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Natural plant extracts and compounds to improve the condition of gut microbiota

BACHELOR'S THESIS

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BACHELOR THESIS ASSIGNMENT

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Thesis title

Natural plant extracts and compounds to improve the condition of gut microbiota

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Objectives of thesis

The main aim of this thesis will be to identify natural plant extracts and compounds in specific plant species, which could be used to improve the condition of animals' gut microbiota. Subsequently to create a comprehensive overview of such plants and their respective properties for potential use as health-promoting feed additives.

Methodology

This thesis will be written in the form of a literature review. Scientific databases such as Web of Science, ScienceDirect, ResearchGate, Scopus, and PubMed will be used to compile and research information, as well as scientific books and textbooks. All relevant data was summarized in a comprehensive table.

The proposed extent of the thesis

30-50

Keywords

microflora, antimicrobial, selective effect, combinatory effect

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Declaration

I hereby declare that I have done this thesis entitled Natural plant extracts and compounds to improve the condition of gut microbiota independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 14. 04. 2023

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Sára Drozenová

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Abstract

The gut microflora plays a crucial role in the health of everyone, including both humans and animals. Therefore, improving the condition of gut microbiota has become an important aspect of animal health. In recent years, natural plant extracts and compounds have gained attention for their potential to improve gut health. This literature review aimed to identify natural plant extracts and compounds in specific plant species, which could be used to improve the condition of animals' gut microbiota. The plant species identified include *Thea sinensis, Withania somnifera, Astragalus membranaceus, Panax ginseng*, and *Scutellaria baicalensis*. These plants have been shown to possess various bioactive compounds such as flavonoids, alkaloids, saponins, and polysaccharides, which can promote the growth of beneficial gut microorganisms and inhibit harmful ones. These properties make these plant extracts and compounds potential candidates as health-promoting feed additives for livestock. Further research is needed to confirm the efficacy of these natural plant extracts and compounds in improving gut microbiota, as well as their safety and practicality as feed additives.

Key words: microflora, antimicrobial, selective effect, combinatory effect

Abstrakt

Mikroflóra trávicího traktu hraje klíčovou roli v zdraví jak člověka, tak zvířat. Zlepšení stavu mikroflóry trávicího traktu se proto stalo důležitým krokem pro zajištění zdraví zvířat. V posledních letech si získaly pozornost přírodní rostlinné extrakty a látky v nich obsažené, pro své potenciální využití ke zlepšení zdravotního stavu trávicího traktu. Tato literární rešerše měla za cíl identifikovat přírodní rostlinné extrakty a látky v nich obsažené v konkrétních druzích rostlin, které by mohly být použity ke zlepšení stavu mikroflóry trávicího traktu zvířat. Mezi identifikované druhy rostlin patří *Thea sinensis*, *Withania somnifera*, *Astragalus membranaceus*, *Panax ginseng* a *Scutellaria baicalensis*. Tyto rostliny obsahují různé bioaktivní látky, jako jsou flavonoidy, alkaloidy, saponiny a polysacharidy, které mohou podporovat růst prospěšných střevních mikroorganismů a potlačovat růst mikroorganismů škodlivých. Tyto vlastnosti dělají tyto rostlinné extrakty a látky v nich obsažené potenciálními kandidáty pro podpůrné přídavné látky pro krmení dobytka. K zapotřebí je další výzkum, pro potvrzení účinnosti těchto extraktů a látek ve zlepšování stavu mikroflóry trávicího traktu, stejně jako k ověření jejich bezpečnosti a praktičnosti jako přídavných látek pro krmení.

Klíčová slova: mikroflóra, antimikrobiální látka, selektivní účinek, kombinační účinek

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List of the abbreviations used in the thesis

As	arsenic
Ba	barium
Cd	cadmium
Cr	chromium
HPV	human papillomavirus
HSV	herpes simplex virus
IBD	inflammatory bowel disease
IL-6	interleukin-6
MRSA	methicillin resistant *Staphylococcus aureus*
MSSA	methicillin sensitive *Staphylococcus aureus*
NAFLD	non-alcoholic fatty liver disease
Ni	nickel
Rb	rubidium
RNS	reactive nitrogen species
ROS	reactive oxygen species
Sr	strontium
TNF-α	tumour necrosis factor-alpha
WIE	whole ingredient extract

1. Introduction

The gut microflora plays a crucial role in maintaining the overall health and well-being of both humans and animals. Disruptions of the gut microflora have been linked to several diseases, including metabolic diseases, leading to severe economic losses in the livestock industry (Colombino et al. 2021; Chen et al. 2021). The use of antibiotics in animal feed has been banned in many countries due to the development of antibiotic-resistant bacteria (European Commission 2005). Therefore, finding natural alternatives to improve animal gut microbiota's condition and promote animal health without the use of antibiotics is of great interest.

