Cowpea

Research Progress and Management Challenges

Božica Anđelo Nikolić
Editor

Agriculture Issues
and Policies

COWPEA

RESEARCH PROGRESS AND MANAGEMENT CHALLENGES

AGRICULTURE ISSUES AND POLICIES

Additional books and e-books in this series can be found
on Nova's website under the Series tab.

AGRICULTURE ISSUES AND POLICIES

COWPEA

RESEARCH PROGRESS AND MANAGEMENT CHALLENGES

BOŽICA ANĐELO NIKOLIĆ
EDITOR

nova
science publishers
New York

NOTICE TO THE READER

Library of Congress Cataloging-in-Publication Data

ISBN: 978-1-53614-283-9 (eBook)

Published by Nova Science Publishers, Inc. † New York

CONTENTS

In: Cowpea
Editors: Božica Anđelo Nikolić
ISBN: 978-1-53614-282-2

Chapter 4

BIOLOGICAL AND PHARMACOLOGICAL ACTIVITIES OF COWPEA (*VIGNA UNGUICULATA* L.) AND ITS BIOACTIVE COMPONENTS

Heba I. Mohamed[1,*], Hossam S. El-Beltagi[2,3], Hany N. Yousef[1] and Eman M. Fawzi[1]

[1]Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Cairo, Egypt
[2]Department of Biochemistry, Faculty of Agriculture, Cairo University, Giza, Cairo, Egypt
[3]Cairo University, Research Park (CURP), Giza, Cairo, Egypt

ABSTRACT

Cowpea (*Vigna unguiculata* L.) is an important legume crop that is widely grown in many countries of sub- Saharan Africa and Latin America. The seeds and leaves are a major source of plant proteins and vitamins for man and they serve as food for animals as well. The little leaves and immature pods are eaten as vegetables. It forms part of the human diet due

[*] Corresponding author: hebaibrahim79@yahoo.com.

to the fact that it has a high amount of carbohydrates (56 - 67%), protein (20.5 - 31.7%), fiber (4%) and fats (1.14 - 3.03%) that can satisfy the human essential nutritive necessities when complemented with cereals. Cowpea is rich in potassium with a good amount of calcium, magnesium and phosphorus. It also has a small amount of iron, sodium, zinc, copper, manganese and selenium. Cowpea is rich in vitamin A & C and also has an appreciable amount of thiamin, riboflavin, niacin, vitamin B6 and pantothenic acid as well as small amount of foliate. Cowpea roots and leaves are rich sources of calcium, phosphorous and Vitamin B. These nutrients provided by cowpea make it extremely valuable especially where many people cannot afford animal proteins such as meat and fish. We can evaluate nutritional composition and functional properties of some recombinant inherited lines of Cowpea to establish potential usage. This versatile crop has various uses: a good source of nutritious food, animal fodder, and a source of income through the trade of seeds. It also increases soil nitrogen levels and prevents soil erosion. Furthermore, an infusion of the seed can be taken orally to treat amenorrhea whilst powdered roots eaten with porridge is believed to treat painful menstruation. Leaves are applied to burns and can be used as a snuff to treat headaches. Some health benefits of cowpea include toning the spleen, stomach and pancreas, helping induce urination and relieveing damp conditions like licorice. Cowpeas contain the highest concentration of antioxidant compounds. Plant flavonoids, anthocyanins, polyphenols and tannins are a diverse group of phytochemicals that occur in legume seeds and have gained attention due to their antioxidant capacity that benefits human health by preventing oxidative stress. Cowpea contains isoflavone as the fitoestrogen, which is a main component of daidzein (4',7-dihydroxyisoflavone), genistein (4',5,7-trihydroxyisoflavone), and glycetin (7,49-dihidroxy-6-methoxyisoflavone), flavonoid such as quercetin, kaempferol and isorhamnetin. Also, phenolic aglycons including *p*-coumaric acid and caffeic acid have also been isolated from cowpea leaves and it has been shown that *p*-coumaric acid, caffeic acid and kaempferol do exhibit anticancer activity and antimicrobial activity against various bacterial and fungal pathogens. Besides its health-related benefits, beans are inexpensive. Due to their physicochemical and functional attributes, legume starches can be used as nutritional ingredients in the same way as starches from cereals and tubers are used. Medicinally, they have been used as an astringent, appetizer, laxative, aphrodisiac, diuretic, anti-hyperglycemic, antinociceptive, galactagogue, and liver tonic. They exhibit antioxidant and free radical scavenging activities. They also exhibit antibacterial activity against both the Gram-positive and Gram-negative organisms. Besides, they are useful in jaundice, menstrual disorders, epilepsy, anorexia, and constipation.

Keywords: cowpea, antioxidant, anticancer, anti-inflammatory, antimicrobial, pharmacological activities

1. INTRODUCTION

Cowpea (*Vigna unguiculata*) is one of the most popular legume seeds with an estimated world production of 5.39 million metric tonnes (FAO, 2010). It is an annual herbaceous legume from the genus *Vigna*. Due to its tolerance for sandy soil and low rainfall, it is an important crop in the semi-arid regions across Africa and other countries (Lattanzio et al., 2000). It has similar physical and chemical properties to common beans (*Phaseolus vulgaris*), except for its fat content. Cowpeas contain around 1.14 - 3.03% fat, whereas common beans have only 0.5 – 1.5% lipids (Samman et al., 1999). These legume seeds are staples in many parts of the world. *Vigna unguiculata* is a member of the *Vigna* (peas and beans) genus. Unguiculata is Latin for "with a small claw", which reflects the small stalks on the flower petals (Small 2009). All cultivated cowpeas are found within the universally accepted *V. unguiculata* subspecies *unguiculata* classification, which is then commonly divided into four cultivar groups: *Unguiculata*, *Biflora*, *Sesquipedalis*, and *Textilis* (Perrino et al., 1993; Padulosil and Ng, 1997). Some well-known common names for cultivated cowpeas include black-eye pea, southern pea, yardlong pea, catjang, and crowder pea (Timko et al., 2007).

Cowpea contains phenolic compounds including phenol acids, flavonoids, and tannins (Cai et al., 2003). Due to their antioxidant, anti-inflammatory, and anticarcinogenic effects, these bioactive compounds pose an added health benefit to humans and animals (Folmer et al., 2014; Ojwang et al., 2015). In animal studies, flavonoids (Middleton et al., 2000) and condensed tannins (Min et al., 2004) have been reported to have benefits for ruminant health and productivity. For example, feeding tannin-rich forages to animals attenuates blotting, increases their resistance to gastrointestinal nematodes (Min et al., 2004) and improves nutrition through increased availability of high-quality proteins to the small intestines (Barry et al., 2001). The health promoting benefits of cowpeas for human consumption have been studied using human cell lines and laboratory animal models against chronic diseases such as cancer (Gutiérrez-Uribe et al., 2011). In the realm of this chapter, we will focus on the biological and pharmacological activities of cowpea and its bioactive components.

2. Plant Description and Cultivation

There is a large morphological diversity found within the crop, and the growth conditions and grower's preferences for each variety vary from region to region (Padulosil and Ng, 1997). Cowpeas can either be short and bushy (as short as 20 cm) or act like a vine by climbing supports or trailing along the ground (to a height of 2 m) Figure (1) (Sheahan 2012). The tap root can penetrate to a depth of 2.4 m after eight weeks (Davis et al., 1991). The size and shape of the leaves vary greatly, making this an important feature for classifying and distinguishing cowpea varieties (Pottorff et al., 2012). Another distinguishing feature of cowpeas is the long 20–50 cm peduncles which hold the flowers and seed pods. One peduncle can support four or more seed pods (Davis et al., 1991). Flower colour varies through different shades of purple, pink, yellow, white and blue. Seeds and seed pods from wild cowpeas are very small, while cultivating varieties can have pods between 10 and 110 cm long (Rawal, 1975). A pod can contain 6 – 13 seeds that are usually kidney shaped, although the seeds become more spherical the more restricted they are within the pod (Figure 2). Their texture and colour are very diverse. They can have a smooth or rough coat, and can be speckled, mottled or blotchy. Colors include white, cream, green, red, brown and black or various combinations (Davis et al., 1991).

Cowpeas thrive in poor dry conditions, growing well in soils up to 85% sand (Obatolu, 2003). This makes them a particularly important crop in arid, semi-desert regions where not many other crops will grow. As well as an important source of food for humans in poor arid regions, the crop can also be used as feed for livestock (Quin, 1997). Its nitrogen fixing ability means that as well as functioning as a sole-crop, the cowpea can be effectively intercropped with sorghum, millet, maize, cassava or cotton (Blade et al., 1997). The optimum temperature for cowpea growth is 30°C (86°F), making it only available as a summer crop for most of the world. The ideal soils are sandy and it has better tolerance for infertile and acid soil than most other crops. The grain can be harvested after about 100 days or the whole plant can be used as forage after approximately 120 days. Leaves can be picked from 4 weeks after planting (DAFF, 2011).

