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Review

The Glucosinolates: A Sulphur Glucoside Family of Mustard Anti-Tumour and Anti-Microbial Phytochemicals of Potential Therapeutic Application

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Abstract: This study reviewed aspects of the biology of two members of the glucosinolate family, namely sinigrin and glucoraphanin and their anti-tumour and anti-microbial properties. Sinigrin and glucoraphanin are converted by the β-sulphoglucosidase myrosinase or the gut microbiota into their bioactive forms, allyl isothiocyanate (AITC) and sulphoraphanin (SFN) which constitute part of a sophisticated defence system plants developed over several hundred million years of evolution to protect them from parasitic attack from aphids, ticks, bacteria or nematodes. Delivery of these components from consumption of cruciferous vegetables rich in the glucosinolates also delivers many other members of the glucosinolate family so the dietary AITCs and SFN do not act in isolation. In-vitro experiments with purified AITC and SFN have demonstrated their therapeutic utility as antimicrobials against a range of clinically important bacteria and fungi. AITC and SFN are as potent as Vancomycin in the treatment of bacteria listed by the World Health Organisation as antibiotic-resistant "priority pathogens" and also act as anti- cancer agents through the induction of phase II antioxidant enzymes which inactivate potential carcinogens. Glucosinolates may be useful in the treatment of biofilms formed on medical implants and catheters by problematic pathogenic bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* and are potent antimicrobials against a range of clinically important bacteria and fungi. The glucosinolates have also been applied in the prevention of bacterial and fungal spoilage of food products in advanced atmospheric packaging technology which improves the shelf-life of these products.

Keywords: glucosinolate; sulphopharane; allyl isothiocyanate; phase II detoxification enzymes; anti-tumour agents; anti-bacterials.

1. Introduction

Plants produce a myriad of phytochemicals and many of these have valuable nutritive, medicinal and health promoting properties [1-3] (Table 1-3). Anecdotal evidence often points to these beneficial properties however in this report we will concentrate on two members of the glucosinolates (Fig 1), Glucoraphanin and Sinigrin with a very extensive scientific and nutritional literature illustrating their potential therapeutic applications [3-12] (Table 2).

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Cruciferous plants such as those listed in Table 1 represent important components of a healthy diet and have characteristic spicy flavor profiles which are appealing to many and have important effects in a number of physiological processes.

Table 1.

Examples of Glucosinolate rich Cruciferous plants of the *Brassicacea* family order Capparales

Brocolli, Brocolli Sprouts	White Mustard (*Sinapis alba*)
Cabbage	Yellow Mustard (*Brassica juncea*)
Brussell Sprouts	Bok Choi
Cauliflower	Arugula, Rocket (*Eruca sativa*)
Daikon (Japanese radish), Daikon sprouts	Collard Greens
Garden Cress (*Lepidum sativum*)	Horseradish
Kale	Kohlrabi
Rapeseed (*Brassica napus*)	Radish
Wasabi (*Wasabia japonica*)	Rutabaga/turnip
	Watercress
	Mustard Greens

2. The natural anti-microbial activity of glucosinolate rich foods.

When plant tissues are damaged, myrosinase, a β-thioglucosidase converts the glucosinolates (Fig 1) to nitriles, thiocyanates and isothiocyanates (Fig 2) which have potent anti-microbial activity with the isothiocyanates in particular displaying potent antibacterial and anti-fungal activity profiles (Fig 3). The inclusion of dietary cruciferous vegetables rich in the glucosinolates may counter antibiotic resistant bacteria in the food chain arising from the overuse of antibiotics in animal rearing practices. The traditional use of mustard derived flavoring condiments while contributing desirable flavor profiles to cooked food items also provide food preservative properties which traditional societies have relied upon in the prevention of microbial spoilage of foods [13, 14]. This is particularly important in climatic conditions and ambient temperatures conducive to microbial growth leading to food spoilage[15]. Until relatively recently, these societies did not have access to refrigerated storage facilities thus mustard seed products played an important role in food preservation. Mustard seed oil is a potent source of bioactive glucosinolates[15] and represents approximately 30% of the edible oil market in SE Asia, the widespread use of this oil has positively contributed to food storage properties and protection from microbial infection [13, 14]. The glucosinolates and their derivatives are volatile compounds and this property has been applied in modern gaseous food packaging technology to extend the shelf-life of food products[14, 16]. All cereals have fungal spores associated with the grain surface and the husk thus whole milled cereal flours used for bread production contain fungal spores. These are inactivated during the baking process however fungal spoilage of bread and bakery products can still occur in the post baking storage and or processing of bakery products. Rape seed oil or mustard flour have been evaluated in bread production to minimize fungal spoilage [17], the major active glucosinolates in rape seed Brown mustard (*Brassica juncea*) oil are AITC (85%) and butenyl isothiocyanate (10%) [13] and these have broad fungicidal activity (Fig 3). In the bakery environment, 2 ppm AITC inhibited the growth of *Penicillium roqueforti,*

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P. corylophilum, Eurotium repens, A. flavus and Endomyces fibuliger on rye bread stored in an airtight environment [18]. Modified atmospheric packaging formats (85% CO_2, 1% O_2) combined with mustard oil vapour packaging has been used to extend the storage properties of bread and bakery products [19] and enhances the potency of AITC as a preservative [19, 20].

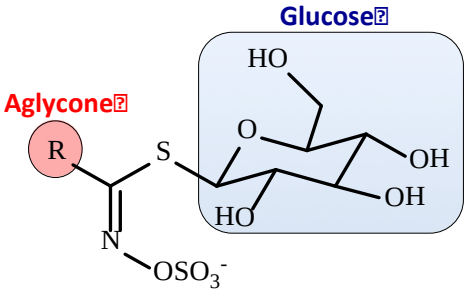

Figure 1. Generic structure of the Glucosinolates showing glucose, sulphation and the aglycone side chain (R) used to categorize the aliphatic, indolic or aromatic glucosinolates.