Tropical plant extracts are a rich source of natural compounds that have been used in traditional medicine for centuries to treat various illnesses. These plant extracts contain diverse bioactive compounds, including polyphenols, alkaloids, flavonoids, and terpenoids, that have antimicrobial, anti-inflammatory, and immunomodulatory properties (Darwish & Aburjai 2010; Miraj & Kiani 2016; Parihar 2022). Recent research has shown that tropical plant extracts can improve gut microflora's condition in animals by promoting beneficial bacteria's growth and inhibiting harmful bacterial colonization (Perez-Burillo et al. 2020). The use of tropical plant extracts as health-promoting food additives for animals offers several advantages over traditional antibiotics, including lower risk of antibiotic resistance development, improved animal welfare, and reduced environmental impact. However, more research is needed to determine the optimal dosage, safety, and efficacy of tropical plant extracts as feed additives in different animal species and production systems.

Thea sinensis, Astragalus membranaceus, Withania somnifera, Panax ginseng, and Scutellaria baicalensis are well-known medicinal plants with a long history of traditional use in different parts of the world. These plants contain a variety of bioactive compounds that have shown potential in improving the condition of gut microbiota and promoting animal health. The choice of these plants for the thesis is based on their known health benefits.

2. Aims of the Thesis

The main aim of this thesis will be to identify natural plant extracts and compounds in specific plant species, which could be used to improve the condition of animals' gut microbiota. Subsequently to create a comprehensive overview of such plants and their respective properties for potential use as health-promoting feed additives.

3. Methodology

This thesis was written in the form of a literature review. Scientific databases such as Web of Science, ScienceDirect, ResearchGate, Scopus, and PubMed were used to compile and research information, as well as scientific books and textbooks. All relevant data was summarized in a comprehensive table.

4. Literature Review

4.1.1. Thea sinensis

4.1.1.1. Description

Thea sinensis, also known as *Camellia sinensis*, or more commonly known as tea, is a widely cultivated plant species used for the production of tea, one of the most consumed beverages worldwide. The plant, belonging to the Theaceae family, is native to China and has been used for medicinal purposes for thousands of years (Wang et al. 2022). *Thea* can grow up to 16 m tall but is usually pruned to a manageable size (Duke 1983). Tea is rich in bioactive compounds, particularly catechins, which have been associated with numerous health benefits, including antioxidant, anti-inflammatory, and anti-cancer properties (Fan et al. 2017). The use of *Thea sinensis* in traditional medicine and the potential health benefits of its consumption have led to growing interest in its bioactive components and their mechanisms of action. This has also led to the development of various pharmaceutical and nutritional products derived from *Thea sinensis* (Khan & Mukhtar 2007).

Figure 1 Thea sinensis (Miller 1771)

4.1.1.2. Biological activity

Thea sinensis has been shown to indicate antimicrobial activity against various microorganisms, which includes bacteria, fungi, and viruses. The reason why this is possible, is because is it a plant rich in a wide range of secondary metabolites, those being namely catechins and polyphenols. Studies have shown the potential of *Thea sinensis* extract to enhance activity of antibiotics against resistant and standard *Escherichia coli* strains (Darwish & Aburjai 2010).

The phenolic compounds in tea vary, depending on whether the tea is fermented (green) or unfermented (black). Green tea production aims to preserve as many polyphenols contained in fresh tea leaves, mainly flavan-3-ols, known as catechins. On

the contrary, the flavan-3-ols in fermented tea are fully oxidized. Over 5,000 various characteristic black tea polyphenols have been detected.