Figure 1. *Vigna unguiculata* (L) Walp.

Figure 2. A cowpea plant with some pods ready for harvest.

3. Physical Properties of Cowpea Seed

Cowpeas have black colored seed coats, a test weight of 83.4 kg/hl and a 1000 seed weight of 136.8 g. The kidney shaped seeds (Figure 3) have an average length and width of 8 and 6 mm respectively. Mechanically-removed coats constitute 9.86% of the seed weight. Sosulski and Dabrowski (1984) reported that Canadian cowpeas only containe 4.2% seed coats. Other common legume seeds, such as soybeans and pinto beans, range from 6.6% to 9.2% (Kay, 1985).

4. Uses of the Different Parts of Cowpea

Cowpea has various uses; for example, it can function as a good source of nutritious food, an animal fodder and a source of cash through trading the seeds. It also increases soil nitrogen levels and prevents soil erosion (Singh et al., 1997). Roasted seeds are used to treat neuritis, insomnia, weakness of memory, dyspepsia, indigestion, needles in the limbs and sensation of pins, periodic palpitation, congestive cardiac failure, etc. They are an admirable medicine for stomatitis, corneal ulcers, coleic diseases, kwasiorkar, marasmus (Battu et al., 2011). Table (1) shows that cowpea seeds are a rich source of amino acids and proteins, and some of the amino acids play an important role in the management of sickle cell disease (EGBA et al., 2011). Furthermore, Cowpea seeds have cardioprotective potency and prevent cardiovascular diseases (Khusniyati et al., 2014). An infusion of the seeds can be taken orally to treat amenorrhoea whilst powdered roots, eaten with porridge, are believed to treat painful menstruation, epilepsy and chest pain by the indigenous people of South Africa. Cowpea seeds – cooked with the roots of *Lannea edulis* (Sond.) Engl. *Euclea divinorum* Hiern or *Terminalia sericea* Burch ex DC. – are used to treat blood in the urine and bilharzias by South Africans (Nyazema, 1987; Van Wyk and Gericke, 2000). Also, the

Figure 3. Cowpea seeds with a distinctive black spot on their hilum.

Table 1. Amino acid content of the seed of cowpea (EGBA et al., 2011)

Amino acid	% Composition
Aspartate	27.8
Threonine	3.3
Serine	2.6
Glutamine	43.5
Proline	17.6
Glycine	9.5
Alanine	18.7
Cysteine	3.6
Valine	0.8
Methionine	3.2
Isoleucine	5.3
Leucine	5.4
Tyrosine	0.5
Phenylalanine	5.5
Histidine	4.5
Lysine	0.5
Arginine	14.3
Tryptophan	0.5

seeds are used as astringent, antipyretic, diuretic and in cardiovascular diseases. Globulin fraction of the seed shows hypolipidaemic effects in rats. Ayurvedic pharmacopoeia of India recommend the decoction of dry seeds in calculus and amenorrhoea.

Leaves are applied to burns and can be used as a snuff to treat headaches. The Zulus (a South African tribe) make emetics from the plant that is taken to relieve fever (Hutchings et al., 1996). Cowpea has also been identified as a plant that traditional healers use to treat urinary shistomiasis (bilharzia) in Zimbabwe (Ndamba et al., 1994). Also, leaves are used to treat hyperacidity, nausea and vomiting (Caswell and Clifford, 1958). The presence of vitamin A in the green pods makes them a valuable diet for children. In addition, green leaves may be used in vitamin C deficiency syndrome (Khare, 2008).

5. Chemical Composition of Cowpea

5.1. Leaf and Stem

According to Gutierrez-Uribe et al. (2011), a whole cowpea contains 11.6% moisture, 28.2% protein, 10.5% fat, 4.9% crude fiber, 4.2% ash and 40.6% nitrogen free extract. Seed coats contain more crude fiber (12.7%) and ash (12.7%), whereas cotyledons contain high quantities of protein (35.2%) and fat (15.2%). Moreover, whole cowpeas contain about 70% free and 30% bound phenolics, whereas seed coats contain approximately equal percentages.

In cowpeas, the phenolic acid present in the highest amount is coumaric acid followed by ferulic acid (Sosulski and Dabrowski, 1984). The major chemical constituents in a cowpea leaf and stem are genisteine, dalberegioidin, kievitone, phaseollidin, coumesterol, psoralidin, lectin like glycoprotein dolichin A and dolichin B, Isoferreirin (Figure 4). *Vigna unguiculata* have high concentrations of malaic, malonic, citric and oxalic acids that are used in medicine. Also found is a calcium content that ranges from 58 to 287 mg/100g, while the iron content is about 2.5 to 10.5 mg/100g (Agugo et al., 2013).

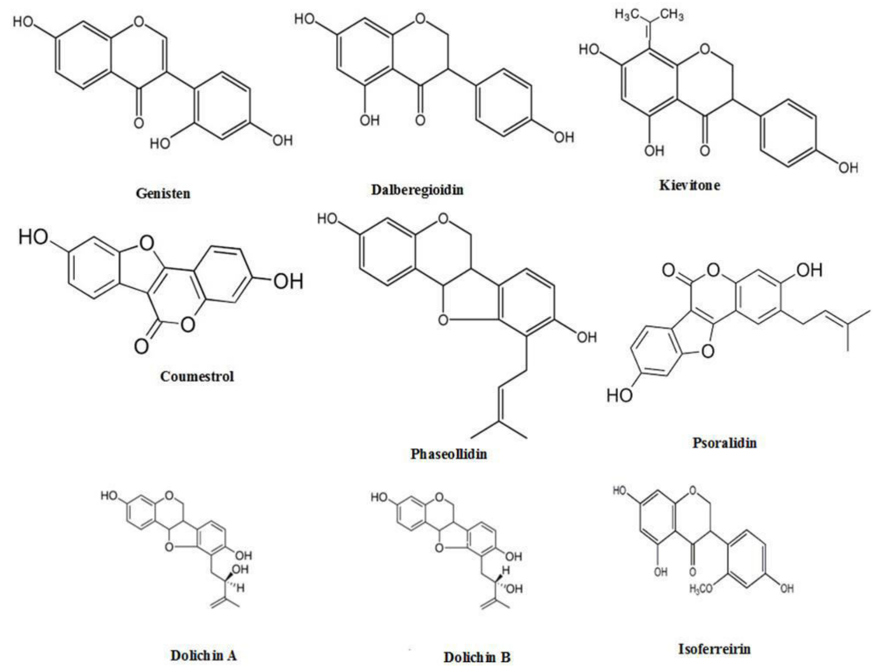

Figure 4. Structures of cowpea leaf and stem major chemical constituents.

5.2. Seeds

Seed coats have at least 5 to 10 times more free and bound phenols than the whole seeds. The quantities of free and bound phenolics are 107.3 and 737.7 mg gallic acid equiv/100 g for whole cowpeas and seed coats respectively. Also, seed coats and cotyledons of a cowpea contain about 50% and 95% of free phenolics respectively (Gutierrez-Uribe et al., 2011). The major phenolics associated to seed coats are gallic and protocatechuic acid, whereas in cotyledons *p*-hydroxybenzoic acid is prevalent. Cai et al., (2003) concluded that acid hydrolysis during fermentation converts esterified phenolics into simple acids. These authors found that the protocatechuic acid present in 17 varieties of *V.*

unguiculata varied from trace amounts to 92.7 mg/100 g after hydrolysis. Protocatechuic acid was identified as the major phenolic acid present in esterified moieties. Other phenolics, such as *p*-hydroxybenzoic, caffeic, *p*-coumaric, ferulic, 2,4-dimethoxybenzoic, cinnamic acid, vanillic acid, gallic acid, and syringic acid were also identified (Nderitu et al., 2013) (Figure 5). The total phenolics differed among 17 cowpea varieties values ranged from 34.6 to 376.6 mg/100 g indicating large intervarietal differences (Cai et al., 2003).

Seed coats contained approximately 10 times more flavonoids than the whole seeds, while cotyledons were practically free of flavonoids. After acid hydrolysis, myricetin, quercetin and kaempferol were identified as flavonol in seed coats. Most of the antioxidant activity determined with the 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and oxygen radical absorbance capacity (ORAC) assays was exerted by free phenolics.

