy (NMR) from the isolates of the leaf of *A. pachyphloia* and *A. sutherlandii* are Australian members of subgenus *Acacia,* both considered to contain proacacipetalin (32). The cyanogenic glycosides of *A. globulifera* proved to proacacipetalin and epiproacacipetalin (35).

According to Jaroszewski et al. (36) the co-occurrence of heterodendrin and proacacipetalin in *Acacia* may be quite general. These glucosides were also identified by NMR spectroscopy, from the leaves of *A. hebeclada* and/or *A. giraffae.*

Fractionation of the ethanolic extract of the immature pods freed from the non-cyanogenic seeds of *A. sieberana* var. *woodii* resulted in the isolation of 3-hydroxyheterodendrin (37). Chromatography of pod extracts of the same plant yielded another new compound, proacaciberin (38). According to Seigler et al. (39) *Acacia rigidula* was not reported to be cyanogenic and no cyanogenic glycoside was reported by another in a further experiment (9).

3.3.4. Terpenes

The composition of flower essential oils of *A. rigidula* (subgenus *Acacia*) and *A. berlandieri* (subgenus *Aculeiferum*) was examined (30). The major components of the essential oil of *A. rigidula* were *p*-anisaldehyde, jasmone, kaur-16-ene, cis-3-hexenyl benzoate, methyl 2,6-dihydroxybenzoate and citronellyl acetate. Those of *A. berlandieri* were linalool oxide B, 1-octanol, eugenol, and benzyl benzoate (40).

The essential oils of the flowers of *A. farnesiana* (cassie ancienne or sweet acacia) have long been used in perfumery (41). *A. farnesiana* flowers contains methyl salicylate (47.5%), anisaldehyde (17.3%), geraniol (9.8%), benzaldehyde (6%), geranyl acetate (3.3%), geranial (2.8%), 3-methyldec-3-en-1-ol (1.9%), (Z)-3-nonen-1-ol (0.7%), β-ionone (0.7%), myrcene (0.5%), 3-methyldec-4-en-1-ol (0.5%), benzylalcohol (0.5%), linalool (0.4%), α-ionone (0.4%), and a number of other volatile components (40).

3.3.5. Fatty acids from seed oils

Most species e.g. *A. farnesiana* have 3–10% oil in the seeds (30). Oleic and linoleic acids predominate in the seed oil triglycerides of most *Acacia* species, although a some species (e.g. *A. caven*, *A. farnesiana*, *A. lenticularis*, *A. macrothyrsa*, *A. tortilis*) contain relatively large (> 50% in the oil composition) percentage of linolenic acid (42). The remaining portion of most oils is comprised of palmitic and stearic acid.

3.3.6. Flavonoids

Many flavonol and flavone glycosides, aglycones, flavan-3-ols, and flavan-3,4-diols are found in the leaves, barks, and heartwoods of *Acacia* species (30). These flavonoids often lack the 5-hydroxy group, which is characteristic to the family *Legumosidae*. Generally, barks contain much more complex flavonoid mixtures than heartwoods (43). Cavazos et al. (44) confirmed the presence of flavonoids in the leaves of *A. berlandieri* and *A. rigidula* via qualitative phyto-chemical tests supported by NMR, ultraviolet–visible spectroscopy (UV–Vis) and infrared spectroscopy (IR).

3.3.7. Hydrolysable tannins and condensed tannins (proanthocyanidins)

Hydrolysable tannins, like 1,3-di-*O*-galloyl-4,6-(–)-hexahydroxydiphenoyl-β-glucopyranose, 1-*O*-galloyl-β-glucosylpyranose, 1,6-di-*O*-galloyl-β-glucosylpyranose and 1,3,6-tri-*O*-galloyl-β-glucosylpyranose are found in the leaf material of a number of *Acacia* species of subgenera *Acacia* and *Aculeiferum* (30,45–47). Structures of hydrolyzable tannins have been reported from *A. raddiana* (46). Based on the potassium iodate method for gallotannins, the leaves, and to a lesser extent the bark, of many species contains 1–8% hydrolysable tannins. Bark and leaves of *A. rigidula* are relatively rich in tannins (3-10%) (47).

Proanthocyanidins from *A. mearnsii* (black wattle) constitute an important commercial product (48). Six proanthocyanidin dimers were isolated from the steamed bark of *A. mearnsii* (fisetinidol-(4β-8)-catechin, fisetinidol-(4α-8)-catechin, robinetinidol-(4β-8)-catechin, robinetinidol-(4α-8)-catechin, robinetinidol-(4β-8)-gallocatechin, and robinetinidol-(4α -8)-gallocatechin (49).

The leaves of *A. berlandieri* and *A. rigidula* contain high levels of condensed tannins (50). In a phytochemical study, blackbrush acacia contained the highest amount of tannins, compared to *Prosopis glandulosa* and *Celtis pallida* (51).

3.3.8. Other compounds

A. rigidula and *A. berlandieri* are used during the dry season as an important feed, since these plants are rich in proteins, energy content, vitamins and minerals (50). Fluoroacetate is a relatively common compound in a number of Australian and South African plants; however, it has been described from only one *Acacia* species, *A. georginae*. The foliage (52) and the seeds (53) of this plant are highly toxic to livestock and people (54).

3.4. Biological activities

3.4.1. Weight loss effect

Extracts of *A. rigidula* leaves and other plant parts are used in weight loss products with no evidence regarding their efficacy or potential mechanisms of action (3). The slimming effect of *A. rigidula* is partly attributed to the amphetamine-derivatives of the plant (27).

