

Article

Extraction and Identification of Effective Compounds from Natural Plants

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Abstract: Most botanical species contain various types of bioactive compounds. This study focusses on the extraction and identification of bioactive compounds from *Calicotome spinosa* (Gorse), including flavones, α -linolenic acid and sugar. During the investigation of gorse flowers, leaves and bark, flavones were isolated from the bark and leaves. *Calicotome spinosa* showed a total isoflavonoid content of 1.5% from the bark of gorse and 1.3% from the leaves. To find the best conditions for flavone extraction, samples of *Calicotome spinosa* were extracted with different solvents (methanol, water and acetonitrile). Methanol was found to be a suitable solvent to selectively extract flavone. An unsaturated cis fatty acid (α -linolenic acid, C18:3 Δ 9, 12, 15) was identified as the principal component of the triacylglycerol fraction from the flowers. Hydrolyses process conditions were used to study Gorse wood. The results indicated that the wood of gorse is not a suitable substance for making paper. The extracted bioactive compounds were analysed using NMR, GCMS, UV, TLC and Fibre Analyser techniques. The extracted compounds offered uses as antioxidants and agricultural chemicals in addition to other benefits.

Keywords: gorse; α -linolenic; flavones



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1. Introduction

Humans and animals have depended on plants for more than just food. Plants have provided shelter and medicine. Some of the most famous drugs that have had a powerful impact on the health of people were initially obtained from plants. For instance, Quinine saved millions from death caused by malaria. It was initially obtained by boiling the bark of the Cinchona tree. Another example is aspirin, which is a derivative of salicylic acid that was initially extracted from the bark of the willow bush. Aspirin is used as an analgesic, antipyretic, anti-inflammatory and anti-coagulant. It is even being investigated as an anti-cancer agent [1]. In the modern era, plants have become the source of new materials and chemicals. The usefulness of plants today goes beyond herbal remedies. Latex was used to make plastic materials before the introduction of synthetic polymers. Wool was used to weave textiles and clothing materials. Most of the useful plant-derived compounds are classed as secondary metabolites. These are small molecular weight organic compounds which do not play a major role in the normal homeostatic metabolism of the plant. They are used by the plant for defence or survival [2] Most chemical plant metabolites are trapped in the cell by the tough cellulose cell wall. Various methods have been developed to extract them. The simplest of these, and probably the oldest, is aqueous extraction.

The plant is either immersed in cold water for an extended period of time or boiled for a brief period. A crude mixture of many plant secondary metabolites is obtained. Another common extraction method involves the use of alcoholic spirits. Sort-chain alcohols or ethers such as methanol, ethanol or diethyl ether are often used. For more hydrophobic plant components, aliphatic hydrocarbon solvents such as hexane or petroleum ether are used. These organic solvents have the advantage of being easily distilled off to leave a concentrated crude product.

Gorse is an important plant that contains many bioactive products; it is a leguminous plant. The botanical binomial nomenclature is *Ulex europaeus*. Like other legumes, it belongs to the taxonomical family Fabaceae. This family is also sometimes referred to as Leguminosae [3,4]. Although there are several species of the *Ulex* genus, the name gorse normally refers to three species. These are *U. europaeus*, also known as the common gorse, *U. gallii*, the Western gorse or Furze, and *U. minor*, the Dwarf gorse or Dwarf Furze.

Legumes have been of very important economic value to humans. The family consists of 19,325 species in 727 genera [5]. Common food plants such as peas, beans, lentils, soy and peanuts are well known members of this family. They are the main source of plant proteins and are sometimes referred to as the 'poor man's meat' [6]. Another legume, alfalfa (*Medicago sativa*), is cultivated mainly for feeding livestock.

Probably the most significant role of legumes is their ability to enrich soil by fixing nitrogen. The root nodules of most leguminous plants contain symbiotic bacteria that transform inert atmospheric nitrogen gas into ammonia. The plants can then use this in the synthesis of amino acids [7].

U. europaeus is an evergreen shrub that grows to a height of between 60 cm and 2 m. It has a thick thorny stem which is also green throughout the year. The spines are typically about 1 to 3 cm long. Its leaves are non-circular and also needle sharp. They are actually shorter than the spines that grow on the stem. The flowers of *U. europaeus* are between 1.5 to 2.5 cm long, much larger than the other two gorse plants, *U. minor* and *U. gallii*. These flowers have a scent much like the smell of coconut. *U. europaeus* produces a non-edible and black fruit (Figure 1).



Figure 1. A gorse plant in full blossom.

Ulex plants are believed to have originated in the Iberian Peninsula, along the Mediterranean Coast [8]. Today, they are found in about 15 countries with similarly moist and cool maritime climatic conditions to its ancestral origins. It is widely dispersed, in the Nordic states, North America, Latin and South America, North Africa, Australia and New Zealand [9–12]. Gorse grows well in nutrient-poor soils such as roadsides and over-grazed pastures [13]. Soils with a depth of 20–50 cm and enriched with phosphorus promote its growth [14]. A high soil calcium content was reported to retard growth by Richardson and

Hill [15]. A soil pH of 4.5–5.0 is required for optimal growth. The plant acidifies the soil by harvesting litter and depleting the calcium in the soil [16,17].

There are several reports describing the pros and cons of the Gorse Plant. For example, Gorse is legally classed as a noxious weed [18]. Wherever it takes root, Gorse suppresses the growth of native trees [19]. It does this by either acidifying the soil or growing in large and dense thickets. Clearing these bushes can be financially costly [20]. The dense growth is a major fire hazard, especially because the branches have a high content of oil [21]. The sharp spines prevent penetration. Thus, they restrict the movement of animals. The gorse plant has also been put to good use. As a legume, it is able to fix nitrogen in the soil. It has been used to regenerate nutrient-depleted forests or abandoned old mines [22,23]. A major use of gorse has been as hedgerows. Because of its high oil content, it is also used as firewood. The gorse flower pollen is a major source of honey production [24]. In a study by Moar in 1985, pollens from gorse were found to make up as much as 25% of all the pollens in a sample of honey [25].

The *Ulex* plants were initially introduced into some regions as forage for livestock [26]. The animals showed a preference for the shrub when it is not fully mature and the spines are still short and soft [27]. There is dispute as to its use as fodder. In a study of 11 shrubs as potential livestock feed, wild gorse was among the best three [28]. However, it is not clear how digestible it is [29]. Krause et al. argued that allowing gorse to grow on a pasture for feeding purposes hinders the ability of cattle and sheep to graze [30]. On the other hand, much of the research into gorse has been agricultural, looking into controlling the weed [26]. The use of gorse as a biomass source has also been investigated [31]. It produces more biomass than other plants growing on unfertilised land. Nunez-Regueira et al. studied gorse as a source of renewable energy. They investigated the variation in the calorie content of the gorse plant in different seasons [32] by measuring the high and low heating values of the plant along with its flammability. The high heating value was reported to be approximately 21,000 kJ/kg, while the low heating value was about 6000 kJ/kg. There was no significant seasonal variation. The flammability, however, was highest in the summer. Compared to other sources of biomass, gorse was the least flammable.

Gorse has also been investigated as a source of useful biochemical and medical products. Legumes are the main source of isoflavonoids. Isoflavonoids are often pigmented groups of compounds with an aromatic ring nucleus. They were first isolated from the roots of the legume *Ononis spinosa* [33]. This first compound was called ononin, and over 1600 have been isolated to date. Those that show biological activities are sometimes referred to as bioflavonoids. They are water soluble and are antioxidants. In the relationship between plants and microorganisms, isoflavonoids play a role as signal molecules for both beneficial and pathogenic microorganisms. They induce genes that initiate the formation of nodules by the *Rhizobium* bacteria [34]. The resulting symbiotic relationship allows the leguminous plant to fix atmospheric nitrogen. The isoflavonoids daidzein and genistein help the soybean pathogen *Phytophthora sojae* locate its host target [35].

Isoflavonoids are also widely used in medicine. They have both steroidal and anti-cancer activities [36,37]. When given in the diet of experimental rats, genistein provided prophylaxis against mammary cancer [38]. Quercetin has a high antioxidant activity that is capable of protecting against cardiovascular illness. The *Ulex* plants are particularly rich in isoflavones and pterocarpanes. Several have been isolated from these plants, and some have insecticidal and cytotoxic effects [39,40]. The extraction of the isoflavonoids from the *Ulex* plants is relatively easy. The isoflavonoids in the figure above were extracted from the dried, fine powder of the plant shoot with separate petroleum ether and dichloromethane solvents at room temperature. The purification of the crude extract was carried out by normal silica column chromatography eluting with hexane-ethyl acetate solvent systems.

This manuscript focuses on the development of new methods for extracting natural products from plants such as Gorse, which could be used in composite processing, such as cellulose fibre extraction. To date, almost no work has been done in this respect on this plant. Moreover, the extraction of effective molecules involved a simple and economically

inexpensive method from which analytical methodologies could be developed to extract and identify effective compounds from natural plants [40].

The aim of this work is to find an alternative use, allowing for environmentally friendly control, to: (1) extract the active compounds from all parts of the gorse, (2) identify the compounds isolated from the gorse and, finally, (3) test the chemical extracts from the gorse for biological activity such as bacterial inhibition.

2. Experimental

2.1. General

All of the chemicals used were purchased from Alfa Aesar. Crude products were dried over anhydrous magnesium sulphate. They were purified via column chromatography using silica gel from Aldrich (particle size 30–70 μm). Solvents in the crude products were removed via vacuum evaporation at 14 mm Hg.