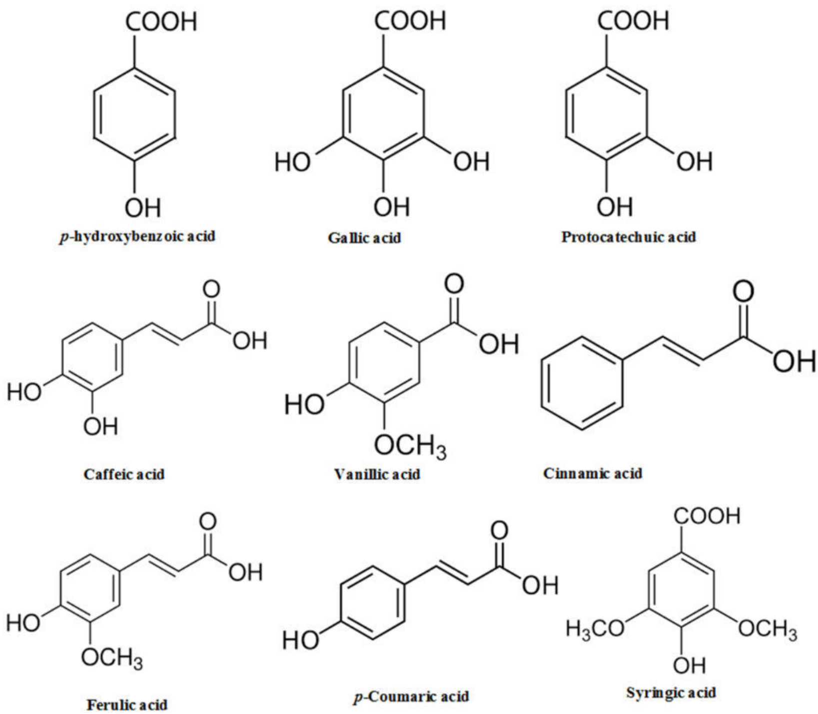

Figure 5. Chemical structures of cowpea seed phenolic compounds.

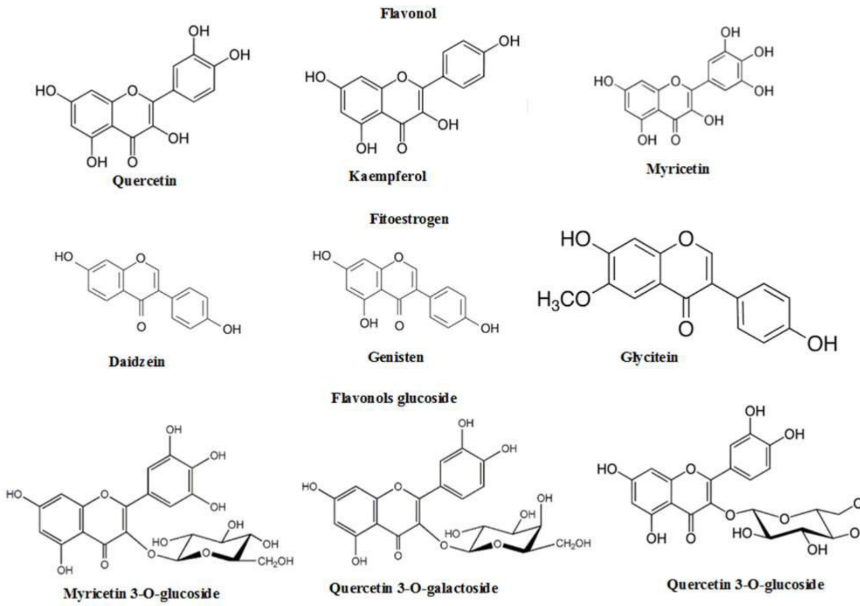

Figure 6. Chemical structures of cowpea seed flavonoid compounds.

A cowpea contains isoflavone as the fitoestrogen, with main component of daidzein (4',7- dihydroxyisoflavone), genistein (4',5,7-trihydroxyisoflavone), and glycitein (7,4'-dihidroxy-6- methoxyisoflavone) (Kaur and Murphy, 2010). Dueñas et al., (2005) identified hydroxybenzoic acids and flavonols such as myricetin 3-O-glucoside, quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin feruloyldiglycoside and another diglycoside of quercetin in cowpeas (Figure 6).

The whole seeds of cowpea are reported to contain about 0.18%–0.59% tannins, phenolic acids, such as p-hydroxybenzoic acid, protocatechuic acid, 2,4-dimethoxybenzoic acid, and cinnamic acid derivatives, such as p-coumaric acid, caffeic acid, cinnamic acid and ferulic acid (Khusniyati et al., 2014)._Urease, protease (Rao and Suresh, 2007), strepogenin, β-sitosterol, genistein, 2' –hydroxygenistein, dalbergioidin, kievitone, phaseollidin, isoferreirin, coumesterol, psoralidin, 5-O-α-L rhamnopyranosyl (1→2)-O-β-D-glucopyranoside, phyto-haemagglutinins, β-N-acetyl glucosaminidase, α & β-galactosidase, α-mannosides, β-glucosides, β-sitosterol.28

5- hydroxy-7,3,4-trimethoxy-8-methyl isoflavone - 5-neohesperidoside, D-glucose, D-galactose, L-rhamnose, D-arabinose and L-ascorbic acid and amino acids viz., glycine, alanine, cysteine, serine and aspartic acid (Figure 7) (Battu et al., 2011). Chlorogenic acid and Caffeic acids were present in methanolic extract of *V. unguiculata* (Zia-Ul-Haq et al., 2013). Amino acid analysis of the seed extract of *V. unguiculata* showed the presence of the following amino acids; aspartate (27.8%), threonine (3.3%), serine (2.6%), glutamine (43.5%), proline (17.6%), glycine (9.5%), alanine (18.7%), cysteine (3.6%), valine (8.0%), methionine (3.2%), isoleucine (5.3%), leucine (5.4%), tyrosine (0.5%), phenylalanine (5.5%), histidine (4.5%), lysine (0.5%), arginine (14.3%) and tryptophan (0.5%). The proximate analysis of the extract indicated appreciable content of protein (23.65%), moisture (12.85%), ash (3.4%), fats and oil (4.5%) and fiber (4.8%) (EGBA et al., 2011).

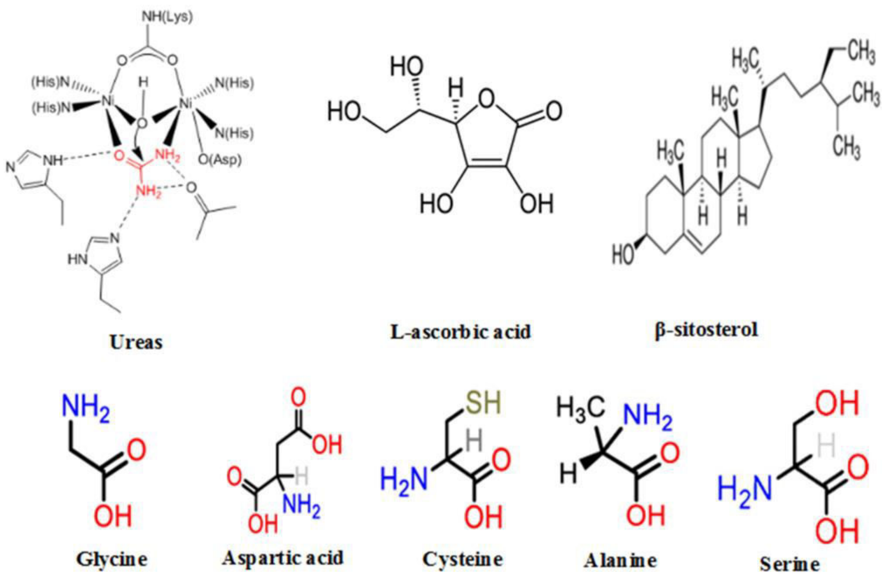

Figure 7. Structures of Chemical Constituents (Battu et al., 2011).

6. Pharmacological Activities of Cowpea

6.1. Anthelmintic Activity

Through a study carried by Maisale et al., (2012) *Vigna unguiculata* seeds were coarse powdered and exhaustively with hot solvent (Soxhlet) extraction by ethanol and maceration with chloroform water. Five concentrations (10-100 mg/ml) of ethanolic and aqueous extracts were studied for anthelmintic activity by using Edriluseuginiae earthworms. Both aqueous and ethanolic extracts showed paralysis and death of worms in concentration (10-100mg/ml) dependent manner. Alcoholic extract of *V. unguiculata* showed significant activity than aqueous extract. Piperazine citrate (10mg/ml) and distilled water were included in the assay as standard drug and control respectively. The result showed that seeds of *Vigna unguiculata* possessed potential anthelmintic activity.