3. The Brassicaceae family of plants

As already indicated, the Brassicaceae are a rich source of sulphur glucoside glucosinolates, these impart a characteristic spicy flavor profile to these vegetables. Glucosinolates have been classified into three categories on the basis of their amino acid precursors (i) aliphatic (e.g., glucoraphanin; Ala, Leu, Ileu, Val, Met), (ii) indole (e.g., glucobrassicin; Trp), and (iii) aromatic (e.g., gluconasturtiin; Phe, Tyr). While ~130 glucosinolates have been identified to date, in a survey of 2,121 German participants in the European Prospective Investigation into Cancer and Nutrition (EPIC study), only five of these glucosinolates were commonly found in the human diet, glucobrassicin, sinigrin, glucoraphasatin (dehydroerucin), glucoraphanin, and glucoiberin [21].

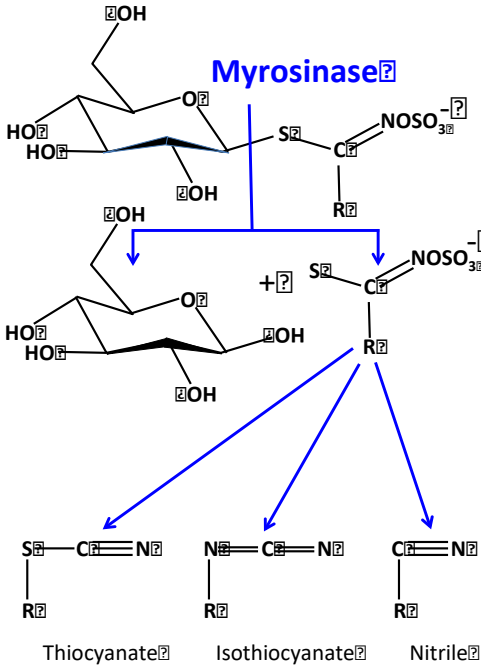

Figure 2. Enzymatic processing of the glucosinolates by myrosinase into bioactive components.

Glucosinolates have only been found in dicotyledonous plants occurring mainly in the Capparales order (Fig 1, 2) including cruciferous vegetables and the mustards *Brassica juncea* (brown

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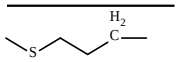
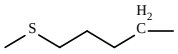
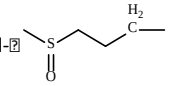
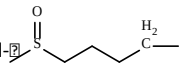
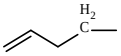
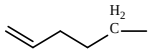
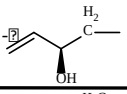
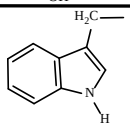
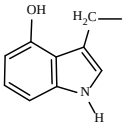
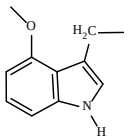
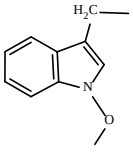
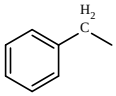
mustard) [22], *Brassica napus*. (rape seed) and the popular Japanese condiment horseradish Wasabi (*Eutrema japonicum* or *Wasabia japonica*) [23, 24](Table 1). The glucosinolates are stored in a concentrated form in the seed heads and are extracted in cold pressed oils but are also components of the stem and leaves of these plants (Fig 4).

Table 2.Examples of The Aliphatic, Indolic and Aromatic Glucosinolate Forms.*
Trivial and Chemical names, structures of the glucosinolates and their contents in *Brassica* vegetables of the Capparales order (mmol/100g wet weight tissue).

Trivial name	Chemical name	Structure (R)	Vegetable source	Glucosinolate Content mmol/100g
A. Aliphatic	**Aglycone**			
Glucoibervirin	3-Methylthiopropyl-		Green cauliflower White cauliflower	0-11.8 1.5-7.1
Glucoerucin	4-Metylthiobutyl-		Rocket	52-109
Glucoiberin	3-Methylsulfinylbutyl-		Brocolli sprouts Savoy cabbage	59-181 24-50
Glucoraphanin	4-Methylsulfinylbutyl-		Brocolli sprouts Brocolli	233-676 24-285
Sinigrin	Prop-2-enyl-		Brussels sprouts White cauliflower	46-91 57-121
Gluconapin	But-3-enyl-		Pak choi	24-157
Glucobrassicanapin	Pent-4-enyl-		Chinese cabbage Pak choi	2.3-25 27-69
Progoitrin	(2R)-2-Hydroxybut-3enyl-		Turnip Chinese brocolli	18-41 49
B. Indolic				
Glucobrassicin	Indol-3-ylmethyl-		Many vegetables eg Brocolli White cauliflower	13-29 11-33
4-Hydroxy-Glucobrassicin	4-Hydroxy-indol-3-ylmethyl-		Many vegetables eg Brocolli White cauliflower	0.1-3.3 0.2-2.8
4-Methoxy-Glucobrassicin	3-Methoxy-indol-3-ylmethyl-		Many vegetables eg Brocolli White cauliflower	0.9-2.8 0.7-3.2
Neo-Glucobrassicin	4-Methoxyindol-3-Ylmethyl		Many vegetables eg Brocolli White cauliflower	1.8-13 0.9-3.0
C. Aromatic				
Glucotropaeolin	Benzyl-		Garden cress	
Gluconasturtiin	Phenylethyl-		Water cress	

* data modified from [25-27].