Thin layer chromatography was conducted using Merck silica gel 60 F254 plates. Separated components were detected using GCMS, UV light or I_2 .

NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer in CDCl_3 or CD_3OD if not differently indicated. All chemical shifts in CDCl_3 are quoted in δ relative to the resonance of the solvent for ^1H -NMR (δ 7.27 ppm) and for ^{13}C -NMR (δ 77.0 ppm). For CD_3OD , these were δ 3.39 and δ 49.3, respectively. Infra-red spectra were obtained on a Perkin–Elmer 1600 series FT-IR spectrometer as liquid films, and UV were obtained on a Perkin–Elmer.

2.2. Experimental

General: Preparation of gorse (*Ulex*) flower for extraction.

The ripe flowers of gorse (*Ulex*) were collected and dried at 50 °C for 48 h to remove the moisture. The flower was milled using a hammer mill, and the milled flower was then transferred to dry plastic containers to minimise exposure to atmospheric moisture until use.

2.2.1. Soxhlet Extraction of Dried Prepared Gorse Flower with dichloromethane

The gorse flower milled (9.23 g) was added to a cellulose extraction thimble and extracted in a Soxhlet apparatus using dichloromethane (300 mL) at 75 °C for 24 h. Some yellow coloration was firstly observed in the extraction, at which point the solvent was running clear. The solvent was removed in vacuo to recover a yellow oily residue (0.9 g, 10%) of triglyceride fatty acid, which showed δ_{H} (500 MHz, CDCl_3): 7.27 (1 H, s), 5.43–5.29 (12 H, m), 5.29–5.25 (1 H, m), 4.30 (2 H, dd, J 12.0, 4.3), 4.15 (2 H, dd, J 11.9, 5.9), 2.81 (7 H, dd, J 8.1, 4.2), 2.32 (7 H, td, J 7.6, 3.4), 2.11–2.03 (9 H, m), 1.62 (11 H, d, J 4.9), 0.98 (7 H, t, J 7.5), 0.89 (9 H, t, J 6.9). $\delta^{13}\text{C}$ (126 MHz, CDCl_3) 173.26, 172.80, 131.93, 130.20, 130.18, 128.28, 128.28, 128.22, 128.21, 127.75, 127.74, 127.09, 77.25, 77.00, 76.75, 68.89, 62.08, 34.18, 34.04, 34.01, 31.91, 29.68, 29.65, 29.59, 29.58, 29.46, 29.35, 29.26, 29.18, 29.16, 29.11, 29.07, 29.03, 27.19, 25.61, 25.52, 24.86, 24.82, 22.68, 21.91, 20.54, 14.25, 14.09.

2.2.2. Preparation of Methyl Ester from Crude of Gorse Flower from DCM Extraction

The crude triglyceride extract (0.12 g) was reacted in methanol (20 mL), and sulphuric acid (0.5 mL, 98%) was added to the mixture as a catalyst. The mixture was heated at reflux for 5 h and then quenched by the addition of an aqueous NaHCO_3 (100 mL) to stop the reaction. The aqueous layer was separated, and the combined organic layer was washed with aqueous NaHCO_3 (3×50 mL). The aqueous layer was drawn off, the organic layer was dried with magnesium sulphate and filtered and the solvent was removed in vacuo to give a green oily product of methyl ester fatty acid (0.04 g, 33%). This was identified by GC MS m/z , as shown in Section 2.2.1.

2.2.3. Soxhlet Extraction of Dried Prepared Gorse Flowers with Methanol

The milled gorse flower (8.80 g) was extracted in a Soxhlet apparatus using methanol (250 mL) at 80 °C for 48 h, at which point the solvent was running clear. Then, the solvent was removed in vacuo to recover a yellow, thick and oily residue (3.52 g, 40%). The oil was dissolved in acetone (150 mL) by refluxing at 40 °C for 4 h. The mixture was left to settle for 20 min. The solvent was filtrated through a Buchner funnel under reduced pressure, and the solid on the filter paper was washed with acetone (3 × 40 mL), transferred to a round bottle flask with acetone (150 mL) and removed in vacuo; this was repeated with the addition of further acetone (3 × 50 mL), the solvent removed each time in order to dry the solid. The solid was then ground to a fine powder and placed under a vacuum for 16 h in order to remove any remaining moisture, giving a green powder (3.5 g, 34%). ¹H-NMR analysis appears to indicate a sugars region as δ_{H} (500 MHz, MeOD): 5.36 (1 H, d, *J* 3.6 Hz), 5.09 (1 H, d, *J* 3.6 Hz), 4.88 (1 H, s), 4.83 (5 H, s), 4.46 (1 H, d, *J* 7.8 Hz), 4.06 (1 H, s), 4.00 (1 H, dd, *J* 17.9, 9.9 Hz), 3.90–3.52 (4 H, m), 3.45 (1 H, dd, *J* 10.0, 7.8 Hz), 3.38–3.20 (2 H, m), 3.17 (1 H, s), 3.14–3.08 (1 H, m). δ_{C} (126 MHz, MeOD) 103.10, 99.20, 98.10, 93.87, 84.78, 83.16, 77.98, 77.91, 76.20, 74.79, 74.16, 73.74, 73.66, 73.35, 72.93, 72.49, 71.90, 71.79, 71.76, 71.64, 71.13, 69.31, 65.76, 64.48, 64.07, 62.76, 62.68, 49.51, 49.34, 49.17, 49.01, 49.00, 48.83, 48.66, 48.49; ν_{max} (CH₃OH)/cm⁻¹: 3436, 2924, 2861, 1608, 1494, 1451, 1384, 1073, 824; UV λ_{max} nm, 261 br, 213 s, 206 s.

2.2.4. Small Scale Acid Hydrolysis of Flower Milled and Extracted with Methanol

Crude flower extract (1 g) was dissolved in 5% hydrochloric acid solution (50 mL) and left to reflux at 90 °C for 5 h. The solution was allowed to cool and filtrated through a Buchner funnel under reduced pressure and washed with water (3 × 30 mL). The solid was dissolved in the minimum amount of methanol and refluxed at 90 °C for 2 h. The mixture was then cooled down using an ice bath for 5 min. The solvent was evaporated to give brown solid that was collected to give (0.11 g, 11%), the ¹H-NMR δ_{H} (500 MHz, MeOD) showed no significant change for the sugar region.

2.2.5. Small Scale Acid Hydrolysis of Flower Milled and Extracted with Methanol

Crude flower extract (1 g) was dissolved in 10% sulphuric acid solution (40 mL) and left to reflux at 80 °C for 65 h. The solution was allowed to cool and was filtered through a Buchner funnel under reduced pressure and washed with water (3 × 30 mL), removing any remaining acid, and then dried using a Buchner funnel under reduced pressure for 2 h, at which point it was oven dried at 50 °C, giving a dark brown solid (0.21 g, 21%) and showing δ_{H} (500 MHz, MeOD): 5.36 (1 H, d, *J* 3.6 Hz), 5.09 (1 H, d, *J* 3.6 Hz), 4.88 (1 H, s), 4.83 (5 H, s), 4.46 (1 H, d, *J* 7.8 Hz), 4.06 (1 H, s), 4.00 (1 H, dd, *J* 17.9, 9.9 Hz), 3.90–3.52 (4 H, m), 3.45 (1 H, dd, *J* 10.0, 7.8 Hz), 3.38–3.20 (2 H, m), 3.17 (1 H, s), 3.14–3.08 (1 H, m); UV λ_{max} nm: 202.63 s, 362.37 br.

2.2.6. Small Scale Acid Hydrolysis of Milled Flowers Extracted with Methanol

Crude flower extract (0.24 g) was dissolved in 10% hydrochloric acid solution (40 mL) and left to reflex at 120 °C for 10 h. The solution was allowed to cool and was filtrated through a Buchner funnel under reduced pressure to give a brown solid that was washed with water (3 × 30 mL). The solid was firstly dried on the Buchner funnel under reduced pressure and then using an oven at 50 °C for 24 h, affording a brown powder product (0.11 g, 50%). δ_{H} (500 MHz, CDCl₃) δ_{H} (500 MHz, CD₃OD) 4.87 (5 H, s), 3.36 (1 H, s), 3.32 (5 H, dt, *J* 3.3, 1.6 Hz), 2.29 (1 H, t, *J* 7.4 Hz), 1.37–1.23 (1 H, m), 1.21 (1 H, d, *J* 2.0 Hz), 1.12 (1 H, d, *J* 2.3 Hz), 1.04–0.96 (1 H, m), 0.93–0.91 (1 H, m), 0.90 (1 H, d, *J* 3.0 Hz), 0.84 (1 H, s); UV λ_{max} nm: 202.63 s, 362.37 br.

2.2.7. Small Scale Base Hydrolysis of Milled Flower Extracted with Methanol

Crude flower extract (1 g) was added to a round bottom flask with a solution of sodium hydroxide (50 mL, 1 M) and heated to 75 °C under reflux for 4 h. The mixture

was allowed to cool and was transferred to a separating funnel, and a solution of sodium hydroxide (10 mL, 1 M) was used to wash the flask. The solution was acidified to pH 7 with 10% sulphuric acid solution to give a green precipitate. The mixture was then transferred into a centrifuge tube for 30 min. The supernatant solution was decanted, and the solid was collected and dried at 50 °C for 24 h to give green powder (0.12 g, 12%) of the base hydrolysis product, which was identical to ¹H-NMR and UV, showing no sugar region. δ_{H} (500 MHz, DMSO): 3.51 (1 H, s), 3.33 (2 H, s), 2.78 (1 H, s), 2.52–2.49 (5 H, m, *J* 3.7, 1.8 Hz), 2.18 (1 H, t, *J* 7.3 Hz), 1.67 (1 H, d, *J* 9.4 Hz), 1.27 (1 H, d, *J* 27.4 Hz), 1.16–1.05 (1 H, m), 0.98 (1 H, s), 0.92 (1 H, d, *J* 7.5 Hz), 0.89 (1 H, s), 0.85 (1 H, d, *J* 5.8 Hz), 0.81 (1 H, d, *J* 5.0 Hz), 0.76 (1 H, s). δ_{C} (126 MHz, DMSO) 40.02, 39.85, 39.69, 39.52, 39.35, 39.19, 39.02. UV λ_{max} nm: 202.63 s, 362.37 br.