Tea polyphenols are agreed to have effect upon gut microbial activity. Their abilities include increasing microbial diversity, promoting beneficial bacteria and inhibiting pathogenic bacteria (Liu et al. 2022). Polyphenols in *Thea sinensis* have anti-inflammatory and anti-oxidation properties, which have been shown to reduce oxidative stress and inflammation in the gut (Fan et al. 2017).

In human hosts, multiple health benefits have been observed. These benefits are as follows: improvement of inflammatory bowel disease (IBD) and non-alcoholic fatty liver disease (NAFLD), anti-obesity, gut barrier protection and bile acid regulation (Liu et al. 2022). Several studies claim that green tea consumption has protective properties on stomach and colorectal cancer, whereas consuming black tea seems to have little to no effect. Furthermore, other studies claim there to be no correlation between drinking green tea and improvement of stomach and colorectal cancer and suggest these changes may be due to some other factors such as lifestyle (McKay & Blumberg 2002).

4.1.1.3. Microorganisms

Thea sinensis polyphenols have been shown to interact with various microorganisms in the gut. *Bifidobacterium*, *Lactobacillus* and *Bacteroides*, which are types of beneficial bacteria commonly found in the gut, have positive reaction in growth after interacting with tea polyphenols. Furthermore, these polyphenols have been found to inhibit the growth of pathogenic bacteria such as *Clostridium* spp. and *Escherichia coli*, which can lead to various diseases (Liu et al. 2022).

Researchers also propose that tea polyphenols contribute to extending the shelf life of food products, by inhibiting microorganisms and oxidative processes (Gonçalves Bortolini et al. 2021).

4.1.1.4. Toxicity

Thea sinensis and its extracts are considered to be safe when consumed in moderation. Higher than normal doses can cause side effects such as nausea, headache, anxiety, and a few others (Bedrood et al. 2018). Chan et al. (2010) have evaluated the toxicity of green tea extract in rats. Their research has found that 1,000 mg/kg may cause

liver necrosis and subsequent death. There is also the potential for toxic elements to leach into tea leaves. High concentrations of barium (Ba), rubidium (Rb), strontium (Sr) and chromium (Cr) can be absorbed through soil parent material. Higher concentrations of cadmium (Cd), arsenic (As) and nickel (Ni) can be attributed to anthropogenic activities, such as fertilizer application and the manufacturing process (Zhuang et al. 2022).

4.1.2. Astragalus membranaceus

4.1.2.1. Description

Astragalus membranaceus, commonly known as milk vetch, also known in traditional Chinese medicine as Huang Qi, is a perennial plant that belongs to the Fabaceae family. It is native to China, Mongolia, and Korea and is widely used in traditional Chinese medicine. The plant typically grows up to 1.5 meters in height and has erect stems, pinnate leaves, and yellowish-white flowers that bloom in the summer (Durazzo et al. 2021). The root of the plant is the most commonly used part for medicinal purposes.

Figure 2 Pressed specimen of Astragalus membranaceus Moench (Takeda 1906)

4.1.2.2. Biological activity

Polysaccharides, saponins and flavonoids from *Astragalus membranaceus* have been shown to enhance phagocytosis to eliminate harmful pathogens and stimulate the immune system (Sun et al. 2008).

Studies have shown that *Astragalus membranaceus* extract can enhance the production and function of immune cells, natural killer cells, T and B cells. Astragalosides, extracted from the root of *Astragalus membranaceus* was shown to have anti-inflammatory, anti-fibrotic, diuretic, anti-diabetic, antioxidant, antiviral, analgesic properties (Gui et al. 2006; Jin et al. 2014). Popular methods of extraction are water extracts, whole ingredient extracts (WIE) and ultrafine powder, with whole ingredient extract seeming to be more efficient (Li et al. 2019).

A decoction from the root of *Astragalus* with rhizoma of *Curcumae longae* has been shown to exhibit combinatory anti-tumour and anti-cancer properties against a variety of cancers, such as stomach, colon, gastric and colorectal cancer. The decoction can inhibit growth and proliferation of cancer cells; however, this mechanism has not yet been well studied (Miraj & Kiani 2016; Bian et al. 2022). The polysaccharides contained within the root have been proven to be one of the major active agents (Jin et al. 2014).