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The Antibacterial and Antifungal Profiles of the Activated Glucosinolates

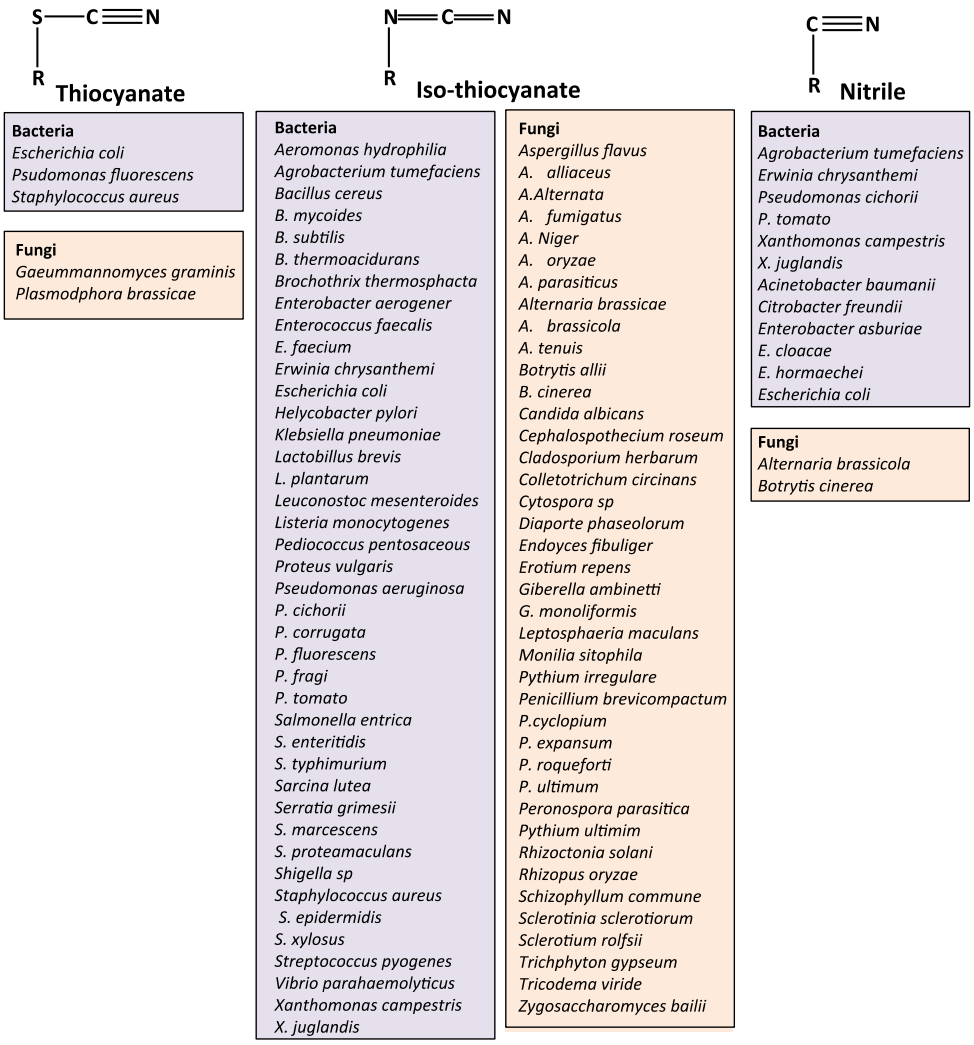

Figure 3. The anti-microbial activities of glucosinolate thiocyanate, iso-thiocyanate and nitrile derivatives.

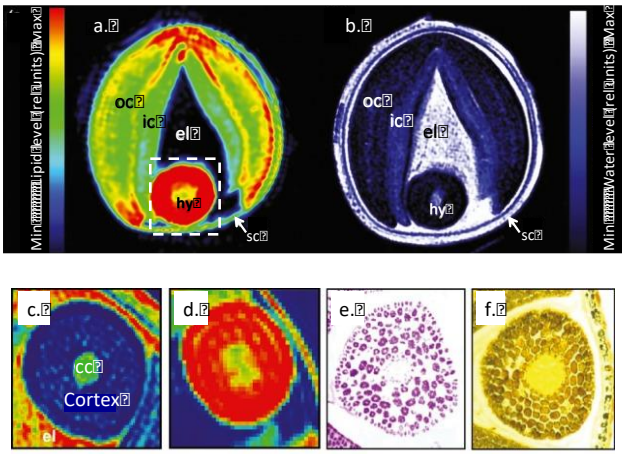

Figure 4. Lipid and moisture storage in *Brassica napus* seeds (a, b) and hypocotyl (c, d) visualized by non-invasive MRI. The concentration of water and oil are colour coded red (high); blue, (low). Crucifern immunolocalisation (e) and iodine stained starch (f) modified from [28] under Creative Commons Deed Attribution licence 2.5. oc,/ic outer/inner cotyledon; el, endosperm; hy, hypocotyl; sc, seed coat; cc, central cylinder.

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The mustard plant, Rape seed, yellow, white and brown mustards are widely distributed and have a characteristic yellow flower head (Fig 5). Rapeseed (*Brassica napus*), also known as rape, oilseed rape [29] is a member of the *Brassicacea*, mustard or cabbage family named from the Latin word for turnip, *rapum* [30]. This is an ancient plant known of since Biblical times and has even been identified in the fossil record of the Mesozoic era/mid-Devonian period in Western China. Identification of fossil remains in food cooking implements suggest that mustard seeds may have been the first ever condiment used to flavor food by Prehistoric man [31]. Plant evolutionary studies show that the mustard seed plant was of fundamental importance to the subsequent evolution of most other modern day cultivated plants. The leaves, seeds, and roots of wild mustard *Cleome viscos*a have all been widely used in traditional and folkloric medicine for generations. In Ayurvedic medicine mustard was reported to have many beneficial properties, subsequent scientific and pharmacological studies verified it's antimicrobial, analgesic, anti-inflammatory, antipyretic, anti-diabetic and hepatoprotective qualities [32-35]. Subsequent studies have identified the phytochemicals responsible for these activities as shown in the present study, glucosinolates are prominently represented on this list of bio-active compounds.

Brassica napus was botanically described and published in *Species Plantarum* by Carl Linnaeus who introduced the binomial name *Brassica napus* for the first time in 1887 [29] (Fig 5).

Figure 5. Anatomical description of a mustard (*Brassica napus*) plant showing its characteristic four petal flower head, stamen, seed pods, leaf arrangements and seeds. Image from Franz Eugen Koehler archive, Kohlers Medicinal Plants, Germany 1887. Image reproduced from Wikimedia Commons Repository through Open Access. [File:Brassica napus - Köhler–s Medizinal-Pflanzen-169.jpg|Brassica napus - Köhlers Medizinal-Pflanzen-169].