2.3. Preparation of Gorse Leaves (Needle) for Extraction

Ripe leaves of gorse were collected from the plant by hand and left to dry at room temperature for 24 h. These leaves were then placed in a 50 °C drying oven for 72 h to remove moisture. The needles were then milled using an electric mill fitted with a 2 mm sieve plate, transferred to dry plastic containers and sealed with screw cap lids and parafilm to minimise exposure to atmospheric moisture until use.

2.3.1. Soxhlet Extraction of Dried, Prepared and Milled Gorse Leaves with Dichloromethane

Milled gorse leaves (10.49 g) were extracted in a Soxhlet apparatus using a cellulose extraction thimble with industrial dichloromethane (300 mL) and refluxed at 75 °C for 24 h, at which point the solvent was running clear (no further colours). The heating was stopped, and the solvent was removed in vacuo to recover a green oily residue (0.32 g, 3%), which showed δ_{H} (500 MHz, CDCl₃): 7.27 (1 H, s), 6.37–5.75 (23 H, m), 5.43–4.89 (62 H, m), 5.13 (13 H, d, *J* 7.1 Hz), 4.69 (8 H, d, *J* 5.2 Hz), 4.18 (11 H, ddd, *J* 17.9, 11.4, 5.6 Hz), 3.98 (25 H, dt, *J* 21.9, 13.5 Hz), 3.31 (12 H, d, *J* 87.3 Hz), 2.90–2.24 (72 H, m), 2.24–2.11 (14 H, m), 2.24–1.67 (111 H, m), 1.64 (17 H, d, *J* 18.6 Hz), 1.14 (21 H, s), 1.05–0.95 (61 H, m), 1.67–0.59 (772 H, m), 1.17–0.59 (296 H, m), 0.92–0.78 (135 H, m), 0.68 (49 H, s); δ_{C} (126 MHz, CDCl₃) 131.94, 131.93, 130.30, 130.18, 128.92, 128.28, 128.20, 127.76, 127.73, 127.22, 127.09, 77.25, 77.00, 76.75, 34.01, 31.90, 31.89, 31.50, 29.67, 29.63, 29.58, 29.34, 29.32, 29.15, 29.09, 27.95, 27.18, 25.61, 25.60, 25.51, 22.66, 22.60, 22.55, 20.53, 14.25, 14.09.

2.3.2. Preparation of Methyl Ester Fatty Acid

Gorse leaf extract (from Exp 8, 0.5 g) was dissolved in methanol (20 mL) with continuous stirring. To this mixture, sulphuric acid (5 mL, 98%) was added and then left to reflux for 5 h, and the reaction was quenched by the addition of an aqueous NaHCO₃ (100 mL). The aqueous layer was separated, and the combined organic layer was washed with aqueous NaHCO₃ (3 × 50 mL). The aqueous layer was drawn off, and the organic layer was dried with magnesium sulphate and filtered. The solvent was then removed in vacuo to afford a green oily product (0.3 g, 60%). This was identified by GC MS *m/z*, as shown in Section 2.2.1.

2.3.3. Soxhlet Extraction of Dried and Milled Gorse Leaves with Methanol

The milled gorse needles (10 g) were added to a cellulose extraction thimble and extracted in a Soxhlet apparatus using methanol (250 mL), refluxing at 95 °C (oil bath) for 48 h, at which point the solvent was running clear. Heating was stopped, and the solvent was allowed to cool and then was removed in vacuo to recover a green, thick and oily residue, appearing as mixture spots by the TLC of crude flavonoids extract (2.56 g, 25%). The crude product (0.55 g) was then purified by column chromatography on silica using chloroform/methanol (5:2) to give a crystal product (0.13 g, 23% of crude, 1.3% total), appearing as a single spot by TLC *R_f* 0.4 and showed δ_{H} (400 MHz, MeOD) 7.99 (1 H, s), 7.34 (2 H, d, *J* 8.3), 6.84 (2 H, d, *J* 8.3), 6.77 (1 H, s), 6.69 (1 H, s), 5.08 (1 H, d, *J* 6.5), 4.19 (2 H, d, *J* 7.8), 3.98–3.80 (8 H, m), 3.75–3.64 (4 H, m), 3.63–3.48 (9 H, m), 3.45–3.24 (12 H, m), 3.18

(2 H, t, *J* 8.4). δ_C (101 MHz, $CDCl_3$) 176.29, 162.18, 161.18, 159.49, 157.17, 151.49, 130.19, 125.75, 122.87, 114.71, 109.78, 104.00, 100.39, 97.01, 95.99, 77.11, 76.65, 76.53, 76.47, 73.66, 73.35, 70.23, 69.98, 63.02, 61.34, 61.14, 55.95, 55.35, 48.49, 48.28, 48.07, 47.85, 47.64, 47.43, 47.21, 47.00: MALDI MS *m/z* $[M + Na]^+$ 469, UV λ_{max} nm: 256 br, 204 s.; m.p. 217–220 °C: ν_{max} (CH_3OH)/ cm^{-1} : 3435.8 w, 2926.9 s, 2861.2 s, 1637.1 w, 1493.8 s, 1050 w, 824.3.

2.4. Preparation of Gorse Bark for Extraction

The bark of gorse was collected from a mature plant and dried at room temperature for 24 h. The bark was then placed in a 50 °C oven for 72 h to remove moisture. The bark was cut into small pieces and milled using an electric mill fitted with a 2 mm screen. The milled bark was then transferred to dry plastic containers and sealed with screw cap lids and parafilm to minimise exposure to atmospheric moisture until use.

2.4.1. Soxhlet Extraction of Dried, Milled Gorse Bark with Dichloromethane

The milled gorse bark (9.22 g) was extracted in a Soxhlet apparatus using 300 mL of dichloromethane at 75 °C for 24 h, at which point the solvent was running clear. The solvent was removed in vacuo to recover a yellow oily residue as a crude product (0.26 g, 3%). This showed δ_H (500 MHz, $CDCl_3$) 8.09 (1 H, d, *J* 2.0 Hz), 7.61 (3 H, d, *J* 15.8 Hz), 7.41 (1 H, d, *J* 8.5 Hz), 7.33–7.20 (5 H, m), 6.91 (1 H, d, *J* 8.2 Hz), 6.82 (2 H, d, *J* 8.4 Hz), 6.40–6.23 (4 H, m), 5.64 (1 H, d, *J* 9.9 Hz), 5.30 (1 H, s), 5.30 (3 H, s), 4.74–4.64 (2 H, m), 4.24–4.11 (5 H, m), 4.09–4.03 (2 H, m), 3.93 (2 H, s), 3.49 (34 H, s), 2.43–2.23 (14 H, m), 2.18 (1 H, s), 1.98–1.15 (253 H, m), 0.88 (58 H, t, *J* 6.8 Hz), 0.68 (1 H, s). δ_C (126 MHz, $CDCl_3$) 129.89, 115.86, 77.27, 77.01, 76.76, 50.78, 31.92, 29.69, 29.65, 29.60, 29.35, 29.25, 29.10, 22.68, 14.10.

2.4.2. Soxhlet Extraction of Dried, Milled Gorse Bark with Methanol

The milled gorse bark (8.95 g) was extracted in a Soxhlet apparatus using methanol (250 mL) at 95 °C for 48 h. After this time, the solvent appeared to be running clear. The heating was turned off and the thimble allowed to cool. The solvent was removed in vacuo to recover a light brown solid which gave a mixture of spots by the TLC of crude flavone (1.26 g, 15%). The crude product (0.5 g) was then purified by column chromatography on silica using chloroform/methanol (5:2) to give a crystal (0.15 g, 30% of crude, 1.5% total), which appeared as a single spot by TLC *R_f* 0.4 and showed δ_H (400 MHz, MeOD) 8.01 (1 H, s), 7.34 (2 H, d, *J* 8.5), 6.82 (2 H, d, *J* 8.5), 6.79 (1 H, d, *J* 1.9), 6.71 (1 H, d, *J* 1.8), 5.07 (1 H, d, *J* 7.2), 4.85 (16 H, s), 3.98–3.87 (3 H, m), 3.70 (1 H, dd, *J* 12.1, 6.2), 3.70 (1 H, dd, *J* 12.1, 6.2), 3.58–3.47 (3 H, m), 3.42–3.35 (1 H, m), 3.31 (7 H, s). δ_C (101 MHz, MeOD) 177.62, 163.52, 162.52, 160.82, 158.54, 152.83, 131.55, 127.09, 124.25, 116.07, 111.12, 101.77, 98.35, 97.35, 78.48, 77.85, 74.73, 71.36, 62.51, 56.70, 49.85, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36: MALDI MS *m/z* $[M + Na]^+$ 469, UV λ_{max} nm: 256 br, 204 s.; m.p. 217–220 °C: ν_{max} (CH_3OH)/ cm^{-1} : 3435.8 w, 2926.9 s, 2861.2 s, 1637.1 w, 1493.8 s, 1050 w, 824.3.