6.2. Antidiabetic, Antihyperglycemic and Antinociceptive Activities

The seed oil of *V. unguiculata* was investigated by Ashraduzzaman et al., (2011) for its anti-diabetic activity against alloxan monohydrate induced diabetes in rats. Levels of blood glucose, total cholesterol (TC), triglycerides (TGs), low density (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) decreased and high density lipoprotein (HDL) increased in alloxan induced diabetic rats after treatment with 200 mg/kg barbati seed oil for 21 days. The study reported that the seed oil of cowpea may be very useful for the improvement of the complications of diabetes. Kidney beans, navy beans and green beans are good sources of omega 3 fatty acids (alpha linolenic acid, ALA). It is also reported that omega-3 fatty acids improve blood sugar levels in those with diabetes and lowers cholesterol levels (Duyff, 2006; Pollan, 2008).

The antihyperglycemic effects and antinociceptive activity of the methanolic extract of *Vigna unguiculata* beans were evaluated by (Tazin et al., 2014). For determination of antihyperglycemic activity, oral glucose tolerance test (OGTT) was used and antinociceptive activity was examined through the observation of decrease in abdominal constrictions in intraperitoneally administered acetic acid-induced pain model in mice. Administration of methanol extract of beans resulted in dose-dependent and significant decreases in blood glucose levels in glucose-loaded mice. The tests for antinociceptive activity showed that, the methanolic extract decreased the number of abdominal constrictions in all the used doses. This study concluded that the beans can be a good source for alleviating pain and for lowering blood sugar in diabetic patients.

6.3. Hypocholesterolemic Activity

The seeds of cowpea were investigated for its Hypocholesterolemic activity in Wistar rats. Rats were grouped and fed a high fat diet with 20% Bombay (BO), 20% MI 35 (MI), 20% Cowpea extract, 20% Dawala (DA) in comparison with 20% casein (HFD). Serum total cholesterol, non-HDL cholesterol, triacylglyceride and glucose concentrations were analyzed. Serum lipids and glucose concentrations in cowpea fed rats were significantly lower ($P < 0.05$) than HFD. Therefore, raw cowpea produced significant hypolipidemic and hypoglycemic effects in Wistar rats (Weththasinghe et al., 2014).

6.4. Antimicrobial and Antiviral Activities

Bacterial and fungal pathogens play a negative role with regard to the nutritional and economical value of various important crop plants. These pathogens cause severe damage to the roots and aerial parts of the plants. Many pesticides as well as various other chemical formulations used to control plant pathogens are inaccessible to small scale farmers due to financial constraints. Furthermore, since chemicals pose a danger to the environment and non-targeted organisms, the use of plant extracts as an alternative means of controlling plant fungal and bacterial pathogens has been widely exploited (Poswal et al., 1993). Moreover, bacterial and fungal pathogens are also capable of causing serious diseases in humans and animals (Worthington and Bigalke, 2001). Some fungal pathogens infest the seed during storage and produce toxic secondary metabolites (mycotoxins) which, when ingested, can cause acute and chronic toxicities in humans and animals (Barrett, 2000). Due to problems with the toxicity of existing antimicrobial agents as well as the emergence of drug-resistant strains, the use of plant extracts can be exploited as an alternative way to control these pathogens. Many of these plant extracts contain secondary compounds that have an inhibitory effect on harmful bacterial and fungal human pathogens (Lall and Meyer, 2000; Mathekga et al., 2000).

Acetone and ethanol extracts of the leaves of White (BW) and Kpodjiguégué (Kpod) cultivars of cowpea plants were investigated by Kritzinger et al., (2005) for their antimicrobial properties against bacterial and fungal pathogens. With the exception of *Fusarium equiseti*, all the extracts significantly inhibited growth of the fungal pathogens at 5.0 mg ml^{-1}. *Alternaria alternate* was significantly reduced by both BW extracts at 2.5 mg ml^{-1} whereas only the ethanol extract showed antifungal activity against *Fusarium proliferatum* at the same concentration. The acetone extract from Kpod significantly inhibited the growth of *A. alternata* at 2.5 mg ml^{-1}. BW acetone extracts inhibited growth of the Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis* at 2.5 mg ml^{-1} and *Bacillus cereus*, *B. subtilis* and *Enterobacter cloacae* at 5.0 mg ml^{-1}. Ethanol extracts of the same cultivar only showed antibacterial activity against *Enterococcus faecalis* and *Enterobacter cloacae* at 5.0mg ml^{-1}. The Kpod extracts exhibited no inhibitory effect on the bacteria. Lattanzio et al. (1997) found three flavonoid aglycones, namely quercetin, kaempferol and isorhamnetin, always to be present in the leaves of cultivated cowpea lines. Quercetin, a naturally occurring bioflavonoid, is known to inhibit the growth of various fungi and bacteria (Aziz et al., 1998). Further phenolic aglycons including pcoumaric acid and caffeic acid have also been isolated from cowpea leaves (Lattanzio et al., 2000) and it has been shown that pcoumaric acid, caffeic acid and kaempferol do exhibit antimicrobial activity against various bacterial and fungal pathogens (El-Gammal and Mansour, 1986, Aziz et al., 1998).

Nóbrega et al., (2005) showed that roots from cowpea seedlings contained b-1,3- Glucanases, chitinases and lipid transfer proteins (LTPs), all of which may potentially function as plant defense proteins. Immunolocalization of one of these proteins, chitinase, revealed its presence in the xylem cell wall vessel elements. These exudates also demonstrated an inhibitory effect on the growth of the fungus, *Fusarium oxysporum*, *in vitro*. The results suggest that plant roots may exude a variety of proteins that may function to repress the growth of root pathogenic fungi. Also, Sandeep (2014) found that the aqueous and ethanolic extracts of seeds of *Vigna unguiculata* possess excellent antibacterial activities which support the traditional myths. In addition, the antimicrobial activity of *Vigna unguiculata (L)Walp* seed oil was investigated against five Gram positive bacteria (*Bacillus megaterium*, *Bacillus subtilis*, *Sarcinalutea*, *Salmonellatyphi* and *Staphylococcus aureus*) and four Gram negative (*Escherichia coli*, *Shigelladysenteriae*, *Shigellasonnei*, *Shigellashiga*) and four fungi (*Penicilium spp.*, *Mucor spp.*, *Candida albicans and Aspergillusfumigatius*). Oil at the concentration of 400 µg/disc showed the highest activity against *Sarcinalutea* (19±0.1 mm) and *Staphylococcus aureus* (16±0.1 mm). Oil is active against the three tested fungi namely *Penicilium spp.*, *Mucor spp.* and *Candida albicans* but showed no sensitivity against *Aspergillus fumigatius* (Ashraduzzaman et al., 2016).

Cowpea seeds were examined for the presence of various proteins and amino acids with antiviral and antifungal potency. The two proteins, designated α- and β-antifungal proteins according to their elution order from the CM-Sepharose column, were capable of inhibiting human immunodeficiency virus (HIV) reverse transcriptase and one of the glycohydrolases associated with HIV infection, α-glucosidase, but β-glucuronidase was not repressed. The ability of the proteins was also demonstrated in order to retarding mycelial growth of a variety of fungi, and α-antifungal protein being proved more potent in most cases. β-Antifungal protein was highly active in only one instance. Both antifungal proteins had low cell-free translation-inhibitory activity (Ye et al., 2000).

6.5. Antisickling and Anti-Hypertensive Properties

The cowpea extracts have shown to be therapeutically beneficial in the management of sickle cell disease and, thus, it is strongly recommended to be developed into supplements for the management of sickle cell disease (EGBA et al., 2012).

Regarding anti-hypertensive properties, the inhibition of angiotensin I-converting enzyme (ACE) is used as an indicator for potential protection against hypertension. The principal function of ACE is to control blood pressure (Kamath et al., 2007; De Leo et al., 2009). At low blood pressure, renin converts angiotensinogen to angiotensin I which is converted to a strong vasoconstrictor (angiotensin II) by ACE (De Leo et al., 2009), leading to an increase in blood pressure. ACE also induces breakdown of bradykinin (a vasodilator) and this augments the increase in blood pressure (Pihlanto-Leppälä, 2000). Sreerama, Sashikala, and Pratape (2012) reported that phenolic extracts from cowpea inhibited angiotensin I-converting enzyme (ACE-1) in a dose-dependent manner. Segura-Campos et al. (2011) determined ACE inhibitory potential of hydrolysates produced by hydrolysing cowpea protein isolate with three protease enzymes (Alcalase, Flavourzyme and pepsin-pancreatin) and their peptide fractions produced using ultrafiltration.