Rapeseed oil is one of the oldest known vegetable oils, but historically has been used in limited quantities as a food item due to its high levels of erucic acid, natural rapeseed oil can contain up to 54% w/v erucic acid [36]. Rapeseed cultivated for food production typically contains ~0.5-5% w/v

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erucic acid. Erucic acid ($C_{22}H_{42}O_2$) is a C22 chain mono-unsaturated omega-9-fatty acid. A strain of mustard subsequently developed with low erucic acid and glucosinolate levels, Canola, a contraction of the terms "*Canada*" and "*ola*", is a low erucic acid, low glucosinolate rapeseed [37]. Canola oil is limited by government regulation to a maximum of 2% w/v erucic acid in the USA and 5% w/v in the EU. In 1992, the health promoting properties of Rapeseed oil gained publicity in the George Miller feature film "Lorenzo's Oil" starring Nick Nolte and Susan Sarandon which documented the work of a British chemist, Don Suddaby, and Augusto Odone in 1985 who developed a blend of rapeseed and olive oils which halted the progression of Adrenoleukodystrophy, a genetic disorder characterized by an enzyme abnormality resulting in the build up of toxic fatty acid levels in the brain damaging the myelin sheaths impairing neuronal function and resulting in convulsions, seizures and hyperactivity. The anti-oxidant properties of activated glucosinolate compounds are also conducive to the maintenance of brain health [38-48]. The brain is a fatty acid rich tissue and particularly prone to redox ROS mediated mitochondrial damage during neuroinflammation [49, 50].

4. Public health concern over the impact of antibiotic resistant bacteria.

There is considerable current day public concern about the over-use of antibiotics in husbandry practice in order to maintain animal health and commercial output levels. The emergence of antibiotic resistant organisms in humans is related to this agricultural practice. This has been acknowledged by the WHO and by the publication of government guidelines on the use and abuse of antibiotics in agricultural practice. The publication of a list of antibiotic resistant pathogenic bacteria of particular concern by the WHO (Table 3) and the allocation of major research funds to national agencies in the USA, Canada and Australia to address the problem of antibiotic resistant bacteria testifies to the significant threat these organisms represent to human health.

4.1 Treatment of antibiotic resistant bacterial infections

Antibiotics and antimicrobial agents, have been used for the last 70 years to treat human infectious diseases. Since the 1940s, these drugs greatly reduced illness and death from infectious diseases. However, these drugs have been used so widely and for so long that the infectious organisms the antibiotics are designed to kill have adapted to them, making these drugs far less effective. Each year in the USA, at least 2 million people become infected with bacteria that are resistant to antibiotics with at least 23,000 deaths recorded as a direct result of these infections. Multi drug resistant bacterial infections were also responsible for an estimated 25,000 deaths per year in the EEC in 2015-2017 and these cost €1.5 billion per year in healthcare treatment costs and lost productivity. If these current infection rates are not reversed then 10 million deaths globally per year are predicted by 2050, (317,000 in USA; 392,000 in S.America; 392,000 in EEC; 4.1 million in Africa; 4.7 million in Asia and 22,000 in Australia). Moreover it is estimated that additional hospital costs per patient will be in the order of 10-40,000 $US in OECD countries. Furthermore, the associated impact of lost economic output due to increased mortality, prolonged sickness and reduced labour efficiency may effectively double this figure. In-vitro studies on the activated thiocyanates, isothiocyanates and nitrile compounds generated from the glucosinolates by myrosinase demonstrate these are suitable compounds for anti-bacterial and anti-fungal evaluations

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in the treatment of such infections (Fig 3). Furthermore, some of these plant compounds synergise with existing antibiotic treatment protocols (gentamycin, vancomycin) and may represent a useful adjunct to these treatments [51]. *Listeria monocytogenes* and *Staphylococcus aureus* in particular were significantly inhibited by benzylisothiocyanate and 2-phenylethylisothiocyanate in isolation or in phytochemical-antibiotic combinations.

Despite the fact that bacterial infections are already one of the leading causes of death globally and that mortality rates are escalating at alarming rates, no new antibiotics have been produced by the pharmaceutical industry in more than a decade. The WHO has warned of the possibility that we may be entering a "post-antibiotic era" within this century. Bacteria resistant to all known antibiotics are becoming increasingly common and already producing untreatable infections.

The repurposing of anticancer drugs for the treatment of bacterial infections has been suggested since some of these have proven to be effective in-vitro for the elimination of recalcitrant, multidrug tolerant bacteria while other antibiotics are useful as anti-cancer compounds [52-55]. Among the most harmful human pathogenic bacteria, *Staphylococcus aureus (Golden Staph)* stands out as one of the most virulent and troublesome due to its ability to cause life-threatening infections and to readily adapt to changing environmental conditions [56, 57]. The ability of *S.aureus* to establish itself in various community home and hospital environments, and its resistance to antibiotic treatment make this an important healthcare threat [58]. The emergence of methicillin resistant *S.aureus* (MRSA) almost 5 decades ago demonstrates the serious nature of such infections. Hospital environments are conducive to *S.aureus* colonisation and its virulence is a major threat particularly to patients with reduced immune function. Particularly virulent strains of *Enterococcus*, resistant to conventional antibiotic treatment, have also emerged in hospitalized patients [59]. Of particular concern are the vancomycin-resistant enterococci (VRE), that lead to infections of the urinary tract associated with prolonged catheter use or to catheter mediated bloodstream infections [60]. There is therefore an increasing global interest in the identification of bioactive compounds from plant sources, which display antibacterial and antifungal properties that are pharmacologically effective but which display limited or no side effects. The glucosinolates produced by the *Brassicacea* family, order Capparales contain compounds with potent anti-bacterial, anti-fungal, anti-nematodicidal, anti-viral and insecticidal properties making them obvious candidates in the search for compounds to counter bacterial infections [4, 10, 11, 61-67]. Morever, many of the glucosinolates act synergistically with existing antibiotic regimens improving their effectiveness [51, 64]. **A list of antibiotic-resistant "priority pathogens" published by WHO in 2017 covers 12 bacterial families posing the greatest threat to human health [68] and highlights gram-negative bacteria resistant to multiple antibiotics which threaten global public health, these have been referred to as Super-bugs [69-71].**