2.5. Fibre Test for Wood

2.5.1. Preparation for the Wood of Gorse for De-Lignification

The raw wood was collected from gorse. The bark was removed and cut to pieces; the average particle size, 2–5 mm, was used in the experiment. Glacial acetic acid and hydrogen peroxide were used for de-lignification.

2.5.2. De-Lignification of Wood

A mixture of acetic acid CH_3COOH 50% and hydrogen peroxide H_2O_2 50% was added to the prepared wood (24.12 g) and refluxed at 70 °C for 3 h with slow stirring. The reaction mixture was allowed to cool and was filtered through a Buchner funnel under reduced pressure and washed with water (3 × 50 mL). The fibre on the filter paper was dried on the filter paper and transferred to the oven at 50 °C, giving (7.30 g, 30%). Then, it was evaluated using a Kajaani FS-200 Fibre Analyser. The results showed Arithmetic AV 0.33.

3. Results and Discussion

3.1. Preparation of Gorse Flower

Flowers of gorse were collected on 3 January 2012 from plants growing in the wild in Bangor during the early morning. The flowers were dried, and the moisture content was calculated as 76%. The dried flowers were then hammer milled to a fine meal and placed in polythene bags to minimise unnecessary moisture entering in and out of direct sunlight. This was then used for extraction by the solvent.

3.1.1. Flower of Gorse Extracted with Dichloromethane

Previous works successively extracted nature products from gorse by using a low polarity solvent, recovering very little yield, which was almost triglyceride fatty acid. The best recovery of triglyceride fatty acid was obtained using dichloromethane as a low polarity solvent [41]. The dry milled flower, was extracted by a Soxhlet apparatus for over 24 h using dichloromethane, and the solvent was removed under vacuum to recover a yellow oil (10%).

$^1\text{H-NMR}$ analysis of the extract confirmed the principal compound to be triglycerides fatty acid (Figure 2). The signal appeared as double doublet at δ 4.30 J 12.0, 4.3 Hz for two protons (CH_2) for triglyceride; at δ 4.15, it appeared as double doublet J 11.9, 5.9 Hz for the other two protons (CH_2) and also for the one proton (CH) for triglyceride, which appeared as multiple peaks at 5.29–5.25. The $^{13}\text{C-NMR}$ spectrum showed a high proportion of olefinic signals in the region of δ 127–131.93 ppm (Figure 3). The principal unsaturated fatty acid of the triglycerides in this region was identified from standards as α -linolenic acid (z-C18:3 Δ 9, 12, 15).

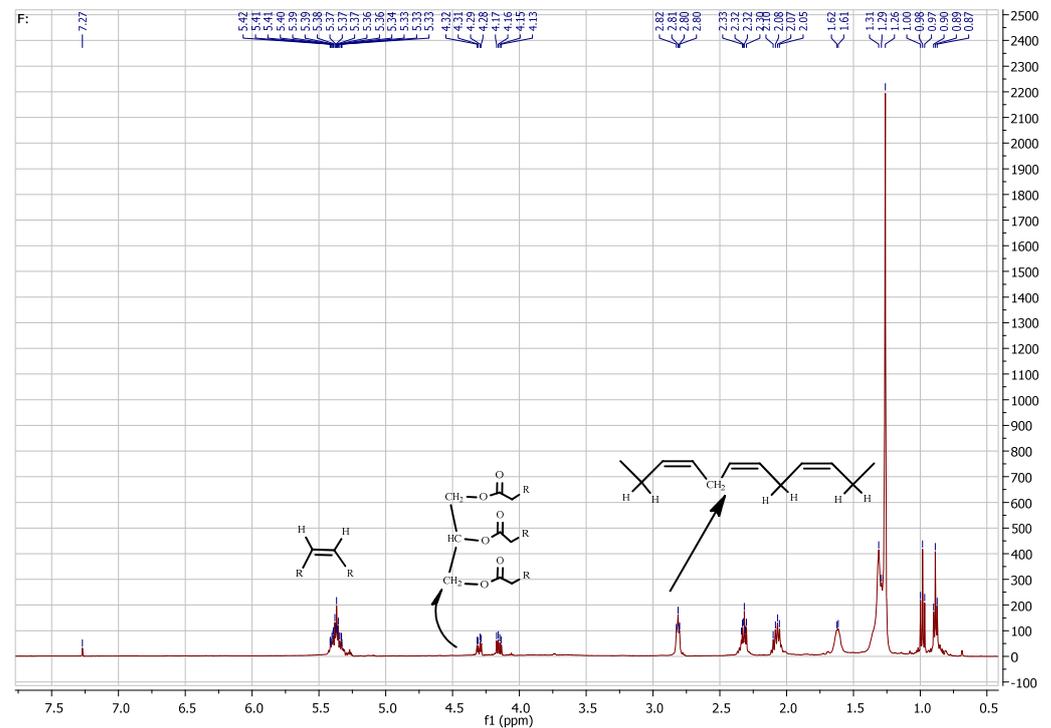


Figure 2. The $^1\text{H-NMR}$ spectrum of the crude gorse flowers extracted with DCM.

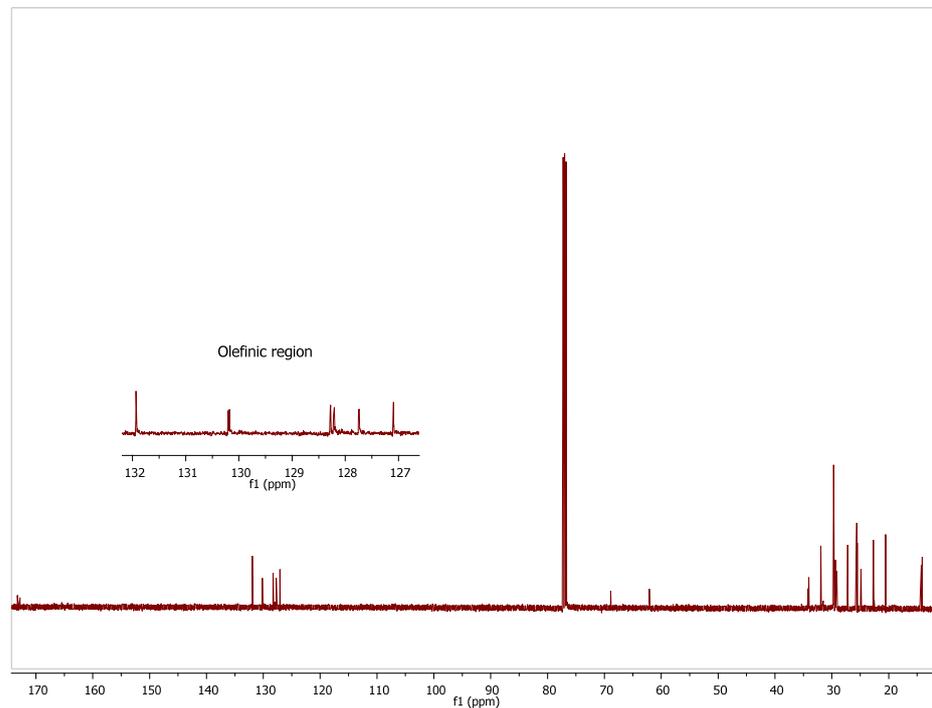


Figure 3. The ^{13}C -NMR spectrum of the crude DCM extracted from gorse flowers.

To confirm this observation and to determine the fatty acid profile, the corresponding methyl esters were prepared. The crude triglyceride mixture was reacted in methanol with a catalytic amount of sulphuric acid (98%) at reflux for 5 h, which yielded a green, oily product of fatty acid methyl ester. The reaction was judged to have reached completion by TLC (R_f of starting oil: 0.47, R_f of product: 0.66, petrol/ethyl acetate, 10:1). By comparing the retention times with those of the standards, GCMS showed linolenic acid as the dominant fatty acid of the mixture and thereby confirmed the observation in ^{13}C -NMR (Figure 4 and Table 1). The results of GCMS are presented in Table 1.

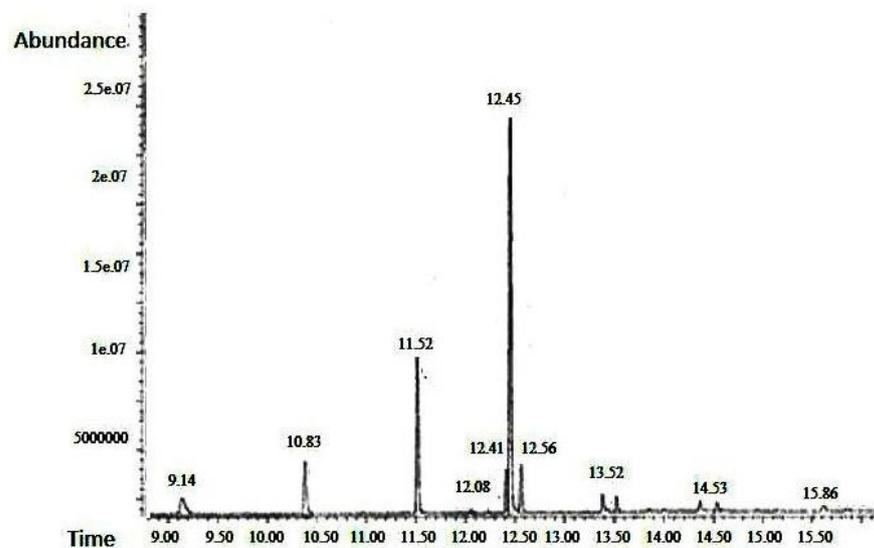


Figure 4. GCMS of the methyl ester fatty acid.