6.6. Antioxidant Activity

The HPLC analysis of cowpea extracts showed the presence of neochlorogenic acid, chlorogenic acid and caffeic acids (Zia-Ul-Haq et al., 2013). EGBA et al. (2011) showed that the phenolic compounds present in the copy, extract showed the antioxidant and antiradical properties using different models like linoleic acid peroxidation model, Ferric-Reducing Antioxidant Power (FRAP), Oxygen Radical-Absorbing Capacity (ORAC), Total Radical-Trapping Antioxidant Potential (TRAP). The raw, dry heated and hydrothermal treated samples of *Vigna unguiculata* were investigated for its antioxidative properties and total phenolic content. The raw, dry heated and hydrothermal treated samples were extracted with 70% acetone and the extracts were freeze-dried. The study reported that all extracts exhibited good antioxidant activity (74.3 – 84.6%) against the linoleic acid emulsion system (Siddhuraju and Becker 2007).

Zia-ul-Haq et al. (2010) reported that cowpea seed oil contains tocopherols. Tocopherols are the most important lipophilic antioxidants and are believed to play a preventive role in diseases associated with oxidative stress like central neurodegenerative diseases, age-related muscular degeneration, cancer, cataracts, cardiovascular diseases and diabetes mellitus (Brigelius-Flohe et al. 2002).

The DPPH• radial scavenging assay was used to assess the scavenging activity of cowpea seed extracts. DPPH• is increasingly used for quickly assessing the ability of antioxidants to transfer the labile H atoms to radicals. The antiradical capacity values of cowpea against DPPH• ranged from 25.1 to 32.5 μmol Trolox/g (Zia-Ul-Haq et al., 2013). These results are close to those reported earlier for cowpea (Siddhuraju and Becker, 2007). Antioxidant potential of the cowpea seed extracts was estimated from their ability to reduce TPTZ-Fe^{3+} to TPTZ-Fe^{2+} complex. FRAP values of cowpea ranged from 13.2 to 19.4 mmol Fe^{2+}/g (Zia-Ul-Haq *et al.*, 2013).

Zia-Ul-Haq et al. (2013) found that extracts of cowpea seeds were assessed for their potential scavenging antiradical activity against some common radicals like hydroxyl, nitric oxide and superoxide and values expressed as IC_{50} μg/mL. Hydroxyl radical is an extremely reactive free radical formed in biological systems and a highly damaging species in free radical pathology as it may damage almost every molecule found in living cells (Hochestein and Atallah, 1988). This radical may join nucleotides in DNA and cause strand breakage which contributes to carcinogenesis, mutagenesis and cytotoxicity (Manian et al., 2008). Hydroxyl radical scavenging capacity of cowpea extract is directly related to its antioxidant activity (Babu et al., 2001). The extracts exhibited significant hydroxyl radical scavenging activity with IC_{50} from 80.6 to 92.4 μg/ml.

The extracts of cowpea seeds exhibited significant superoxide radical scavenging activity (Zia-Ul-Haq et al., 2013). This radical mediates inflammatory tissue injuries in ischemia reperfusion, arthritis, gout and gastric ulceration. Superoxide radical has a low reactivity and a low capacity to penetrate the lipid membrane layer, but it can generate hydrogen peroxide and highly reactive hydroxyl radical, via Haber-Weiss reaction (Zia-Ul-Haq et al., 2011).

Huang and Zhang (2010) showed that genistein supplementation (2.5 mg/kg BW), the daily injected subcutaneous for 30 days, increased SOD serum levels and decreased MDA levels in rats. In addition, analysis of rat brain tissues shows that the SOD activity levels were significantly higher while MDA levels were lower after supplementation using cowpeas extract. Cowpea extract which contains flavonoid compounds such as flavonols group (quercetin, kaempferol, and myricetin) and derived isoflavones (genistein and daidzein) may inhibit the decrease SOD activity and increase MDA levels in brain of rats after exposed to gasoline fume through the inhibition of free radical reactions (Afif et al., 2014).

6.7. Anticancer Activity

Various reports provide some evidence of anti-cancer properties of cowpea which have been demonstrated using *in vitro* and *in vivo* assays. These anti-cancer properties have been shown using such parameters as inhibition of oxidative DNA damage, antiproliferative effects against cancer cells and induction of Phase II detoxifying enzymes (Awika and Duodu, 2017).

The free phenolic extract of whole seeds of cowpea at a concentration of approximately 100 mg gallic acid equiv. (GAE)/l inhibited 65% the proliferation of hormone-dependent mammary (MCF-7) cancer cells. Extracts of seed coats or cotyledons also inhibited cell proliferation (Gutie´rrez-Uribe et al., 2011).

A recent study assessed the inhibitory potential of protein fractions from some legume species (including cowpea) against matrix metalloproteinase (MMP-9) activity in colon cancer cells (HT29) (Lima et al., 2016). The activity of these matrix metalloproteinases is related to cancer growth and metastization and therefore their inhibition is an indicator of potential anticancer effects. Protein extracts from the legume species inhibited activity of MMP-9 and it seemed that the inhibitors of MMP-9 were mainly present in albumin and globulin fractions. These protein fractions also inhibited proliferation of HT29 colon cancer cells.

6.8. Anti-Inflammatory Activity

Genistein has been reported to be the most potent inhibitor of cancer cell growth *in vitro* but also impair the proliferation of vascular endothelial cells in corneal neovascularization. Genistein in cowpea methalonic extract decreased the Matrix metalloproteinase-9 (MMP-9) and VEGF expression on NaOH alkali burn corneal inflammation in rats (Sa'adah and Anandita, 2016). Genistein, grouped in isoflavone serves to inhibit angiogenesis. Fotsis et al. (1993) found that genistein inhibits the vascular endothelial cell proliferation and angiogenesis at concentration of 5 and 150 mM/L (Lee et al., 2012). Genistein has effect to decrease cornea neovascularization and decrease the blood vessel leakage, through inhibiting mechanism of tyrosine kinase protein as important component in the biological tissue control that determine the cell growth and differentiation. The inhibition of tyrosine kinase protein activation for intracellular signal of endothelial cell cause inhibition of MMP-9 (Hamalainen et al., 2007). The giving of genistein also causes the VEGF expression decrease that inhibits cellular proliferation, angiogenesis decrease, and apoptosis (Pugalendhi et al., 2010; Ren et al., 2001).

Cowpea contains relatively high amounts of flavonols (especially glycoside of quercetin and anthocyanins; as well as procyanidins) and that the seed coat color is a major determinant of flavonoid composition (Ojwang et al., 2012). Significant anti-inflammatory effects of these flavonoid classes have been demonstrated by some authors (Gerritsen et al., 1995; De Stefano et al., 2007). Quercetin has been shown to potentially control intestinal inflammation in celiac disease by preventing the activation of transcription factor NF-κB and mitogen activated protein kinases (MAPK) pathways (De Stefano et al., 2007). Anthocyanins are also reported to exert anti-inflammatory property by inhibiting TNF-α-induced endothelial leukocyte adhesion molecule-1 (ELAM-1) and intercellular adhesion molecule-1 (ICAM-1) expression in cultured HUVEC.11 Cell adhesion molecules plays a key role in monocyte recruitment that also plays a role in tumor development. Procyanidins (i.e., condensed tannins), which primarily occur in the form of catechin and epicatechin monomers, their polymers and glycosides, 9 have also been shown as effective in inhibiting the AngII-induced MAPK pathways, leading to reduced adhesion molecule expression in HUVEC (Naito and Yoshikawa, 2009).

6.9. Effect on Bone Formation

The MG-63 human Osteosarcoma cell lines are exposed to different dose concentrations of Daidzein (0.01, 0.1, 1, 2.5, 7.5 and 10 μM), Genestein (0.01, 0.1, 1, C2.5, 7.5 and 10 μM), Vitamin-D (15 to 50 μM), cowpea extract (having 18 μM Daidzein: 6 μM Genestein) and in combinations of Daidzein+Genestein (5 to 30 μM) and the combination of all at 12, 24, 36, 48 hrs respectively. Biochemical parameters like Osteocalcin activity, Alkaline phosphatase and Acid phosphatase activity assays, Intracellular calcium and reactive oxygen species levels were measured. Antioxidant activity of the enzymes like Catalase, Superoxide dismutase, Malonaldehyde, Vitamin C, and Gluthathione reductase were also tested. The results indicated that the cell proliferation of the exposed groups increased when compared with their respective controls. Similarly, antioxidant activity of the enzymes like Catalase, Superoxide dismutase, Gluthathione reductase, Vitamin C and biochemical parameters like Osteocalcin activity, Alkaline phosphatase and Intracellular calcium levels showed a significant increase in their activities in the exposed groups when compared with their respective controls. In contrast, the exposed groups showed a decrease in Reactive oxygen species, Acid phosphatase and Malonaldehyde activity with respect to their controls. Thus, all different parameters through different approaches showed the stimulating effects of

Cowpea isoflavones along with the vitamin D on bone formation (Rishika et al., 2016).