The effective antibiotics available for the treatment of bacterial infections are relatively small in number and in many cases have become largely ineffective. The last time a new antibiotic was released on to the world market was approximately 30 years ago, there is a strong need for antibiotic development and a world market eagerly awaiting this product. The WHO has established three treatment categories based on the urgency for new antibiotics: these are critical,

high and medium priority (Table 3). The most critical group of patients includes those infected with multidrug resistant bacteria that pose a particular threat in hospitals, nursing homes, and among patients whose care requires devices such as ventilators and blood catheters. These include *Acinetobacter*, *Pseudomonas* and various Enterobacteriaceae (*Klebsiella*, *E.coli*, *Serratia*, and *Proteus*). These can cause severe and often deadly bloodstream infections and pneumonia. Such bacteria have become resistant to a large number of antibiotics, including carbapenems and third generation cephalosporins, currently the best antibiotics for treating multi-drug resistant bacteria. The second and third tier bacteria in this list, the high and medium priority categories contain other increasingly drug-resistant bacteria that result in gonorrhoea and food poisoning caused by *Salmonella*. Gonorrhoea is rapidly becoming a condition which will soon become untreatable.

Table 3. World Health Organisation priority pathogen list*

Category	Bacterium
Critical	1. *Acinetobacter baumannii*, carbapenem resistant
	2. *Pseudomonas aeruginosa*, carbapenem resistant
	3. *Enterobacteriaceae*, ESBL** producing carbapenem resistant
High	1. *Enterococcus faecium*, - Vancomycin resistant
	2. *Staphylococcus aureus*, - Methicillin/Vancomycin resistant
	3. *Helicobacter pylori*, - Clarithromycin resistant
	4. *Campylobacter spp.* - Fluoroquinolone resistant
	5. *Salmonellae* - Fluoroquinolone resistant
	6. *Neisseria gonorrhoeae* , Cephalosporin/Fluoroquinolone resistant
Medium	1. *Streptococcus pneumonia*, Penicillin resistant
	2. *Haemophilus influenzae*, Ampicillin resistant
	3. *Shigella sp*, Fluoroquinolone resistant

*http://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

** Certain strains of bacteria are resistant to treatments with commonly used antibiotics such as penicillin and cephalosporins. These bacteria produce enzymes known as **E**xtended **S**pectrum **B**eta-**L**actamases (ESBL). ESBL producing bacteria are resistant to most types of third generation antibiotics and include strains of *Klebsiella pneumoniae, Klebsiella oxytoca and Escherichia coli, Enterobacter* spp., *Salmonella* spp., *Morganella morganii, Proteus mirabilis, Serratia marcescens* and *Pseudomonas aeruginosa* produce ESBLs relatively infrequently.

5. WHO, United Nation and World Bank programmes and co-ordinated inter-agency collaborations designed to combat antibiotic resistant bacteria.

The Global Antimicrobial Resistance Surveillance System (GLASS) is a WHO initiative supporting a standardized approach to the collection, analysis and sharing of data related to antimicrobial resistance at a global level to inform decision-making, and drive local, national and regional action. The Global Antibiotic Research and Development Partnership (GARDP) is a joint initiative of WHO and Drugs for Neglected Diseases initiative (DNDi), GARDP encourages research and development through public-private partnerships. Interagency Coordination Group on Antimicrobial Resistance (IACG), an initiative of the United Nations Secretary-General, was established to improve coordination between international organizations ensuring effective global action against this threat

to health security [72]. By 2023, this partnership aims to develop and deliver up to four new treatments, through improvement of existing antibiotics and acceleration of the entry of new antibiotic drugs. The IACG is co-chaired by the UN Deputy Secretary-General and the Director General of WHO and comprises high level representatives of relevant UN agencies, other international organizations, and individual experts across different sectors.

The Centre for Disease Control (CDC) and related US agencies are also actively involved in a number of measures to combat antibiotic resistant bacterial infections through a collaborative global approach across all government and private sector agencies. CDC has published "CDC. The Core Elements of Human Antibiotic Stewardship Programs in Resource -Limited Settings: National and Hospital Levels. Atlanta, GA: US Department of Health and Human Services, CDC; 2018. Available at: https://www.cdc.gov/antibiotic-use/healthcare/implementation.html" to help improve guidelines for antibiotic use in healthcare settings worldwide; The Food and Drug Administration (FDA) has also announced plans to combat antibiotic resistance through innovative antibiotic developments and the co-ordinated use of antibiotics in human medicine and in animal husbandry practice. CARB-X, a global non-profit partnership, led by Boston University launched in 2016 is dedicated to accelerating antibacterial research to tackle the global threat of drug-resistant bacteria and is now funding 33 projects in 7 countries in N. America, Europe and Asia. CARB-X is funded by the US Department of Health and Human Services Biomedical Advanced Research and Development Authority (BARDA), part of the Office of the Assistant Secretary for Preparedness and Response (ASPR), the Wellcome Trust, a global UK based charity working to improve global health, Germany's Federal Ministry of Education and Research (BMBF), the UK Government's Global Antimicrobial Resistance Innovation Fund (UK GAMRIF), the Bill & Melinda Gates Foundation, the world's largest foundation dedicated to improving the quality of life for individuals around the world, and receives in-kind support from National Institute of Allergy and Infectious Diseases (NIAID), part of the US National Institutes of Health (NIH) and will invest >$500 US million by 2021 into research and development into new classes of antibiotics to battle the deadliest superbugs, vaccines, rapid diagnostics, and other life-saving products. This supports *The National Action Plan for Combating Antibiotic-Resistant Bacteria*, a document released by the U.S. Government AR: https://www.cdc.gov/DrugResistance/us-activities.html. Strategies being developed in Australia to combat bacterial resistant infections involve a unified approach by all government and private agencies to combat the threat of antibiotic overuse and development of antibiotic resistant bacterial infections in Australia following recommendations outlined in "*Responding to the threat of antimicrobial resistance*, *Australia's First National Antimicrobial Resistance Strategy 2015–2019" Australian Government , Department of Health, Department of Agriculture (June 2015).* ISBN: 978-1-76007-191-2 Online ISBN: 978-1-76007-192-9.