Table 1. GCMS for the gorse flower fatty acids.

Name	Formula	Retention Time	Relative Proportion	<i>m/z</i>
Lauric acid	C12:0	9.14	6.1	200
Myristic acid	C14:0	10.38	11.3	228
Palmitic acid	C16:0	11.52	20.9	256
Palmitoleic acid	C16:1	12.08	0.77	254
Stearic acid	C18:0	12.56	5.4	284
Linoleic acid	C18:2	12.41	45.5	280
α -linolenic acid	C18:3	12.45	6.93	278
Eicosanoic acid	C20:0	13.52	2.23	312
docsanoic acid	C22:0	14.53	1.6	340
Tetracosanoil acid	C24:0	15.86	0.4	368

The structures of the fatty acids isolated from gorse flowers are illustrated in Figure 5. The α -linolenic acids C18:3 Δ 9,12, 15 were identified as major components from the gorse flower (Figure 6), although the over-recovery of the triglyceride-containing fraction of fatty acid (0.9 g, 10%) was low. The dichloromethane extraction was more successful than the other low polar solvent (petroleum ether), which recovered (0.4 g, 5%), though that of the dichloromethane probably extracted other components, as it is slightly more polar than that of petroleum ether.

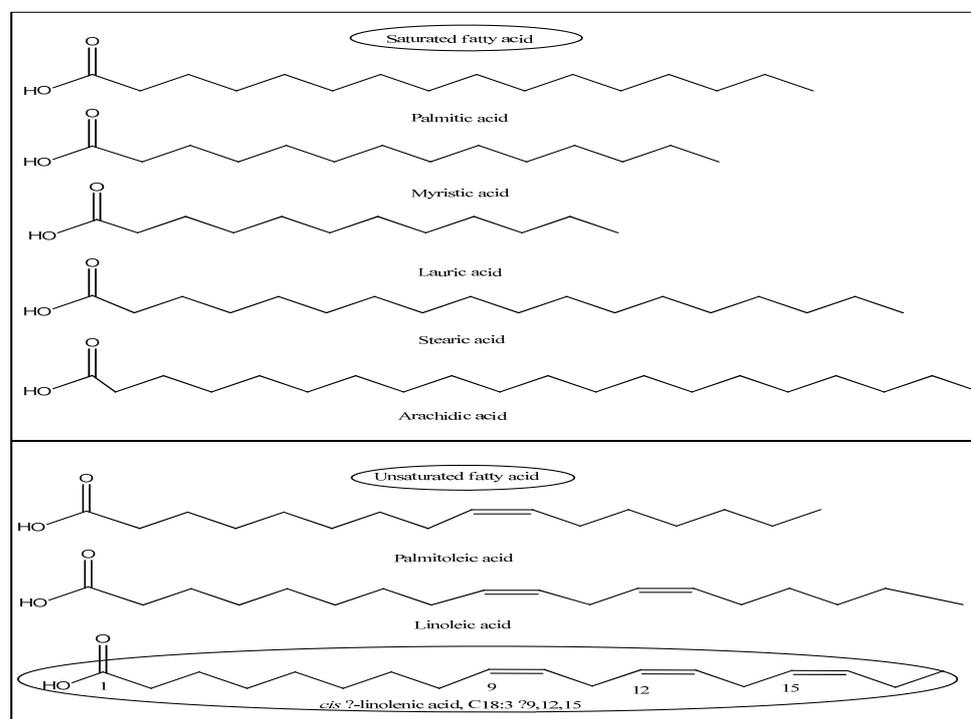


Figure 5. Structures of fatty acids isolated from gorse flower. L weighted AV 0.65 mm, L weighted AV 0.82 mm, where L—Length of an individual fibre.

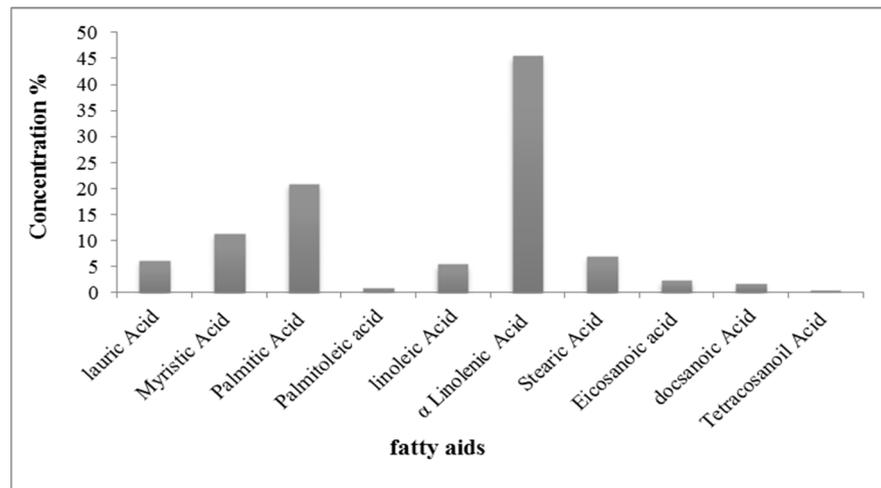


Figure 6. Types of fatty acid extracted from gorse flower.

3.1.2. Milled Flower of Gorse Extracted with Methanol

The milled flowers of gorse were prepared as described above and were extracted in a more polar solvent. Methanol gave a fine white powder in high recovery (3.5 g, 34%). $^1\text{H-NMR}$ analysis of the flower extract showed the characteristic complexity of signals in the region (δ 3.4–4.2 ppm), and it is this region that corresponds to the attached sugars and was quantified by UV spectra. The wavelength scanning of the flower extract indicated UV peaks of the sugar at around 261 and 213 nm, and the IR showed peaks appearing as a signal at 3436 cm^{-1} for the OH group.

The methanol extraction appears to contain a mixture of different sugars; it may be possible that some minor saponins are also present and appear to be linked with the sugar by ester or acetal bond [42,43]. To determine this conjecture, it would be necessary to carry out an acid and base hydrolysis of the crude product from Section 2.2.2 (Figure 7).

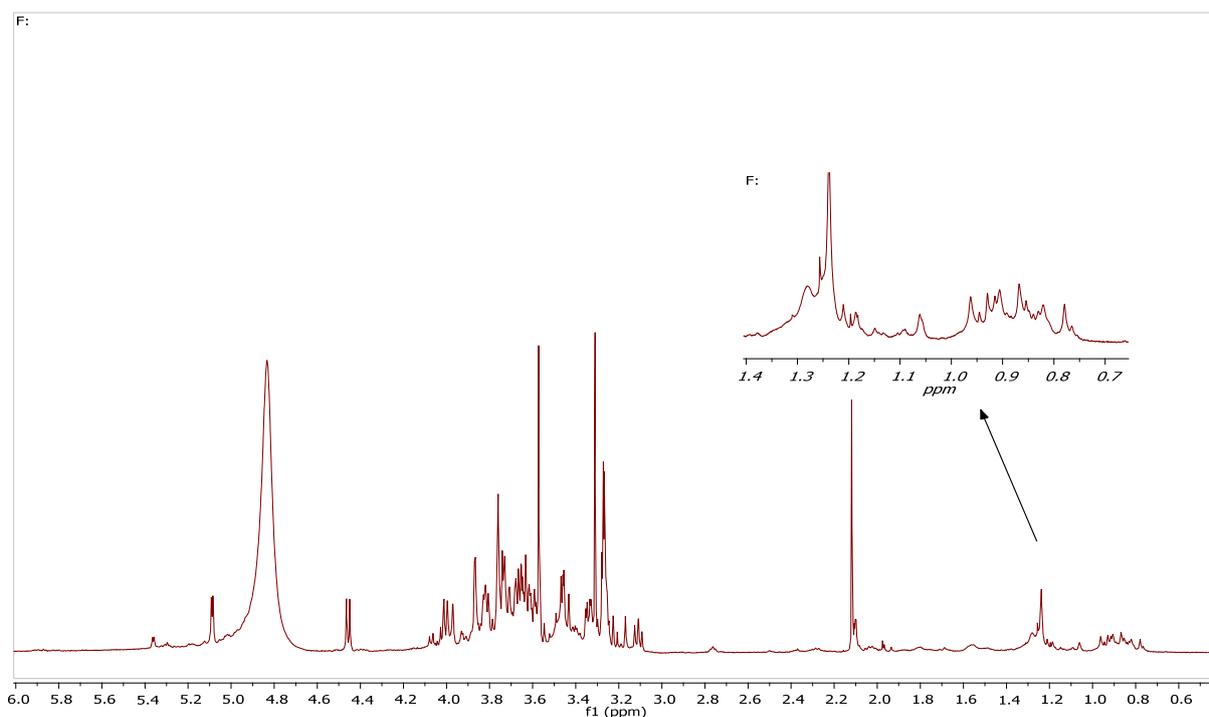


Figure 7. The $^1\text{H-NMR}$ spectrum for methanolic extract including sapiens.

3.1.3. Acid and Base Hydrolysis of the Methanolic Extract

The first attempt to hydrolyse the crude powder from Section 2.2.2 was performed at refluxing with hydrochloric acid (5%) for 5 h. However, the TLC showed no change to the sugar spots signals.