Cowpea contains isoflavone as the fitoestrogen, with main component of daidzein (4’,7- dihydroxyisoflavone), genistein (4’,5,7-trihydroxyisoflavone), and glycetin (7,49-dihidroxy-6- methoxyisoflavone) (Kaur and Murphy, 2010). Daidzein and genestein are natural isoflavonoids of the family leguminosae and these isoflavones are known to mimick the properties of estrogen hormone. Previous reports have indicated that daidzein stimulates early differentiation of osteoblasts and a significant increase in cell proliferation was observed on MC3T3-E1, the preosteoblastic cells (Sugimoto and Yamaguchi, 2000). Genestein have an inhibitory effect on protein tyrosine kinases by interacting with the plasmalemma tyrosine kinase receptors (Wang et al., 2008) and help in reducing free radical secretion by inflammatory cells receptor. Both daidzein and genestein are known to show enhancing effects on cell proliferation, DNA content, Alkaline phosphatase activity, Osteocalcin activity on MC3T3 E1 cell lines. The cells showed significant increase in cell proliferation at a concentration range of 0.01-0.1M (Sugimoto and Yamaguchi, 2000).

CONCLUSION

Vigna unguiculata, a well-known plant for its antioxidant, anticancer and anti-inflammatory potential, because of its phenolic and flavonoid content, was explored for its antimicrobial potential against various gram positive and gram negative bacteria and fungal pathogens. This chapter evidently reveals that the cowpea (leaves and seeds) extracts are effective antimicrobial and pharmacological agents. Further, detailed study on their mechanism and safety profile may develop them as good candidates for food preservation or functional foods, as well as for pharmaceutical and natural plant-based products.

Acknowledgment

Authors would like to show appreciation to Faculty of Agriculture, Cairo University, Department of Biochemistry and Faculty of Education, Ain Shams University, Department of Biological and Geological Sciences for continued cooperation to support research that provided facilities necessary to accomplish the most wanted objectives of the research.

References

Afif, Z., Rahmawati, D., Dalhar, M. & Aris Widodo, M. (2014). Effects of ethanolic cowpea extract on MDA, SOD, TNF-α levels and neuron cellvacuolationin Wistar rat brain after exposed with gasoline fume. *IOSR-JESTFT*, *8*, 113-118.

Agugo, U. A., Okere, T. O. & Anya, K. M. (2013). Investigating the nutrient composition and anti-nutritional factors of Akidi (*Vigna unguiculata*). *IOSR*, *5*(4), 32-35.

Ashraduzzaman, M., AshrafulAlam, M., Khatun, Sh. & Absar, N. (2016). Antimicrobial activity of Vigna Unguiculata (L) Walp. *Int. J. Biotech. Well. Indus.*, *5*(3), 70-75.

Ashraduzzaman, M. D., Alam, M. A., Khatun, S., Banu, S. & Absar, N. (2011). *Vigna unguiculata* Linn. Walp. seed oil exhibiting antidiabetic effects in alloxan induced diabetic rats. *Malay. J. Pharm. Sci.*, *9*, 13-23.

Awika, J. M. & Duodu, K. G. (2017). Bioactive polyphenols and peptides in cowpea (*Vigna unguiculata*) and their health promoting properties: A review. *J Funct Foods*., *38*, 686–697.

Aziz, N. H., Farag, S. E., Mousa, L. A. A. & Abo-Zaid, M. A. (1998). Comparative antibacterial and antifungal effects of some phenolic compounds. *Microbios*, *93*, 43–54.

Babu, B. H., Shylesh, B. S. & Padikkala, J. (2001). Antioxidant and hepatoprotective effect of *Alanthus icicifocus*. *Fitoterapia*, *72*, 272–277.

Barrett, J. R. (2000). Mycotoxins: of molds and maladies. *Environ. Health Perspect.*, *108*, A20-A23.

Barry, T. N., McNeill, D. M. & McNabb, W. C. (2001). Plant secondary compounds; their impact on nutritive value and upon animal production. *Proceedings of the XIX International Grass*, (pp. 445-452). The conference, Sao Paulo, Brazil.

Battu, G., CKVLSN, A., Priya, T. H., Malleswari, V. N. & Reeshm, S. (2011). A phytopharmacological review on Vigna species. *Int. J. Adv. Pharm. Sci.*, *2*, 62-67.

Blade, S. F., Shetty, S. V. R., Terao, T. & Singh, B. B. (1997). "Recent developments in cowpea cropping systems research". In Singh, B.B., Mohan Raj, D.R., Dashiell, K.E., Jackai, L.E.N. *Advances in Cowpea Research*. Ibadan, Nigeria: International Institute of Tropical Agriculture and Japan International Research Center for Agricultural Sciences.

Brigelius-Flohe, R., Kelly, F. J., Salonen, J. T., Neuzil, J., Zingg, J. M. & Azzi, A. (2002). The European perspective on vitamin E: Current knowledge and future research. *Am. J. Clin. Nutr.*, *76*, 703–716.

Cai, R., Hettiarachchy, N. S. & Jalaluddin, M. (2003). High performance liquid chromatography determination of phenolic constituents in 17 varieties of cowpeas. *J. Agr. Food Chem.*, *51*, 1623–1627.

Caswell, G. H. & Clifford, H. T. (1958). Effect of Fumigation and Moisture Content on the Seedling Growth of Cowpea (*Vigna unguiculata*). *Nature*, *182*, 540-541.

DAFF (Department of Agriculture, Forestry and Fisheries). (2011). *Production guidelines for cowpeas*. Pretoria: Directorate Agricultural Information Services. Available at http://www.daff. gov.za.

Davis, D. W., Oelke, E. A., Oplinger, E. S., Doll, J. D., Hanson, C. V. & Putnam, D. H. (1991). "*Cowpea*". *Alternative Field Crops Manual*. www.hort.purdue.edu. University of Wisconsin-Extension, Cooperative Extension. Retrieved 2017-04-13.

De Leo, F., Panarese, S., Gallerani, R. & Ceci, L. R. (2009). Angiotensin Converting Enzyme (ACE) inhibitory peptides: production and implementation of functional food. *Curr Pharm Des.*, *15* (31), 3622–3643.

De Stefano, D., Maiuri, M. C., Simeon, V., Grassia, G., Soscia, A., Cinelli, M. P. & Carnuccio, R. (2007). Lycopene, quercetin and tyrosol prevent macrophage activation induced by gliadin and IFN-[gamma]. *Eur. J. Pharmacol.*, *566*, 192–199.

Dueñas, M., Fernandez, D., Hernandez, T., Estrella, I. & Munoz, R. (2005). Bioactive phenolic compounds of cowpeas (*Vigna sinensis* L.). Modifications by fermentation with natural microflora, with Lactobacillus plantarum ATCC 14917. *J. Sci Food Agr.*, *85*, 297–304.

Duyff, R. L. (2006). *Complete food and nutrition guide*, 3rd edition (Hoboken, NJ: American Dietetic Association and John Wiley & Sons).

EGBA, S. I., Emmanuel, N. T., Ogugua, N. V. & Ndohnui, N. N. (2012). Antisickling potential of the ethanol seed extracts of *Vigna Unguiculata* and *Vigna Subterranean*. *Int. Biochem. Biotechnol.*, *1*, 226-229.

EGBA, S. I., Uzoegwu, P. N., Emmanuel, T. N. & Joshua, P. E. (2011). Amino acid content and proximate analysis of the ethanol seed extract of *Vigna unguiculata* used in the management of Sickle cell disease. *J. Chem. Pharm. Res.*, *3*, 538-541.

El-Gammal, A. A. & Mansour, R. M. A. (1986). Antimicrobial activities of some flavonoid compounds. *Zentrablatt fur Mikrobiologie*, *141*, 561–565.

FAO. (2010). *Statistical database*. Rome, Italy: FAO. <http:// www. faostat. fao.org>.