6. Application of the myrosinase-glucosinolate system in Biomedicine

The bioactivity of glucosinolate hydrolysis products and potential biomedical applications are well documented (Table 3, 4, 5). SFN has roles in cancer prevention, high blood pressure, macular degeneration and stomach ulcers and is a potent inducer of mammalian phase II detoxication enzyme systems which deactivate and excrete many carcinogens. The induction of NAD(P)H quinone reductase, heme oxygenase 1 (HO-1), glutamate-cysteine ligase catalytic subunit, and glutathione S transferases occurs through the Keap1-Nrf2-ARE cell signaling pathway [73-75].

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Under quiescent conditions, KEAP1 protein binds Nuclear factor erythroid 2-related factor-2 (Nrf2) in the cytoplasm and represses its activation. Nrf2 is a master regulator of genes in many diseases [74] and its activation leads to a co-ordinated anti-oxidant and anti-inflammatory response in many disease states including many forms of cancer [6]. Sulphoraphane is a potent inducer of Nrf2 activity [76] inducing cytoprotective genes with key roles in cellular defence mechanisms that regulate redox status and detoxification processes [73] and protection from oxidative damage during traumatic injury and inflammation. The Keap1-Nrf2 pathway is a major regulator of cytoprotective responses to endogenous and exogenous stresses caused by reactive oxygen species (ROS) and electrophiles. Keap1 (Kelch ECH associating protein 1) binds to Nrf2 promoting its degradation by the ubiquitin proteasome pathway regulating cytoprotective responses to oxidative stress in cancer and neurodegeneration [73, 75, 77-80]. Numerous studies in human colon, leukemia, pancreatic, lung, and skin cancer cell lines have demonstrated SFN's inhibitory effects on cell cycle arrest [12, 81-83] and elevated apoptosis in human bladder [84] and prostate [85] cell lines. Sulforaphane's ability to disrupt tubulin and actin polymerization, inhibits mitotic spindle formation and tumour cell growth in animal models of breast cancer [86, 87] and also inhibits histone deacetylase, increasing apoptosis in human colon, prostate, and kidney cell lines [88-91].

6.1 The bioactivity of glucosinolates

The glucosinolates are benign molecules requiring conversion by myrosinase to bioactive thiocyanate, isothiocyanate and nitrile derivatives (Fig 2). Thus glucoraphanin and sinigrin are converted into bioactive SFN and AITCs with fungicidal, bactericidal, nematocidal, anti-oxidant and anti cancer properties (Fig 6). Biofilm formation on medical devices and implants such as catheters, mechanical heart valves, pacemakers, prosthetic joints, and contact lenses pose a critical medical problem. The most common biofilm-forming bacteria include *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus viridans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* [92-97], *S.aureus* and *S.epidermidis* are most commonly found on cardiovascular devices [98-100], it estimated that 40%–50% of prosthetic heart valve infections, and 50%–70% of catheter biofilm infections are due to these bacteria [101, 102]. Despite the evaluation of a wide range of anti-fouling compounds [97, 103, 104] improvements are still required in this area. Glucosinolates have proven useful in the prevention of bio-film development by *Pseudomonas aeruginosa* [5, 105-107].

Cooking of cruciferous vegetables inactivates myrosinase activity however the gut microbiota in humans may provide myrosinase activity and lead to absorption of SFN and AITCs in the intestine. As already noted, when the glucosinolates are converted to their bioactive forms they induce phase II enzymes like glutathione S-transferase (GST) through the KEAP1/Nrf2/ARE pathway, that are critical in mutagen elimination [108]. Sulphoraphane also has chemopreventive properties mediated through its ability to inhibit phase I enzymes responsible for the activation of pro-carcinogens and by induction of phase II detoxification enzymes and also mediates activation of apoptosis, induction of cell cycle arrest, disruption in tubulin assembly and tubular microdynamics and inhibition of NFκB [109]. A diet rich in cruciferous vegetables is associated with a lower risk of developing breast, lung, prostate, and colorectal cancer [110-114], consumption of three to five servings per week is reported to decrease the risk of cancer development by 30%–40% [115]. It is

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important to control the redox balance of oxidant and anti-oxidant species in the human brain since these control neuronal mitochondrial activity which under oxidant stress can diminish neuronal energetics and promote neurodegeneration in Parkinson's and Alzheimers's disease [116]. Brain tissue is very rich in fatty acids and is especially sensitive to the action of excess oxidant activity which can occur focally if GST activity is insufficient [117]. The GSTs are ROS scavengers and are neuroprotective [116, 118, 119], promote microglial activation and proinflammatory astrocyte-microglia communication [120]. GST polymorphisms lead to neuronal dysfunction and pathological changes in glioblastoma, Alzheimer's and Parkinson's disease, stroke, epilepsy [121, 122], multiple sclerosis (MS) [123] and deleteriously impact repair mechanisms following ischemic stroke [124-126]. Induction of GSTs and other phase II detoxification enzymes by bioactive forms of the glucosinolates maintains normal functional properties in the brain. Histone acetylation plays a crucial role in chromatin remodeling and regulates its packing density around chromosomes and their constituent genes. Dense packing can deny transcription factor access to genes thus histone acetylation-deacetylation has an organisational role over chromatin structure and gene accessibility to transcription factors indirectly regulating gene expression [109, 127-129].

Table 4.