By using sulphuric acid (10%), the crude product of Section 2.2.2 was reacted, and the reaction time was extended and judged by TLC be complete after 72 h. The $^1\text{H-NMR}$ spectrum of the acid hydrolysis showed no significant signals for sugar or saponins (Figure 8).

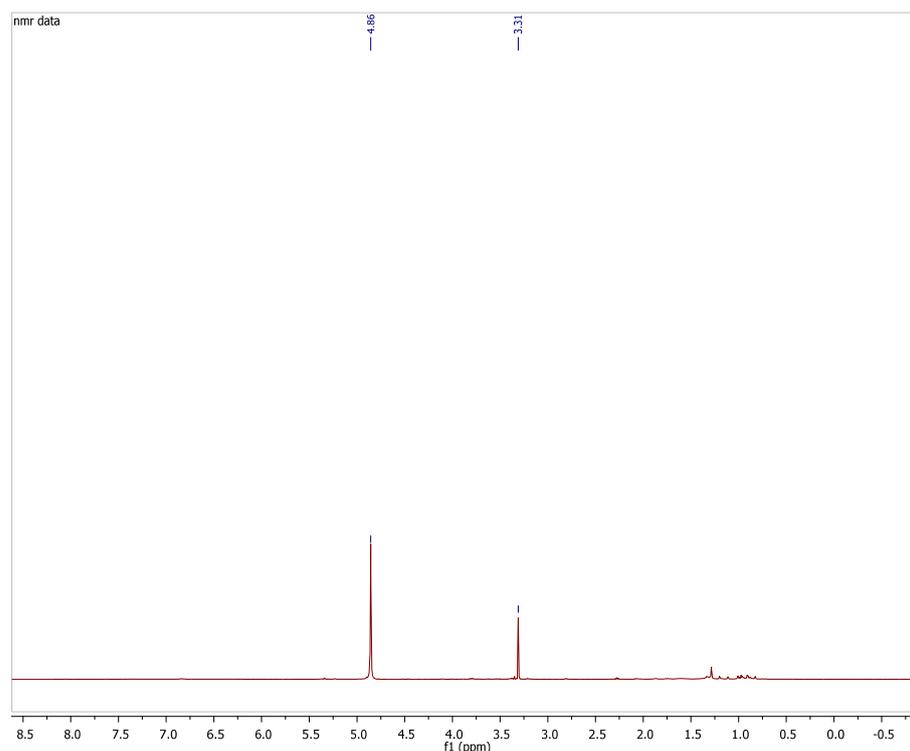


Figure 8. The $^1\text{H-NMR}$ spectrum for the crude acid hydrolysis.

3.1.4. Base and Base Hydrolysis of the Methanolic Extract

Acid hydrolysis cleaves both the acetal and ester bonds of sugars, Because of this, base hydrolysis was used instead, which only cleaved the acetal bonds. The sodium hydroxide (1 M) solution of the crude sugars was heated to 75 °C for 5 h under a reflux condenser when TLC showed that the reaction was complete. The $^1\text{H-NMR}$ spectrum of acid hydrolysis (Section 2.2.3) showed no significant signals for sugar and saponins. It was expected that the base hydrolysis would cleave only the acetal bonds [15]. The $^1\text{H-NMR}$ spectrum showed similar data to the acid hydrolysis. This further supported the belief that minor saponins were linked with the sugar by acetal bond.

3.2. Preparation of Gorse Leaves

Leaves were collected from gorse plants growing in Bangor (Wales) with the assistance of Dr. D. Preskett. The leaves were separated from the plants, dried in an oven at 40 °C overnight and hammer milled as described in the experimental section. The dried leaves were stored and used in the same manner as the flower.

3.2.1. Soxhlet Extraction of Gorse Leaves by Dichloromethane

The dried, milled leaves were extracted in a Soxhlet apparatus using DCM as the solvent to recover dark green residue (3%). The $^1\text{H-}$ and $^{13}\text{C-NMR}$ spectra showed peaks of the triglyceride and olefinic region similar to the result found in Section 2.2.1 (Figures 9 and 10).

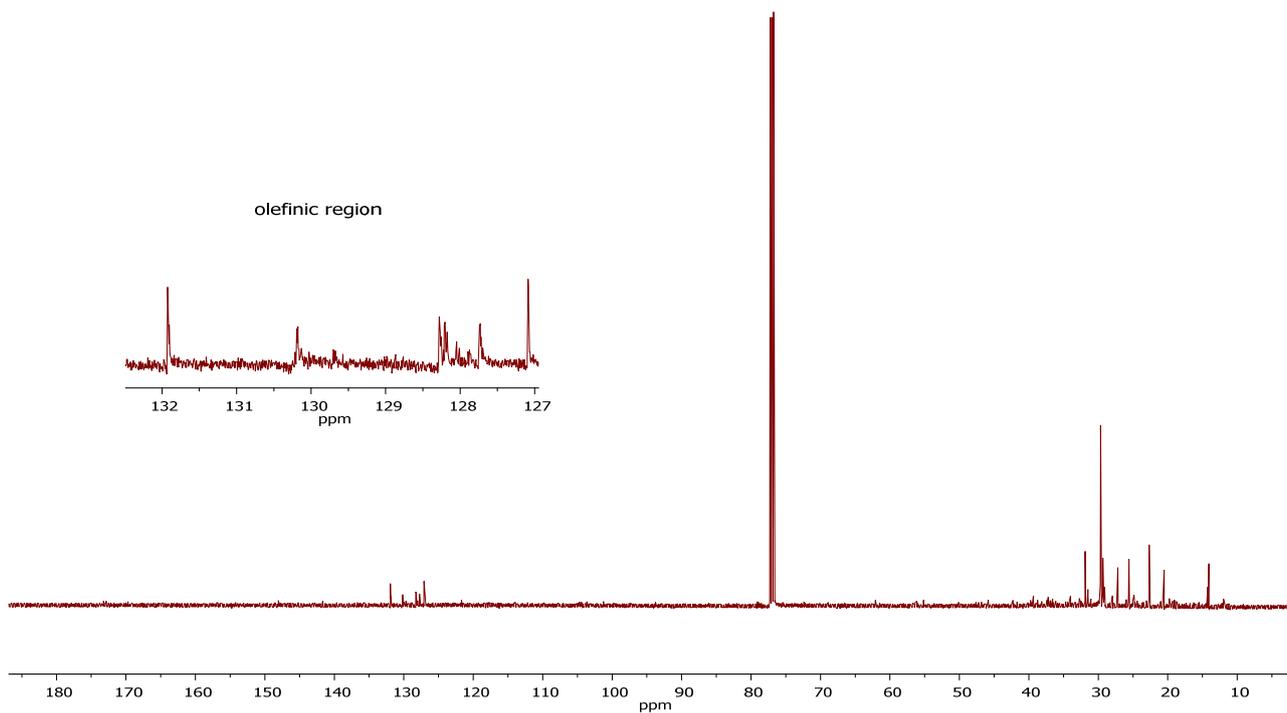


Figure 9. The ^{13}C -NMR spectrum for a DCM extract of leaves.

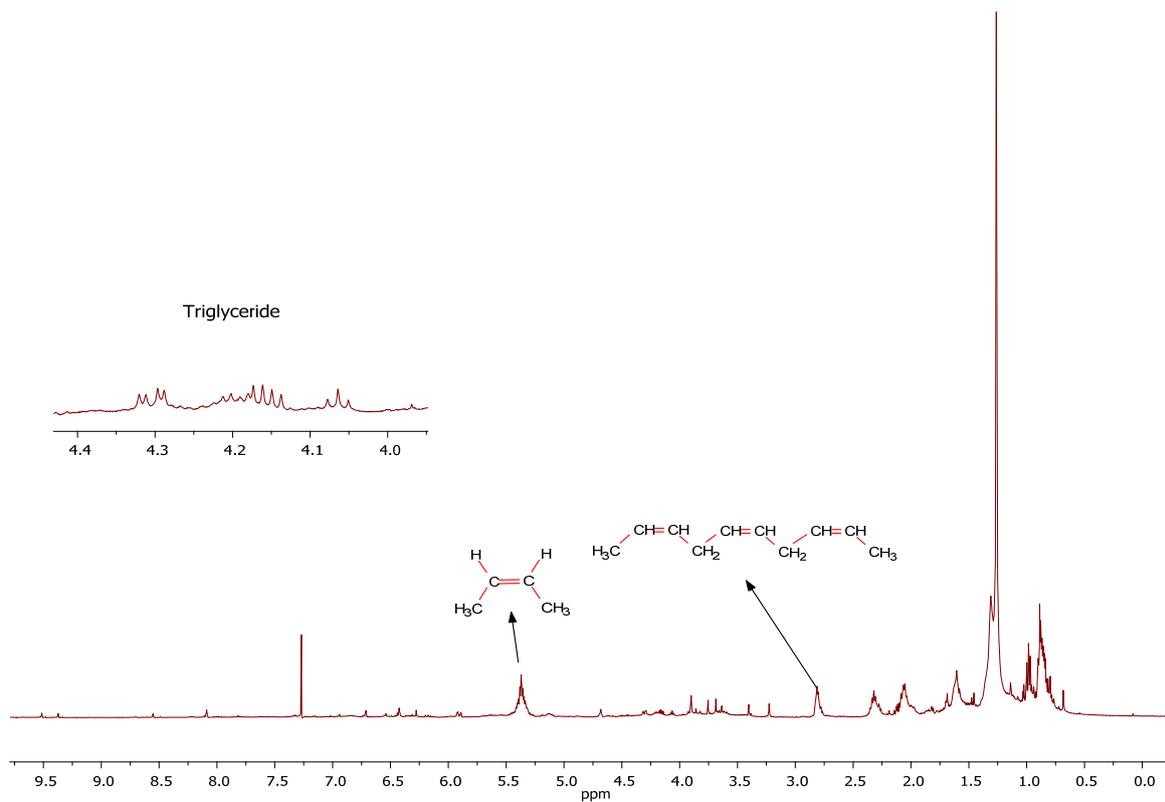


Figure 10. The ^1H -NMR spectrum for a DCM extract of leaves.