Folmer, F., Basavaraju, U., Jaspars, M., Hold, G., El-Omar, E., Dicato, M. & Diederich, M. (2014). Anticancer effects of bioactive berry compounds. *Phytochem. Rev.*, *13*(1), 295-322.

Fotsis, T., Pepper, M., Adlercreutz, H., Fleischmann, G., Hase, T., Montesano, R. & Schweigerer, L. (1993). Genistein, a dietary-derived inhibitor of *in vitro* angiogenesis. *Proc Natl Acad Sci USA*, *90*, 2690–2694.

Gerritsen, M. E., Carley, W. W., Ranges, G. E., Shen, C. P., Phan, S. A., Ligon, G. F. & Perry, C. A. (1995). Flavonoids inhibit cytokine-induced endothelial cell adhesion protein gene expression. *Am. J. Pathol.*, *147*, 278–292.

Gutierrez-Uribe, J. A., Romo-Lopez, I. & Serna-Saldıvar, S. O. (2011). Phenolic composition and mammary cancer cell inhibition of extracts of whole cowpeas (*Vigna unguiculata*) and its anatomical parts. *J. Funct Foods*, *3*, 290-297.

Hamalainen, M., Nieminen, R., Vuorela, P., Heinonen, M. & Moilanen, E. (2007). Anti-Inflammatory Effects of Flavonoids: Genistein, Kaempferol, Quercetin, and Daidzein Inhibit STAT-1 and NF-κB Activations, Whereas flavone, Isorhamnetin, Naringenin, and Pelargonidin Inhibit only NF-κB Activation along with Their Inhibitory Effect on iNOS Expression and NO production in Activated Macrophages. *Mediators Inflamm.*, *10*, 1-10.

Hochestein, P. & Atallah, A. S. (1988). The nature of oxidant and antioxidant systems in the inhibition of mutation and cancer. *Mutat. Res.*, *202*, 363–375.

Huang, Y. & Zhang, Q. (2010). Genistein reduced the neural apoptosis in the brain of ovariectomised rats by modulating mitochondrial oxidative stress. *Br. J. Nutr.*, *104*, 1297–1303.

Hutchings, A., Haxton, S. A., Lewis, G. & Cunningham, A. B. (1996). *Zulu Medicinal Plants: An Inventory*. University of Natal Press, Scottsville, p. 146, ISBN 086–980–893–1.

Iwasaki, K., Maier, P., Fecht, M. & Horst, W. J. (2002). Leaf apoplastic silicon enhances manganese tolerance of cowpea (*Vigna unguiculata*). *J. Plant Physiol.*, *159*, 167-173.

Kamath, V., Niketh, S., Chandrashekar, A. & Rajini, P. S. (2007). Chymotryptic hydrolysates of α-kafirin, the storage protein of sorghum (Sorghum bicolor) exhibited angiotensin converting enzyme inhibitory activity. *Food Chem.*, *100*(1), 306-311.

Kaur, N. & Murphy, J. B. (2010). Cloning, characterization, and functional analysis of cowpea isoflavone synthase (IFS) homologs. *J. Plant Mol. Biol. Biotechnol.*, *1*, 6-13.

Kay, D. E. (1985). *Legumbres Alimenticias*. [Food Vegetables] Zaragoza, Espana: Acribia.

Khare, C. P. (2008). *Indian Medicinal Plants*: *An Illustrated Dictionary*. Springer Science and Business Media., 222-223.

Khusniyati, E., Sari, A. A., Yueniwati, Y., Noorhamdani, N., Nurseta, T. & Keman, K. (2014). The effects of Vigna unguiculata on cardiac oxidative stress and aorta estrogen receptor-β expression of ovariectomized rats. *Asian Pac. J. Reprod.*, *3*, 263-267.

Kritzinger, Q., Lall, N. & Aveling, T. A. S. (2005). Antimicrobial activity of cowpea (*Vigna unguiculata*) leaf extracts. *South Afr. J. Bot.*, *71*, 45-48.

Lall, N. & Meyer, J. J. M. (2000). Antibacterial activity of water and acetone extracts of the roots of *Euclea natalensis*. *J. Ethnopharmacol.*, *72*, 313316.

Lattanzio, V., Arpaia, S., Cardinalli, A., Di Venere, D. & Linsalata, V. (2000). Role of endogenous flavonoids in resistance mechanisms of Vigna to aphids. *J. Agr. Food Chem.*, *48*, 5316–5320.

Lattanzio, V., Cardinalli, A., Linsalata, V., Perrino, P. & Ng, N. Q. (1997). Flavonoid HPLC fingerprints of wild *Vigna species*. In: Singh, B.B., Mohan Raj, D.R., Dashiell, K.E., Jackai, L.E.N. (eds) *Advances in Cowpea Research*. Co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), IITA, Ibadan, Nigeria, pp. 66–74. ISBN 978–131–110–X.

Lee, J. Y., Kim, H. S. & Song, Y. S. (2012). Genistein as a Potential Anticancer Agent against Ovarian Cancer. *Journal of Traditional and Complementary Medicine*, *2*, 96-104.

Lima, A. I. G., Mota, J., Monteiro, S. A. V. S. & Ferreira, R. M. S. B. (2016). Legume seeds and colorectal cancer revisited: Protease inhibitors reduce MMP-9 activity and colon cancer cell migration. *Food Chem.*, *197* (Part A), 30–38.

Maisale, A. B., Patil, M. B., Jalalpure, S. S. & Attimarad, S. L. (2012). Phytochemical properties and Anthelmintic activity of *Vigna Unguiculata*. *J. Pharm. Sci. Innovat.*, *1*, 51-52.

Manian, R., Anusuya, N., Siddhuraju, P. & Manian, S. (2008). The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. *Food Chem.*, *107*, 1000–1007.

Mathekga, A. D. M., Meyer, J. J. M., Horn, M. M. & Drewes, S. E. (2000). An acylated phloroglucinol with antimicrobial properties from *Helichrysum caespititium*. *Phytochem.*, *53*, 93–96.

Middleton, E., Kandaswami, C. & Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol Rev.*, *52*, 673-751.

Min, B. R., Pomroy, W. E., Hart, S. P. & Sahlu, T. (2004). The effect of short-term consumption of a forage containing condensed tannins on gastrointestinal nematode parasite infections in grazing wether goats. *Small Rumin. Res.*, *51*, 279-283.

Naito, Y. & Yoshikawa, T. (2009). Green Tea and Heart Health, *J. Cardiovas. Pharmacol.*, *54*, 385-390.

Ndamba, J., Nyazema, N., Makaza, N., Anderson, C. & Kaondera, K. C. (1994). Traditional herbal remedies used for the treatment of urinary shistosomiasis in Zimbabwe. *Journal of Ethnopharmacology*, *42*, 125–132.

Nderitu, A. M., Dykes, L., Awika, J. M., Minnaar, A. & Duodu, K. G. (2013). Phenolic composition and inhibitory effect against oxidative DNA damage of cooked cowpeas as affected by simulated *in vitro* gastrointestinal digestion. *Food Chem.*, *141*, 1763–1771. http://dx.doi.org/10.1016/j.foodchem.2013.05.001.

Nóbrega, F. M., Santos, I. S., Da Cunha, M., Carvalho, A. O. & Gomes, V. M. (2005). Antimicrobial proteins from cowpea root exudates: inhibitory activity against Fusarium oxysporum and purification of a chitinase-like protein. *Plant Soil*, *272*, 223–232.

Nyazema, N. Z. (1987). Medicinal plants of wide use in Zimbabwe. In: Leewenberg AJM (ed) Medicinal and Poisonous Plants of the Tropics. *Proceedings of Symposium of the 14th International Botanical Congress*, Berlin. Pudoc, Wageningen, pp. 36–43. ISBN 902–200–921–1.

Obatolu, V. A. (2003). "Growth pattern of infants fed with a mixture of extruded malted maize and cowpea". *Nutrition*, *19*, 174–178.

Ojwang, L. O., Banerjee, N., Noratto, G. D., Angel-Morales, G., Hachibamba, T., Awika, J. M. & Mertens-Talcott, S. U. (2015). Polyphenolic extracts from cowpea (*Vigna unguiculata*) protect colonic myofibroblasts (CCD18Co cells) from lipopolysaccharide (LPS)-induced inflammation–modulation of microRNA 126. *Food Funct.*, *6*(1), 145-153.

Ojwang, L. O., Dykes, L. & Awika, J. M. (2012). Ultra performance liquid chromatography–tandem quadrupole mass spectrometry profiling of anthocyanins and flavonols in Cowpea (*Vigna unguiculata*) of Varying Genotypes. *J. Agric. Food Chem.*, *60*, 3735–3744.