Combination Therapies of Sulphoraphane and Conventional Anti-Cancer and Anti-bacterial Drugs

Compound used in Combination Therapy	Ref
SFN-Selenium nanoparticles	[130]
Paclitaxel	[9]
Cisplatin	[131]
Luteolin	[132]
Clofarabine	[133]
Doxorubicin	[134]
5-fluorouracil	[135]
HistoneH3	[136]
Withaferin A	[137]
Hispidulin	[138]
Carboplatin	[139]
Docetaxel	[140]
Lapatinib	[141]
PR-104A	[142]

Table 5. The Diverse Therapeutic Applications of Sulphoraphane

Miscellaneous medical conditions treated with Sulphoraphane	
Spatial learning and memory dysfunction	[143]
Chemotherapy-induced neuropathic pain	[144]
SFN-decorated gold nanoparticle for anti-cancer treatment	[145]
Protection of granulosa cells against oxidative stress	[146]
Epigenetic Nrf2 signaling pathway	[147]
Cadmium-mediated carcinogenesis	[148]
Oxidative stress in cultured adult cardiomyocytes	[149]

Protective effects of glucosinolate hydrolysis products in neurodegenerative diseases	[150]
Clearance of Amyloid-β and Tau protein in a mouse model of AD	[151]
Experimental diabetic peripheral neuropathy	[152]
Joint inflammation in a murine adjuvant-induced mono-arthritis	[153]
Protection against cognitive impairment in AD-like lesions in diabetes	[154]
Anti-inflammatory effect of SFN on human THP-1 macrophages in a murine AD model	[155]
Inhibition of oxidative stress/inflammation improves cardiac function in a Rabbit Model of Chronic Heart Failure	[156]
Inhibition of class IIa histone deacetylase activity	[157]
Apoptosis via microtubule disruption in cancer	[158]
Inhibition of LPS-Induced Inflammation/cytotoxicity/oxidative microglial stress	[159]
Down-regulation of MAPK/NF-κB signaling in LPS-activated BV-2 microglia	[160]
Epigenetic modification of Nrf2 signalling in a model of AD	[161]
Inhibition of oxidative stress in an In-vitro model of age-related macular degeneration	[162]
Prevention of angiotensin II-induced cardiomyopathy by activation of Nrf2 and Akt/GSK-3ß/Fyn pathway.	[163]
Suppression of NLRP3 inflammasome alleviating acute gouty inflammation	[164]
Modification of Histone H3, unpacking of chromatin, to prime defence	[136]
Nrf2-Inducers Counteract Neurodegeneration in Friedreich's Ataxia	[165]
Modulation of oxidative stress and inflammation in rats with toxic hepatitis	[166]
Modulation of oxidative damage in lead exposed rat hippocampus	[167]
Prevention of dexamethasone-induced myotube atrophy via Akt/Foxo1	[168]
Induction of p53 deficient SW480 cell apoptosis by ROS MAPK signaling	[169]
Role of microRNAs in the chemopreventive activity of SFN	[170]
Upregulation of Nrf2 protection in doxorubicin-induced chronic heart failure	[171]
Increased Nrf2 expression protects alveolar epithelial cells against oxidative injury	[172]
Novel phosphonate analogs of SFN with in vitro and in vivo anticancer activity	[173]
Inhibition of PDGF-induced vascular SMC proliferation by targeting mTOR/p70S6kinase signalling independently of Nrf2 activation	[174]
Gastrointestinal protection against *H. pylori* and NSAID-Induced Oxidative Stress	[175]
Protection from cerebral ischemic/reperfusion injury via inhibition of NLRP3 inflammasome activation in rats	[176]
Protection against sodium valproate-induced acute liver injury	[177]
Enhanced SFN cardioprotection against oxidative stress by 17β-Estradiol	[177]
Photoprotective Effects of SFN and Hispidulin	[138]
Differential modulation of mitochondrial biogenesis/dynamics in normal & tumor cells	[178]
Nrf2 targeting by SFN: A potential therapy for cancer treatment	[179, 180]
Improvement of neuronal mitochondrial function in brain tissue	[181]
Protection of pancreatic Acinar cell injury by modulating Nrf2-mediated oxidative stress and the NLRP3 inflammatory pathway	[182]
Improvement in chemotherapy efficacy targeting cancer stem cell-like properties	[183]
Protection against rotenone-induced neurotoxicity via mTOR, Nrf2, and autophagy	[184]

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Chemoprevention of oxidative stress-associated with oral carcinogenesis	[185]
Amelioration of bladder dysfunction via activation of Nrf2-ARE Pathway	[186]
Broccoli sprout homogenate treatment for Sickle Cell Disease	[187]
Treatment of Autism Spectrum Disorder	[188, 189]
Protection against aortic complications in diabetes	[190]
Anti-inflammatory effect against amyloid-β peptide via STAT-1 dephosphorylation and activation of Nrf2/HO-1	[191]
Inhibition of NLRP3 inflammasome signaling dose-dependently attenuating foot swelling and neutrophil recruitment decreasing foot IL-1β levels and caspase-1 activity in animals with acute gouty arthritis	[164, 192]

Table 6. Therapeutic Application of Sulphoraphane in Cancer Models

Cancer type	Ref
Leukemia	[81, 193-198]
Prostate cancer	[85, 89, 199-201]
non-small cell lung cancer cells	[139, 202, 203]
Pancreatic cancer	[182, 204-206]
Breast cancer	[86, 87, 133-135, 137, 140, 141, 207-214]
Bladder cancer	[186, 215-220]
Ovarian cancer	[131]
HepG2 Carcinoma Cells	[221-225]
Gastric cancer	[226, 227]
Squamous cell carcinoma	[228, 229]
Nasopharangeal cancer	[230]
Melanoma	[231]
Glioma	[190, 232-234]
Colon cancer	[142, 235, 236]
Lung cancer	[237, 238]
Schwannoma	[239]
Colorectal cancer	[240]
Cervical cancer	[241]
Oral cancer	[242, 243]