The next step was the preparation of methyl ester fatty acid as described in 2.1.1, which gave a 60% yield of methyl ester fatty acid that was analysed by GCMS (Table 2).

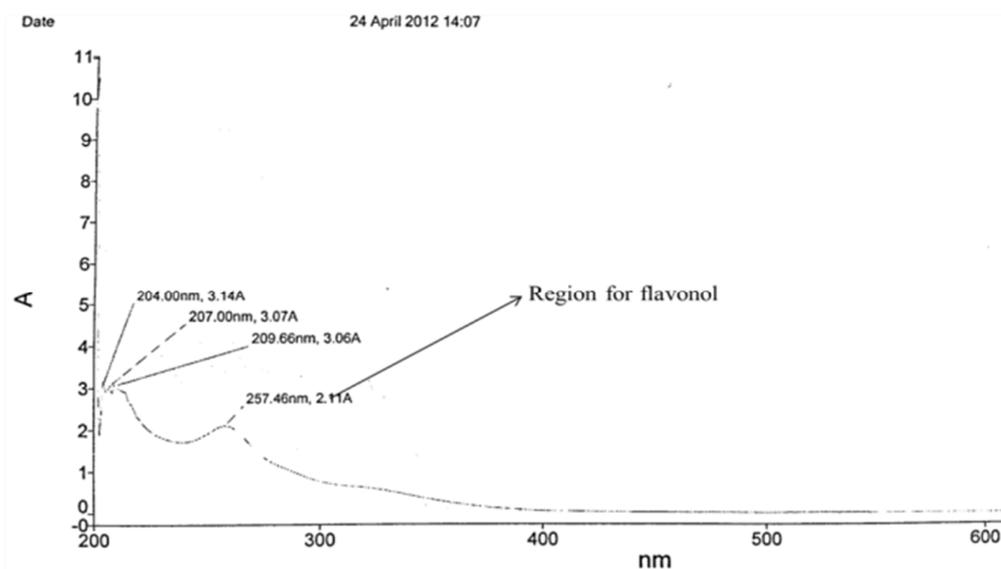
Table 2. Fatty acid profile by the GCMS of triglyceride extracted from leaves.

Name	Formula	Retention Time	Relative Proportion	<i>m/z</i>
Palmitic acid	C16:0	11.52	9.44	256
Palmitoleic acid	C14:0	9.22	1.47	254
Lauric acid	C12:0	6.13	31.65	270
α -linolenic acid	C18:3	12.45	41.75	278
Stearic acid	C18:0	12.07	0.741	284
Oleic	C18:1	12.22	1.477	282
Linoleic acid	C18:2	12.40	2.954	280
Arachidic acid	C20:0	13.89	0.922	312

α -linolenic acid, *z*-C18:3 Δ 9, 12, 15 was shown to be the principal fatty acid. The results also showed a saturated fatty acid (Lauric acid, C12:0) present in the leaves as the second most abundant.

3.2.2. Soxhlet Extraction of Gorse Leaves with Methanol

The extraction was carried out using methanol solvent, giving a surprisingly good yield of 25%. The TLC showed a mixture of compounds; the UV spectrum showed a λ_{\max} : 257 nm that corresponds to the flavonoid compounds (Figure 11) [42]. $^1\text{H-NMR}$ analysis of the methanolic extract of gorse leaves confirmed the isoflavonoid and linked with sugar. The key analytical feature that linked sugar in the region of 4.16–3.17 ppm and for the isoflavonoid at signals 7.99–6.69, respectively, suggested that this could be possible.

**Figure 11.** UV spectrum for the crude of methanolic extracted from gorse leaves.

The crude product was purified by column chromatography on silica (eluting with 5:2 chloroform/methanol), giving a crystal of isoflavonoid glycosides that appeared as one spot by TLC ($R_f = 0.4$), as shown by $^1\text{H-NMR}$ signals (Figure 12). The aromatic region showed two doublets resonated at δ 7.34 and 6.84, with the coupling constant 8.3 Hz, and also showed the aromatic region appear as two singlets resonated at δ 6.77 and 6.96 for the other proton at C-12 and C-7. One proton at signal 7.99 ppm appeared as a singlet for alkene at C-15. The spectrum also showed two protons at C1 glucose sugar. C1 furanose anomeric carbon linked with the isoflavonoid at H1 at 5.08 appeared to be one proton at the duplet *J* constant of 6.5 Hz for glucose, and another anomeric peak appeared as a duplet at 4.19 ppm for two protons at a *J* value of 7.8 Hz. The hypothetical structure for the isoflavonoid glycosides is shown in Figure 13.

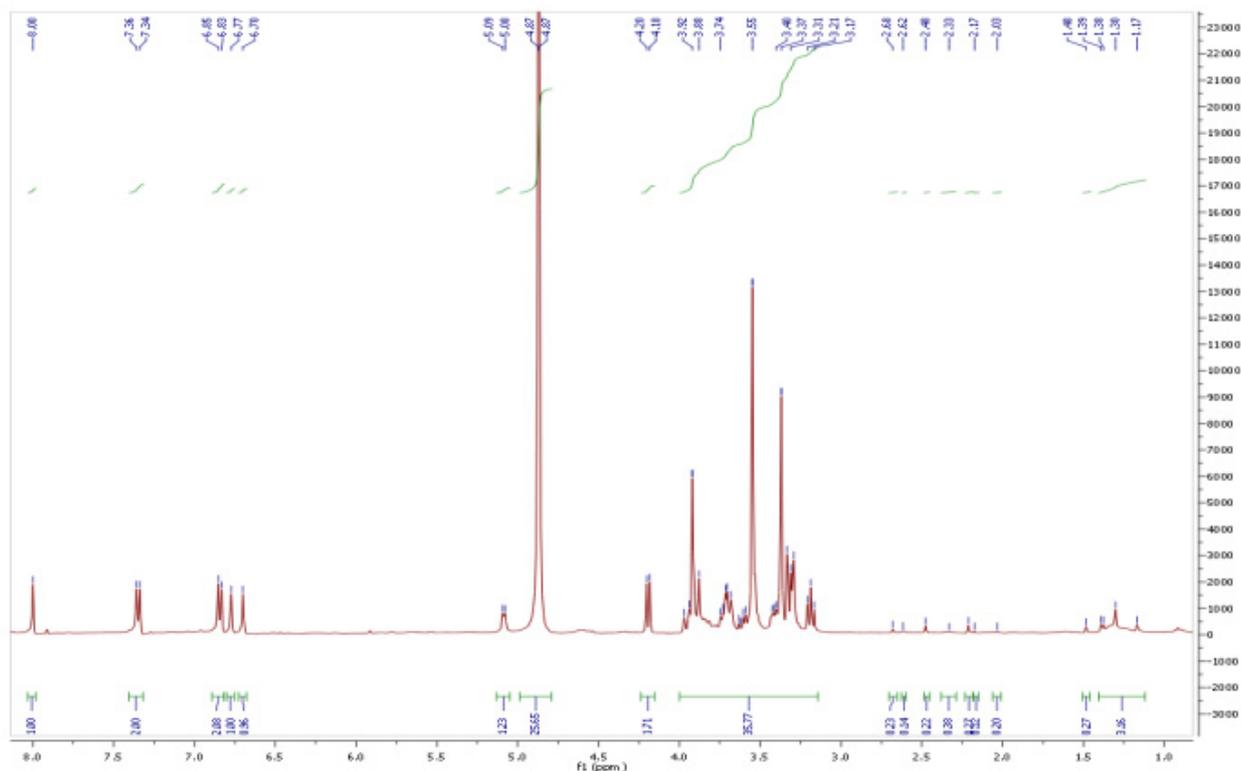


Figure 12. ^1H -NMR spectrum for a methanolic extract of leaves.

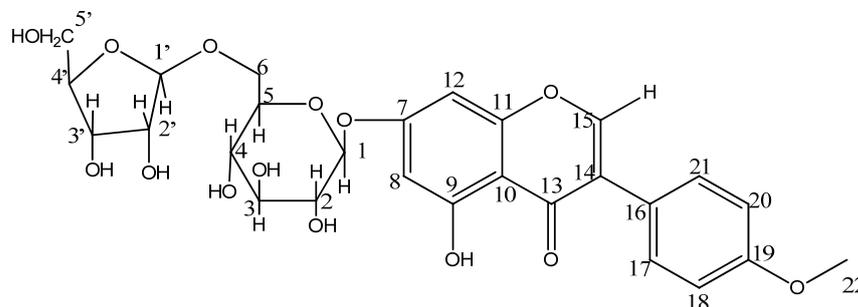


Figure 13. Hypothetical structure for isoflavonoid glucosides isolated from leaves of gorse.

^{13}C -NMR showed δ at 176.25 ppm for the carbonyl group and at δ 104 and 100 ppm [42], respectively, for the anomeric carbon for sugar. In addition, MALDI MS 469 (as described in Section 2.2.4) analysis of this product confirmed the isoflavonoid glucosides isolated from gorse leaves.