Jacobs (55) investigated the acute weight loss effects of a commercially available weight loss product on measures of metabolic and hemodynamic activity (heart rate and blood pressure) in comparison with the effects of caffeine or *A. rigidula*. According to the label, the product contained 'caffeine anhydrous 150 mg', '*Acacia rigidula* extract (leaves) yielding 200 mg phenylethylamine alkaloids, including: methylsynephrine, *N*-methylphenethylamine, *N,N*-dimethylphenethylamine, phenethylamine'; 'synephrine HCl', 'naringin', 'theobromine', 'green tea', '1,3-dimethylamylamine', '5-methoxytryptamine HCl', 'yohimbine HCl'. Apart from caffeine, naringin, green tea and melatonin, the remaining compounds are unauthorized in the European Union (4,56,57). In this small placebo-controlled study, ten recreationally active men (28.5 ± 5 years of age) completed four 3-hour resting metabolic testing sessions in which four treatment conditions, including the weight loss/energy product; 300 mg anhydrous caffeine and 250 mg *A. rigidula* extract (the utilized part was not represented), and cellulose as placebo were examined in randomized order. Physiological activity was determined in 15-minute intervals immediately before and 1, 2, and 3 hours after ingestion. Resting energy expenditure was significantly enhanced with the examined product, caffeine, and *A. rigidula* compared to placebo. Hemodynamic activity (heart rate and blood pressure) was significantly elevated with the examined product in contrast with a modest effects displayed with caffeine or *Acacia* (55).

3.4.2. Antioxidant effects

According to a study, *A. rigidula* leaf extracts can be a source of antioxidant agents. Antioxidant properties of *A. berlandieri* and A. *rigidula* extracts (5 mg/25 mL) were determined by the ferric thiocyanate method. The acetone and methanol extracts of *A. rigidula* exerted more pronounced antioxidant activities that those of *A. berlanieri* (44).

3.4.3. Antimicrobial effects

Ethanolic stem bark extract of *A. nilotica* exhibited antimicrobial activity against *Streptococcus viridans* (MIC value: 40 mg/mL), *Staphylococcus aureus* (MIC value: 40 mg/mL), *Escherichia coli* (MIC value: 45 mg/mL), *Bacillus subtilis* (MIC value: 35 mg/mL), and *Shigella sonnei* (MIC value: 50 mg/mL) (58).

The extract of the whole plant of *A. rigidula* had antifungal activity against 7 strains with minimum inhibitory concentration (MIC) values of 0.93-3.75μg/mL) (59).

In a study, where A. *rigidula* and *A. berlandieri* were studied, the MIC values against *P. alcalifaciens, S. aureus, Y. enterocolitica*, and *E. faecalis* ranged from 37.5 to 75 mg/mL and 37.5 to 150 mg/mL, respectively. Acetone extracts were more active than methanol extracts (44).

3.5. Side effects and toxicity

Phenolic amine derivatives contribute to the toxicity of *A. rigidula* (60). A significant increase in the amount of these compounds was observed in late season foliage (27). Consumption of blackbrush and a related species guajillo (*A. berlandieri*) has been associated with a locomotor ataxia known as limber leg (disease in sheep involving incoordination) by animals (61). A toxic sympathomimetic amine, *N*-methyl-β-phenethylamine (NMPEA), was isolated and identified as the toxic compound responsible for the effects mentioned above (28).

Tannins are commonly occurring plant metabolites in food and feed; however, condensed tannins *in A. angustissima,* cultivated in Africa and in Australia cause toxic reactions in sheep (62).

There was a case report where A 24-year-old man developed hepatotoxicity 1 week following the discontinuation of four food supplements bought over the internet. Three food supplements included a mixture of amino acids and vitamin D, whey proteins and multivitamins; and the fourth included *A. rigidula*, *Camellia sinensis*, white willow bark, grape seed extract, cactus extract, guarana, *Capsicum annuum*, ginseng root and *Cissus quadrangularis*. According to the authors of the report, green tea was the only plant material linked with hepatotoxicity from a literature search, but based on the (deficient) former history of *A. rigidula* it would be useful to investigate and collect the effects of *A. rigidula* in case reports (63).

4. Discussion

The consumption of *A. rigidula* is potentially dangerous because it contains appreciable amounts of toxic nitrogen-containing compounds. Ingestion of the plant can lead to locomotor ataxia partly due to the presence *N*-methyl-***β***-phenethylamine (27). There are no reliable data on the distribution and amounts of toxic compounds in different plant parts or products; moreover, there are no experimental data to support the safety of this plant. The presence of potentially toxic compounds (e.g. cyanogenic glycosides) has not been investigated extensively

Extracts of *A. rigidula* leaves and unknown parts of the plant are used in weight loss products without any clinical evidence, and this plant has no documented history of use as food or traditional herbal treatment (3). *A. rigidula* is an unauthorized novel food in the European Union.

A. rigidula were reported 28 times in the RASFF, and it was one of the most frequently used natural agent for weight loss during the period of 1988–2019 (2). In more than one cases, it was used in combination, which also can be a risk factor, considering dangerous interactions.

5. Conclusions

The taxonomic relationships and nomenclature of the *Acacia* genus are still under debate. A proper appellation would be the basis for the quality control and monitoring of food supplements containing *Acacia* sp.. These issues should be closely monitored, as mislabelling is also a hidden issue in case of *A. rigidula* products (18).

During the last years, the number of prohibited food supplements because of the presence of *A. rigidula* has increased. The safety of this plant is questionable due to the lack of scientific data or empirical evidence.

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