6.2 Cancer and dietary SFN and AITC levels

Meta analyses of clinical trials on dietary glucosinolates have generally provided promising but not compelling evidence of the efficacy of these as anti-oxidants or anti-cancer agents despite positive in-vitro findings in cell culturing experiments and may reflect the inefficiencies of the dietary route for delivery of these compounds. Positive effects are generally achieved in-vitro with concentrations of the active glucosinolate components in the 1-40 μmol range. It is unlikely that this level of therapeutic agent would be delivered successfully to the target tumour cells in-vivo by the diet. Attempts have been made to increase the glucosinolate content of broccoli hybrids, broccoli sprouts are also richer sources of the glucosinolates particularly since these are consumed uncooked

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thus endogenous myrosinase is not inactivated by the cooking process and it has time to convert the glucosinolates to bio-active forms during food mastication. The detection of SFN and AITCs excreted in urine and faecal matter following consumption of cooked cruciferous vegetables where the endogenous myrosinase is inactivated in the initial cooking stages, indicates that the gut microbiota are another source of myrosinase activity. Thus therapeutic doses of SFN and AITCs are likely achievable to target tumour cells in the colon [104, 210, 211], prostate [85, 89, 199-201] and bladder [186, 215-220]. Dietary glucosinolates are also effective in the treatment of gastric *H.Pylori* infections and gastric cancer. The delivery of therapeutic doses of dietary SFN and AITCs through the systemic circulation to pancreatic, ovarian, breast and liver cancer and melanoma however is less likely to be as effective and may explain the relatively poor findings of meta analyses of dietary clinical trials on the glucosinolates as anti-cancer agents. In many cases the statistical power achieved in these analyses has also been recuced by low sample sizes or no associations were established. More high quality cohort studies with larger sample sizes, and well controlled confounding factors is required to confirm the benefit of dietary cruciferous vegetable consumption, initial studies have delivered sufficient evidence to warrant such studies. The bioavailability of glucosinolates following different food processing methods has also been evaluated in order to improve the dietary content of bioactive forms of the glucosinolates [244] Supplementation of the diet with broccoli sprouts or myrosinase containing mustard products have also been examined as a means of increasing the dietary SFN and AITC content [245]. The effective delivery of SFN and AITCs to the target cells in solid tumours is a difficult proposition. Delivery systems based on hyaluronan as a carrier molecule have been developed for a number of steroids and cytotoxic compounds and successfully treated solid tumours however this methodology has yet to be applied to the delivery of SFN or AITCs in these problematic cancers (reviewed in [246]).

6.3 The beneficial bioactivities of sinigrin and their applications in biomedicine.

Although the scientific literature on sinigrin is not as extensive as that of SFN they share similar bioactivities and areas of application and if supplied as a dietary component will not be acting in isolation anyway [107].

Table 7 The Varied Applications of Sinigrin in Biomedicine

Application	Ref
Reduction of liver fibrosis	[247]
Suppression of NF-κB/MAPK and NLRP3 inflammasome activation in macrophages	[248]
Promotion of wound healing	[107, 249]
Anti-cancer properties in methyl glyoxal modification	[250]
.Anti-proliferative activitiy on carcinogen-induced hepatotoxicity	[251]
Biofumigation of potato cyst nematode	[22]
Inhibition of Listeria monocytogenes on bologna sausages	[106]
inhibition of invasion, migration, MMP-2/-9 activities in SK-Hep 1 human hepatoma cells	[252]
Brussel sprout juice mediated effects on cell cycle and adhesion of human colorectal carcinoma cells (HT29) in vitro	[253]
AITC mediated mitotic block, loss of cell adhesion/disrupted cytoskeleton in HT29 cells	[254]
Cytotoxicity and genotoxicity of allyl and phenethyl isothiocyanates, glucosinolates, sinigrin and gluconasturtiin	[255]

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Inhibition of microbial growth	[51, 66, 256]
Effects of dietary sinigrin or indole-3-carbinol on O6-methylguanine-DNA-transmethylase activity and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced DNA methylation and tumorigenicity in F344 rats	[257]

7. Concluding remarks

The myrosinase-glucosinolate system in plants is a sophisticated protective system that developed over several hundred million years of evolution. With a greater understanding of its component parts it is now possible to apply some of these to physiological processes in man of potential benefit in biomedicine. Some of these compounds may be useful in the prevention of fouling of plant equipment, sterilisation of medical implants, wound healing and the prevention of some forms of cancer. An extensive literature documenting the biodiversity of glucosinolate applications in Biomedicine indicate considerable promise in future areas of investigation in:-

1. Antibiotics, anti-fungal and anti-viral agents
2. Biofilm prevention in medical implants, catheters and Industrial plant equipment
3. Nutritive additives with anti-cancer properties
4. Advanced food packaging technology to improve shelf-life of food products.

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Abbreviations

AD	Alzheimers disease
AKT	a serine/threonine-specific protein kinase
ARE	antioxidant response element
EPA	Environment Protection Agency
ESBL	Extended Spectrum Beta-Lactamases
Keap-1-Nrf2-ARE	Kelch-like ECH-Associating protein 1-nuclear factor erythroid 2 related factor 2-antioxidant response element
AITC	Allyl isothiocyanate
GARDP	Global Antibiotic Research and Development Partnership
GSK	Glycogen Synthase Kinase
GST	Glutathione-S-transferase
DNDI	Drugs for Neglected Diseases initiative
IACG	Interagency Coordination Group on Antimicrobial Resistance
LPS	Lipopolysaccharide
MAPK	A mitogen-activated protein kinase
NFκB	Nuclear factor kappa light chain enhancer of activated B cells
NLRPR3	nucleotide-binding domain and leucine-rich repeat–containing protein 3
NSAID	Non Steroidal anti-inflammatory

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PDGF	Platelet derived growth factor
ROS	Reactive oxygen species
SMC	Smooth muscle cell
TNFα⊚⊚	⊚umour necrosis factor-alpha

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