3.3. Preparation of Gorse Bark

Bark was removed from the stem and wood of gorse plants growing in Bangor; the bark was dried and milled by the same method as described above to a moisture content of 6%.

3.3.1. Soxhlet Extraction of Bark from Gorse by Dichloromethane

The dried bark was extracted in a Soxhlet apparatus for 16 h using dichloromethane as described above to give yellow oily residue. NMR spectra showed that there is no significant group. The extraction was repeated using another non-polar solvent (petroleum ether) and showed a similar result. At that point, it was to increase the polarity of the solvent by using methanol, as reported by Russell and his group [43].

3.3.2. Soxhlet Extraction of Bark from Gorse by Methanol

The dried bark was extracted as described above by using methanol as a solvent to recover a very fine brown powder in good yield (15%). This was examined firstly by UV, the spectrum being indicative of flavonoids at λ_{\max} 257–269 nm, as per Russell and his group [44].

The crude product was then purified by column chromatography on silica eluting with 5:2 chloroform/methanol, giving crystals of flavonoid that appeared as one spot by TLC at $R_f = 0.4$, which showed a similar R_f value for the methanolic extract for the leaves (Figure 14).

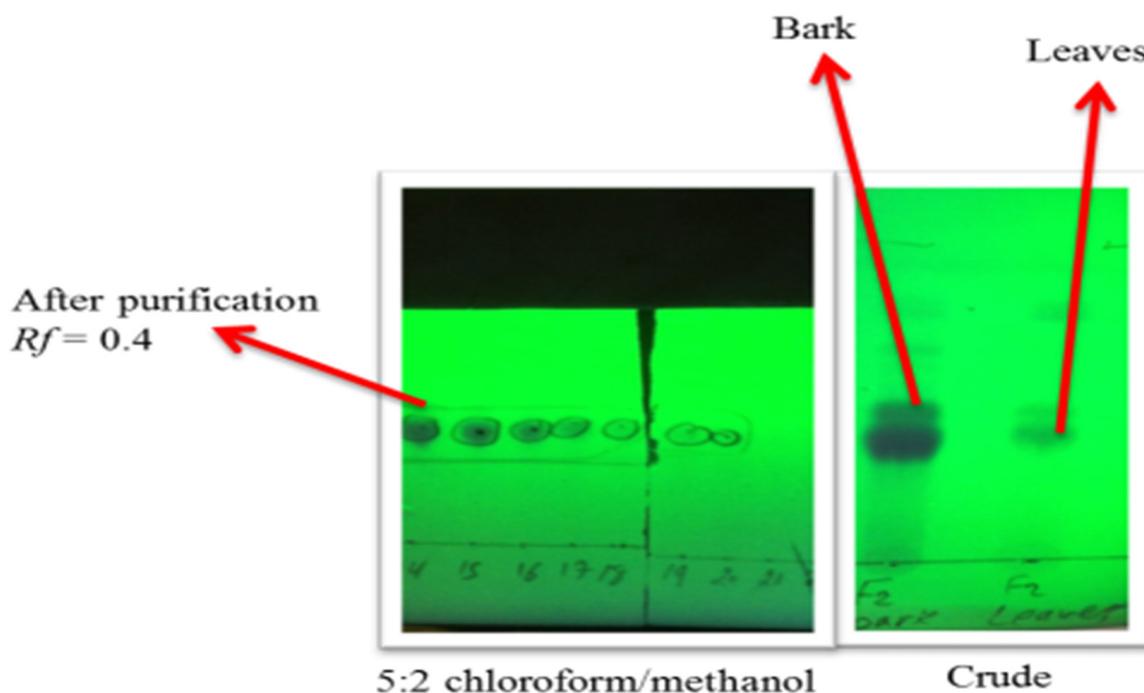


Figure 14. Possible evidence for isoflavonoid isolated from the bark and leaves of gorse.

The $^1\text{H-NMR}$ showed the presence of the signal in the aromatic region at 8.01 and one proton singlet at C15, a doublet signal at 7.34 ppm J 8.5 Hz for two protons at C17 and C21 at signal 6.82 ppm J 8.5 Hz and two protons appear as a doublet at C18 and C20 for the substituted benzene ring. For the substituted benzene ring, one proton appears as a doublet at 6.79 ppm J 1.9 Hz, and at 6.71 ppm, one proton doublet appeared at J 1.8 Hz. At the sugar region, a doublet at 5.07 ppm appears for the anomeric proton. For glucose sugar, the coupling constant 7.5 Hz between H at C1 and H at C2 indicated the β -configuration—showing a similar result to Tamura et al. [45]—which has been isolated from Red Clover Sickness and established to be 7-D- β -glucosyl-5,7-dihydroxy-4'-methoxy-isoflavone and also characteristic of the HMQC spectrum (Table 3).

Table 3. ^1H and $^{13}\text{C-NMR}$ data for compound 2 * methanol CD_3OD .

H/C NO	$^1\text{H-NMR}$ * (δ)	$^{13}\text{C-NMR}$ * (δ)	Multiplicity
C1	5.07 (1 H, d, J 7.2 Hz, H-1')	100.4	CH
C2	3.58–3.47 (3 H, m, H-'2-'5-'4)	74.73	CH
C3	3.35 (1 H, m, H-'3)	76.48	CH
C4	*	71.36	CH
C5	*	78.48	CH

Table 3. Cont.

H/C NO	¹ H-NMR * (δ)	¹³ C-NMR * (δ)	Multiplicity
C6	3.70 (1 H, dd, <i>J</i> 12.1, 6.2, H-6'a) 3.70 (1 H, dd, <i>J</i> 12.1, 6.2, H-6'b)	62.51	CH ₂
C7	-	163.52	C
C8	6.79 (1 H, d, <i>J</i> 1.9, H-8)	98.35	CH
C9	-	162.52	C
C10	-	111.12	C
C11	-	158.54	C
C12	6.71 (1 H, d, <i>J</i> 1.8, H-'12)	97.35	CH
C13	-	177.62	C
C14	-	124.25	C
C15	8.01 (1 H, s, H-'15)	152.83	CH
C16	-	127.09	C
C17–21	7.34 (2 H, d, <i>J</i> 8.5 Hz)	131.55	CH,CH
C18–20	6.82 (2 H, d, <i>J</i> 8.5 Hz)	116.07	CH,CH
C19	-	160.82	C
–OCH ₃	3.98–3.87 (3 H, m)	56.7	CH ₃

* 3.58–3.47 (3 H, m, H-5'-4').

This fraction displayed a λ_{\max} of 256 nm using UV, and MALDI MS analysis showed one molecule present with a mass (469) with Na⁺. However, the IR spectrum showed a broad signal at 3435 cm⁻¹ for the OH group and at 1637.11 cm⁻¹ for the carbonyl group. That result is different from Russell et al. [43]; they showed completely different data for the NMR and MS spectra but similar *R_f* values for the methanolic extraction of leaves.

Figure 15, showed the structure of isoflavonoids compound which isolated from the bark of gorse.

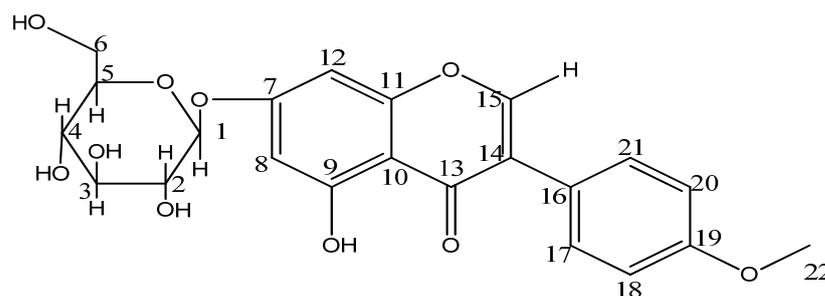


Figure 15. Structure for isoflavonoid glucosides isolated from the bark of gorse.

3.4. Fibre Analysis of Wood

This work was carried out to investigate gorse wood as a possible source for ligno-cellulosic fibre. The raw wood was collected from gorse and cut to matchstick-sized pieces after the removal of the bark, as described in the experiment section. The wood particles were then de-lignified by acetic acid/hydrogen peroxide (50:50) at 70 °C for 3 h to recover the white fibres. A Kajaani FS-200 Fibre Analyser (Figure 16) showed that the fibre lengths appear as Arithmetic AV 0.33 mm, L weighted AV 0.65 mm, L weighted AV 0.82 mm according to El-Hawiet et al. and Ai et al. [42,44], where L = length of an individual fibre.



Figure 16. A Kajaani FS-200 Fibre Analyser.

4. Conclusions

Most of the compounds extracted from gorse flower and leaves were fatty acids of triglycerides which were extracted with low polar solvents. The crude oil content has been established as 10% of flower and 3% of the leaves. The majority of these fatty acids were determined to be unsaturated fatty acids. α -Linolenic acid (C18:3 Δ 9, 12, 15) was the most abundant. It is an essential dietary fatty acid which is an important constituent of the brain and cell membranes. Most of the compounds from the methanolic extract of flower were sugar flavones. Glycosides were isolated from the bark and leaves of gorse. More work is planned to investigate the sugars present in the gorse plant. The wood of gorse was shown to have very short fibres, which are unsuitable for making paper.

Author Contributions: Conceptualization, H.H.M.; Formal analysis, M.A.I.E.; Investigation, M.A.; Methodology, R.T.H.; Resources, L.N.; Supervision, G.F.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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