Leaves of *Astragalus* have also been found to contain a variety of bioactive compounds, including polyphenols, saponins, flavonoids, and other antioxidants. Polyphenols, flavonoids, and proteins are in a higher concentration in dry leaves, than in dry roots. The antioxidant activity is lower in dry roots rather than in dry leaves (Miraj & Kiani 2016; Samuel et al. 2021).

4.1.2.3. Microorganisms

Dry leaves and dry roots have shown inhibition activity against *Salmonella* spp., *Bacillus subtilis*, *Escherichia coli* and yeast. Leaf tea from *Astralagus* shows inhibitory activity against all these strains with the addition of *Aspergillus niger* (Samuel et al. 2021).

4.1.2.4. Toxicity

Astragalus membranaceus has been commonly used as a dietary supplement, mainly in traditional Chinese medicine. The plant is understood to be safe, when consumed in moderation (Dog 2005). Song et al. (2017) have observed the effects of *Astragalus* extract mixture on rats. The mixture, containing extracts of *Astragalus membranaceus*, *Eleutherococcus senticosus* and *Phlomis umbrosa* was tested on rats in amounts up to 5,000 mg/kg, and did not show any adverse effects. However, *Astragalus* extract shows mild activity in relation to aryl hydrocarbon receptor and P-glycoprotein inhibition, which could lead to side effects through medicine interactions (Husain et al. 2023).

4.1.3. Withania somnifera

4.1.3.1. Description

Withania somnifera, commonly known as winter cherry, also known as ashwagandha, is a medicinal plant that has been used in Ayurvedic medicine for centuries (Mishra et al. 2000).

Figure 3 Illustration of Withania somnifera (Smith 1819)

It is native to the dry regions of India, northern Africa, and the Middle East, and is a member of the Solanaceae family. *Withania somnifera* is a perennial shrub, and it produces small, greenish-yellow flowers and red berries. Its roots are tuberous and used for medicinal purposes. It is used to treat a variety of ailments, including stress, anxiety, insomnia, and arthritis. *Withania somnifera* is also believed to have anti-inflammatory, anti-tumour, and immunomodulatory properties (Parihar 2022).