Padulosil, S. & Ng, N. Q. (1997). "Origin, taxonomy, and morphology of *Vigna unguiculata* (L.) Walp." In Singh, B.B., Mohan, D.R., Dashiell, K.E., Jackai, L.E.N. *Advances in Cowpea Research*. Ibadan, Nigeria: International Institute of Tropical Agriculture and Japan International Research Center for Agricultural Sciences.

Perrino, P., Laghetti, G., Spagnoletti Zeuli, P. L. & Monti, L. M. (1993). "Diversification of cowpea in the Mediterranean and other centres of cultivation". *Genet. Resour. Crop Evol.*, *40*, 121–132.

Pihlanto-Leppälä, A. (2000). Bioactive peptides derived from bovine whey proteins: Opioid and ace-inhibitory peptides. *Trends Food Sci Technol.*, *11*(9–10), 347–356.

Pollan, M. (2008). *In defense of food: An eater's manifesto* (New York: Penguin Press).

Poswal, M. A. T., Masunga, G., Javaid, I. & Kwerepe, B. C. (1993). Potential of different toxic and medicinal plant extracts for the control of fungal plant pathogens in Botswana. Mededelingen van de Faculteit Landbouwwetenschappen, *Rijksuniversiteit Gent.*, *58*, 1373–1381.

Pottorff, M., Ehlers, J. D., Fatokun, C., Roberts, P. A. & Close, T. J. (2012). "Leaf morphology in Cowpea [*Vigna unguiculata* (L.) Walp]: QTL analysis, physical mapping and identifying a candidate gene using synteny with model legume species". *BMC Genomics.*, *13*, 234.

Pugalendhi, P., Manoharan, S., Baskaran, N. & Nirmal, M. R. (2010). Effects of genistein and daidzein, in combination, on the expression pattern of biomolecular markers (p53, PCNA, VEGF, iNOS, Bcl-2, and

Bax) during 7,12-dimethylbenz(a)anthracene (DMBA) induced mammary carcinogenesis in Sprague- Dawley rats. *Int. J. Biol. Med. Res.*, *1*, 264-271.

Quin, F. M. (1997). “Introduction”. In Singh, B.B., Mohan Raj, D.R., Dashiell, K.E., Jackai, L.E.N. *Advances in Cowpea Research*. Ibadan, Nigeria: International Institute of Tropical Agriculture and Japan International Research Center for Agricultural Sciences.

Rao, K. N. & Suresh, C. G. (2007). Bowman–Birk protease inhibitor from the seeds of Vigna unguiculata forms a highly stable dimeric structure. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, *1774*, 1264-1273.

Rawal, K. M. (1975). “Natural hybridization among wild, weedy and cultivated *Vigna unguiculata* (L.) Walp”. *Euphytica*, *24*, 699–707.

Ren, M. Q., Kuhn, G., Wegner, J. & Chen, J. (2001). Isoflavones, substances with multi-biological and clinical properties *Eur. J. Nutr.*, *40*, 135-46.

Rishika, J., Vijayalakshmi, V., Rajanna, A., Naresh, V. & Suresh, C. (2016). Possible synergistic role of cowpea isoflavones and vitamin D Combo in the bone formation of MG-63 Osteoblasts. *Vitamin Mineral*, *5*, 146. doi:10.4172/2376-1318.1000146.

Sa’adah, N. L. & Anandita, N. W. (2016). Effect of Kt6 variant Cowpea (*Vigna Unguiculata*) extract on matrix metalloproteinase-9 and VEGF expression of corneal inflammation rat model (Rattus novergicus Strain Wistar). *IJPRIF*, *9*, 119-130.

Samman, N., Maldonado, S., Alfaro, M. E., Farfan, N. & Gutierrez, J. (1999). Composition of different bean varieties (*Phaseolus vulgaris*) of Northwestern Argentina (region OA): Cultivation zone influence. *J. Agr. Food Chem.*, *47*, 2685–2689.

Sandeep, D. (2014). Evaluation of antibacterial activity of seed extracts of *Vigna unguiculata. Int. J. Pharm. Pharm. Sci.*, *6*, 75-77.

Segura-Campos, M. R., Chel-Guerrero, L. A. & Betancur-Ancona, D. A. (2011). Purification of angiotensin I-converting enzyme inhibitory peptides from a cowpea (*Vigna unguiculata*) enzymatic hydrolysate. *Process Biochem.*, *46* (4), 864-872.

Sheahan, C. M. (2012). *"Plant guide for cowpea* (*Vigna unguiculata*)" (PDF). USDA-Natural Resources Conservation Service, Cape May Plant Materials Center, Cape May, NJ.

Siddhuraju, P. & Becker, K. (2007). The antioxidant and free radical scavenging activities of processed cowpea (Vigna unguiculata (L.) Walp.) seed extracts. *Food Chem.*, *101*, 10–19.

Singh, B. B., Mohan, Raj D. R., Dashiell, K. E. & Jackai, L. E. N. (1997). *Advances in Cowpea Research.* Co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), IITA, Ibadan, Nigeria, p x. ISBN 978–131–110–X.

Small, E. (2009). *Top 100 Food Plants.* NRC Research Press., p. 104.

Sosulski, F. W. & Dabrowski, K. J. (1984). Composition of free and hydrolyzable phenolic acids in the flours and hulls of ten legume species. *J. Agr. Food Chem.*, *32*, 131–134.

Sreerama, Y. N., Sashikala, V. B. & Pratape, V. M. (2012). Phenolic compounds in cowpea and horse gram flours in comparison to chickpea flour: evaluation of their antioxidant and enzyme inhibitory properties associated with hyperglycemia and hypertension. *Food Chem.*, *133* (1), 156–162.

Sugimoto, E. & Yamaguchi, M. (2000). Stimulatory effect of Daidzein in osteoblastic MC3T3-E1 cells. *Biochem. Pharmacol.*, *59*, 471-475.

Tazin, T. Q., Rumi, J. F., Rahman, S., Al-Nahain, A., Jahan, R. & Rahmatullah, M. (2014). Oral glucose tolerance and antinociceptive activity evaluation of methanolic extract of *Vigna unguiculata* SSP. Unguiculata beans. *WJPPS*, *3*, 28-37.

Timko, M. P., Ehlers, J. D. & Roberts, P. A. (2007). "Cowpea". In Kole, C. *Pulses, Sugar and Tuber Crops, Genome Mapping and Molecular Breeding in Plants.*, *3*. Berlin, Heidelberg: Springer-Verlag. pp. 49–67.

Van Wyk, B. E. & Gericke, N. (2000). *People's Plants: a Guide to Useful Plants in Southern Africa*. Briza Publications, Pretoria, p 192. ISBN 187–509–319–2.

Wang, J., Shang, F., Mel, Q., Wang, J. & Wang, S. (2008). NO-Donating Genestein prodrug alleviates bone loss in ovarioectomized rats. *Swiss Med Wkly*, *38*, 602-607.

Weththasinghe, P., Liyanage, R., Vidanarachchi, J., Perera, O. & Jayawardana, B. (2014). Hypocholesteolemic and Hypoglycemic effect of Cowpea (*Vigna Unguiculata* (L) Walp) incorporated experimental diets in Wistar rats (*Rattus Norvegcus*). *Agriculture and Agricultural Science Procedia*., *2*, 401-405.

Worthington, R. W. & Bigalke, R. D. (2001). A review of the infectious diseases of African wild ruminants. *Onderstepoort J. Vet. Res*., *68*, 291–323.

Ye, X. Y., Wang, H. X. & Ng, T. B. (2000). Structurally dissimilar proteins with antiviral and antifungal potency from cowpea (*Vigna unguiculata*) seeds. *Life Sci.*, *67*(26), 3199– 3207.

Zia-Ul-Haq, M., Ahmad, S., Chiavaro, E., Mehjabeen. & Ahmed, S. (2010). Studies of oil from cowpea [*Vigna unguiculata* (L.) Walp] cultivars commonly grown in Pakistan. *Pakistan J. Bot.*, *42*, 1333–1341.

Zia-Ul-Haq, M., Ahmad, Sh., Amarowicz, R. & De Feo, V. (2013). Antioxidant activity of the extracts of some cowpea (*Vigna unguiculata* (L) Walp.) cultivars commonly consumed in Pakistan. *Molecules*, *18*, 2005-2017.

Zia-Ul-Haq, M., Cavar, S., Qayum, M., Imran, I. & de Feo, V. (2011). Compositional studies, antioxidant and antidiabetic activities of *Capparis decidua* (Forsk.) Edgew. *Int. J. Mol. Sci.*, *12*, 8846–8861.