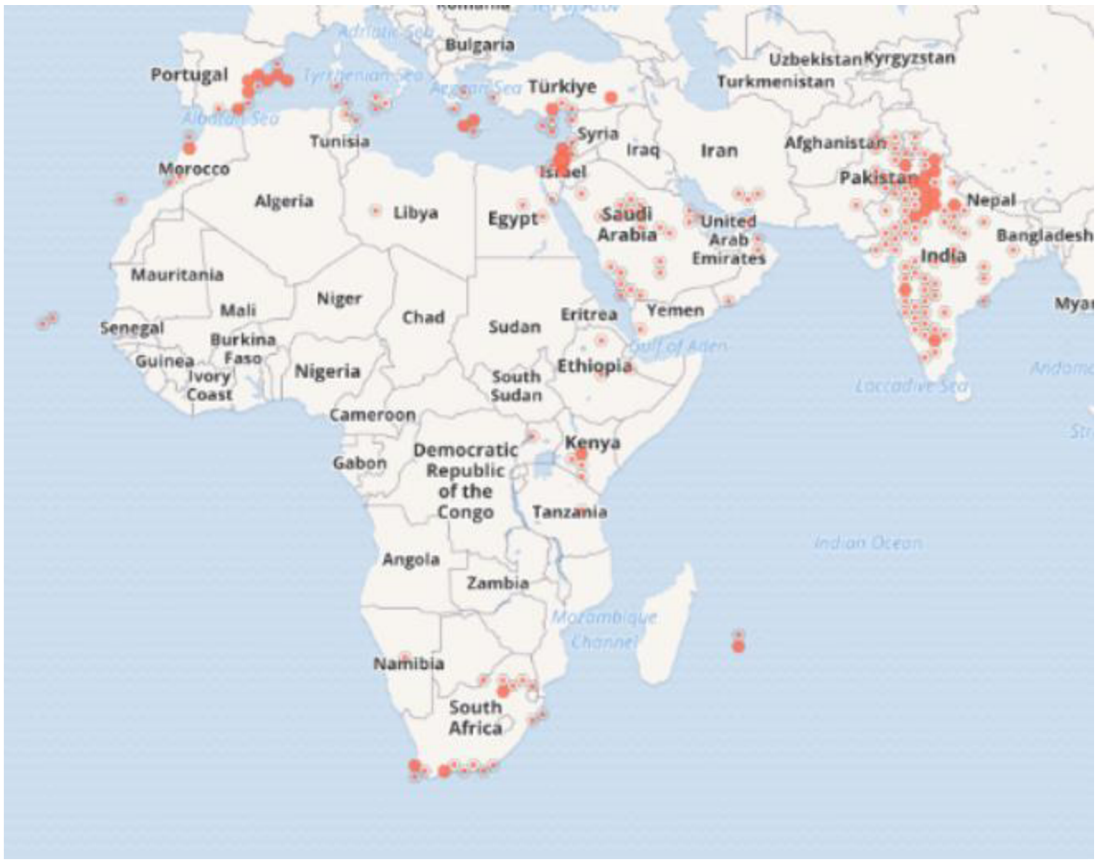

Figure 4 Recorded occurrences of Withania somnifera between 2019-2021 (GBIF et al. 2021)

4.1.3.2. Biological activity

The main active compounds in *Withania* are tannins, polyphenols, terpenoids, flavonoids, saponins, glycosides, alkaloids, and steroids. Of these, isopellertierine, withanolides and withaferins are agreed to be the main reactive compounds (Parihar 2022). Withanolides are steroidal alkaloids and steroidal lactones and are acknowledged to be the origin of *Withania's* pharmacological effects. They are highly concentrated in roots (Mandal 2017; Parihar 2022).

A leaf extract has been shown to exhibit antibacterial, anti-fungal, antitumor properties. A root water extract has anti-inflammatory properties. Some other significant activities are anti-analgesic, anti-aging, aphrodisiac, antivenom, anti-cancer and anti-viral activities (Parihar 2022).

4.1.3.3. Microorganisms

Withania somnifera has been shown to exhibit antibacterial and antifungal properties against a variety of microorganisms, including bacteria, fungi, and viruses. Extract from the plant is able to inhibit their growth. Methanolic leaf extract of *Withania somnifera* especially has been used to combat these microorganisms, however extracts from the root, stem and fruit have also been used (Singh & Kumar 2011). The extract has been shown to be effective against *Staphylococcus aureus*, *Streptococcus thermophilus*, *Enterococcus* spp., *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, as well as *Candida albicans* and *Fusarium verticilloides* from fungal species. *Aspergillus* spp. has shown resistance to *Withania* (Singh & Kumar 2011; Bisht & Rawat 2014; Kumari & Gupta 2015; Sharma et al. 2016; Megawati & Wahyuni 2021; Parihar 2022). Antiviral activities have also been proven to be effective against herpes simplex virus (HSV), human papillomavirus (HPV), parainfluenza-3 and other various pathogens (Parihar 2022).

4.1.3.4. Toxicity

Withania leaf extract shows mild reaction to pregnane xenobiotic receptor and the enzyme CYP3A4. The root extracts exhibit mild reaction to pregnane xenobiotic receptor, weak reaction to CYP3A4 and a strong reaction to aryl hydrocarbon receptor. According to Husain et al. (2023), these reactions could prove to cause health complications through medicine interactions (Husain et al. 2023). *Withania* is considered to have low toxicity and is well tolerated. Rare cases of liver injury however have been reported (Gurley et al. 2022). A review of various toxicity studies on rats claims the lethal doses for various *Withania* extracts to be relatively high, indicating a low risk of acute toxicity. For example, the lethal dose of methanolic extract of seeds was $1{,}750 \pm 41$ mg. Furthermore, treatment using aqueous extract did not have any adverse effects (Paul et al. 2021). Despite these potential risks, this plant has been used for over 3000 years without many, if any, side effects (Mandal 2017).

4.1.4. Panax ginseng

4.1.4.1. Description

Panax ginseng is a popular medicinal herb that has been used in traditional Chinese medicine for thousands of years and is highly used in Korea and Japan. Processed in various forms, it is being used to treat immune disease, liver disease and even cancer (Park et al. 2021).

Panax ginseng belongs to the Araliaceae family. It is a perennial plant with a slender stem and palmate leaves at the top. The plant blooms after 3 years of growth in the summer, and produced seeds are collected after 4 years of growth. The root of *Panax ginseng* is the part that is most commonly used for medicinal purposes. The root can grow up to 10 cm in length. It is harvested between 4 and 6 years of age (Choi 2008). The root has a distinctive aroma and a slightly sweet, bitter taste.

Figure 5 Panax ginseng root (Brücke-Osteuropa 2011)

4.1.4.2. Biological activity

Ginseng has been traditionally used in Asian countries, especially in Korea, China, and Japan. Processed in various forms, it is being used to treat immune disease, liver disease and even cancer. *Panax ginseng* contains several bioactive compounds, such as ginsenosides, polyphenols and polysaccharides. Over 200 different ginsenosides have been found (Park et al. 2021). They are steroid-like saponins and are thought to be the major bioactive compound with pharmacological potential (Kim et al. 2017). A study has reported some effects of ginsenoside Rb1 on gut microflora, this effect however is dependent on the pre-existing composition of gut microflora, therefore, results vary in different experiment subjects (Song et al. 2014). Ethanol and methanol extracts of ginseng have been found to have potential to neutralize free radicals and reactive oxygen species (ROS). These molecules cause oxidative damage to cells and can be highly damaging to gut health. Ginseng's antioxidant activities may improve gut health and health overall (Ratan et al. 2021). There are two methods of preparation of ginseng, which differ in ginsenoside content and even pharmacological effects. Researchers claim that steaming fresh ginseng can increase its bioactivity. The Korean red ginseng is traditionally prepared by steaming, whereas the Korean white ginseng is prepared by steaming and sun-drying (Lim et al. 2015; Amerikanou et al. 2021).

Traditionally, *Panax ginseng* has been used as a natural remedy for obesity and obesity related disorders. There have been many claims that ginseng may help reduce body weight, body fat percentage and waist circumference. Several studies have been conducted on this topic, yet not many studies seem to support these claims (Amerikanou et al. 2021).

4.1.4.3. Microorganisms

Ginsenosides show antibacterial, antifungal, and antiviral activities. Extracts of ginseng have been shown to be effective against *Bacillus cereus, Bacillus subtilis, Clostridium perfringens, Cryptococcus neoformans, Helicobacter pylori, Listeria monocytogenes, Pseudomonas aeruginosa, Porphyromonas gingivalis, Salmonella enterica* and *Streptococcus pneumoniae* (Ratan et al. 2021). Growth can be observed in beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*. Ginseng supplementation may increase the abundance of *Anaerostipes* and *Blautia* in the gut of obese subjects.

Ginsenosides may be able to increase the probiotic effect of *Oscillibacter* and *Eubacterium*_g5 in the gut microflora (Song et al. 2014).

Some studies have shown potential that ginseng may have antiviral effects against many disorders affecting gut health, such as norovirus and rotavirus. So far the effects have been minimal or non-existent, and further research is needed to prove these claims (Im et al. 2015).

4.1.4.4. Toxicity

Panax ginseng is safe in moderation and is a highly used dietary supplement. High concentrations of ginseng will lead to nervousness and excitability. In mice, a lethal oral dose of purified ginseng was found to be higher than 5 g/kg, indicating a relatively low risk of acute toxicity (Lee et al. 2012). Ginseng shows interactions with medicaments such as warfarin and phenelzine, and alcohol. These interactions may cause side-effects like insomnia or high blood pressure (Lee et al. 2012). On the other hand, traditional use of ginseng utilizes the plant to alleviate nausea and vomiting (Kim et al. 2005).

4.1.5. Scutellaria baicalensis

4.1.5.1. Description

Scutellaria baicalensis, also known as Chinese skullcap, or Huang Qin, is a medicinal plant that has been used in traditional Chinese medicine for centuries. It is a

Figure 6 Scutellaria baicalensis plant (Kenraiz 2021)

member of the Lamiaceae family and is native to China, Korea, Mongolia, and Russia. *Scutellaria* is a perennial herb that can grow up to 60 cm in height and has small blue or purple flowers. The roots of *Scutellaria baicalensis* are the most commonly used part of the plant. *Scutellaria* is used to treat a variety of ailments, including inflammation, allergies, infections, and cancer. It is also believed to have antioxidant properties (Muluye et al. 2014; Song et al. 2020).

4.1.5.2. Biological activity

Scutellaria baicalensis is a plant widely used in traditional Chinese medicine. The practice processes the plant's roots, which are said to have anti-inflammatory, antiviral, anti-tumour, antioxidant properties, amongst many others (Xing et al. 2014). *Scutellaria* contains several active compounds including flavonoids, flavonoid glycosides, and other polyphenolic compounds. The flavonoids are the main active compounds and include baicalin, baicalein, scutellarin, wogonin and oroxylin A. Baicalin is the most abundant flavonoid, and along with baicalein and scutellarin, has very high antioxidant activity. A methanolic extract from the root is able to neutralize ROS and reactive nitrogen species (RNS) and protect cells and tissues from oxidative damage. Wogonin has been found to have anti-inflammatory properties. It is able to inhibit the function of lipopolysaccharides, which induce the production of inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). These cytokines are involved in the development of many inflammatory diseases (Meng & Lowell 1997; Wojtunik-Kulesza et al. 2021). Furthermore, baicalein has been found to have the ability to induce apoptosis of inflammatory cells (Song et al. 2020).

4.1.5.3. Microorganisms

Scutellaria baicalensis has been found to have antimicrobial properties against bacteria, viruses, and fungi. A water extract from the roots shows selective inhibitory effects against *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and methicillin resistant (MRSA) and methicillin sensitive (MSSA) strains of *Staphylococcus aureus* (Xing et al. 2014). Similar inhibitory effect from the water extract has been found to work against *Toxoplasma gondii* (Song et al. 2020). Scutellaria has antifungal effects

against *Saccharomyces cerevisiae* (Leach 2011). Antiviral effects have been found to act against influenza A and B, and HSV type 1 (Seong et al. 2018; Luo et al. 2020).

4.1.5.4. Toxicity

Scutellaria is considered to be relatively safe, when consumed in moderation. However, overdosing has showcased very serious side effects. Baicalin shows toxicity in liver and kidney, causing liver fibrosis, kidney damage and allergic reactions. Wogonin in a dose of 40 mg/kg can affect foetus development, as was studied on pregnant mice. Single oral doses of baicalein ranging from 100 to 2,800 mg are generally well tolerated in healthy volunteers, with no observed toxicity in liver or kidney function. Medicine made from baicalein aluminium complexes has been used to treat piglet diarrhoea, by altering the structure and composition of their gut microbiome (Song et al. 2020). *Scutellaria* may have further side effects in relation to medicine interactions. The plant shows mild reaction to increasing pregnane xenobiotic receptor activity, selectively inhibiting enzyme CYP3A4 activity and a strong reaction to increasing aryl hydrocarbon receptor activity. These are receptors and enzymes which interact with medicine, which furthermore interact with the plant's compounds, causing side effects (Husain et al. 2023).

4.2. Known and utilized extracts

Plant extracts are already being used commercially as feed additives in the livestock industry for their beneficial effects on gut health.

For example, garlic extract is commercially used as a feed additive in broiler chicken production. Dietary supplementation of garlic extract has shown an increase in growth, an improvement in in meat quality, a decrease in cholesterol and antimicrobial activities in the gut (Fayed et al. 2011).

Similarly, ginger extract is also used as a feed additive in livestock industry for its beneficial effects on gut health. Dietary supplementation of ginger extract on broiler chickens was found to improve nutrient digestibility and increase immunomodulatory and

antioxidant effects. In combination with black pepper, ginger extract shows an increase in growth of broiler chickens (Herrero-Encinas et al. 2023).

Turmeric extract has been studied for its effect on laying hens. Dietary supplementation of turmeric extract was found to increase weight gain and increase the feed conversion ratio in the starter period of laying hens (Naderi et al. 2014).

While plant extracts have been studied and utilized for their beneficial effects on gut health in livestock, they are also commonly used in human dietary supplements. Ginger, turmeric, and garlic extract, as well as ginkgo biloba, echinacea and aloe vera extract are some of the most commonly used plant extracts that promote human health. Ginger extract is known for its anti-inflammatory and digestive properties. Turmeric extract is often used for its antioxidant and anti-inflammatory properties. Garlic extract is used for its immune-boosting properties. Ginkgo biloba extract is used for cognitive function. Echinacea extract is used for immune system support. Aloe vera extract is used for digestive health and skin care (National Toxicology Program 2023).

These plant extracts are available in various forms, such as capsules, powders, and teas, and can be easily incorporated into a person's daily diet. It is however recommended to consult with a healthcare professional before using supplements, as the efficacy and safety may vary from person to person.

5. Conclusions

In conclusion, the use of natural plant extracts and compounds to improve the condition of gut microflora is an area of growing interest in animal nutrition. Through this review, various natural plant extracts and compounds were identified, which have shown promising results in improving gut microflora condition in animals. These include compounds such as polyphenols, flavonoids, and terpenoids. The identification of specific plant species with potential health-promoting properties provides a cost-effective and sustainable solution for improving animal health and production. These plants were shown to possess properties that could promote the growth of beneficial microorganisms in the gut, inhibit harmful microorganisms, and support the immune system, leading to an overall improvement in microflora and gut health.

Thea sinensis was found to contain compounds that could stimulate the growth of beneficial gut bacteria. *Withania somnifera* had anti-inflammatory properties that could potentially reduce inflammation in the gut and improve overall gut health. *Astragalus membranaceus* was found to contain polyphenols that promote the growth of beneficial gut bacteria and could be used for its anti-cancer properties. *Panax ginseng* had a beneficial effect on gut inflammation. *Scutellaria baicalensis* was found to contain flavonoids that had antioxidant and anti-inflammatory properties and could potentially reduce gut inflammation.

Further research is needed to determine the optimal doses and combinations of these natural plant extracts for different animal species and production systems. Nonetheless, the potential benefits of incorporating natural plant extracts in animal feed cannot be overlooked, and this area of research should continue to be explored.

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Appendices

List of the Appendices:

Appendix I: An Overview of Studied Plants

Appendix 1: An Overview of Studied Plants

Family	Plant species	Part of the plant used	Method of extraction	Toxicity	Microorganism	Reference
Theaceae	*Thea sinensis / Camellia sinensis* / tea	leaf	water extract	1,000 mg/kg	*Bacteroides, Bifidobacterium, Lactobacillus* *Clostridium* spp., *Escherichia coli*	Chan et al. (2010); Liu et al. (2022)
Fabaceae	*Astragalus membranaceus* / milk vetch / Huang Qi	root, leaf	water extract, WIE, ultra-fine powder	no reaction up to 5,000 mg/kg	*Aspergillus niger, Bacillus subtilis, Escherichia coli, Salmonella* spp.	Song et al. (2017); Samuel et al. (2021)
Solanaceae	*Withania somnifera* / winter cherry / ashwagandha	fruit, leaf, root, stem	methanolic extract	1750 ± 41 mg/kg	*Staphylococcus aureus, Streptococcus thermophilus, Enterococcus* spp., *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Candida albicans, Fusarium verticilloides*	Singh & Kumar (2011); Bisht & Rawat (2014); Kumari & Gupta (2015); Sharma et al. (2016); Megawati & Wahyuni (2021); Paul et al. (2021); Parihar (2022)
Araliaceae	*Panax ginseng*	root	ethanol extract, methanol extract	5 g/kg	*Anaerostipes, Blautia*, Bifidobacterium, Eubacterium_g5, *Lactobacillus, Oscillibacter* *Bacillus cereus, Bacillus subtilis, Clostridium perfringens, Cryptococcus neoformans, Helicobacter pylori, Listeria monocytogenes, Pseudomonas aeruginosa, Porphyromonas gingivalis, Salmonella enterica, Streptococcus pneumoniae*	Lee et al. (2012); Ratan et al. (2021)
Lamiaceae	*Scutellaria baicalensis* / chinese skullcap / Huang Qin	root	methanolic extract, water extract	40 mg/kg	Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Staphylococcus aureus (MRSA and MSSA), Toxoplasma gondii	Leach (2011), Xing et al. (2014), Song et al. (2020)