The anti-inflammatory and antioxidant properties of Kenyan leafy green vegetables, wild fruits, and medicinal plants: relation of the health-promoting properties of biodiversity to contribute to kwashiorkor alleviation

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ABSTRACT

The etiology of kwashiorkor, a form of severe acute malnutrition in children, may be linked to inflammation and oxidative stress. Biodiversity can contribute health-promoting properties that may address the inflammation and oxidative stress seen in kwashiorkor. Six medicinal plants, five leafy green vegetables (LGV), and two wild fruits were collected from Kaiti Division, Makueni County, Eastern Province, Kenya and examined for antioxidant and anti-inflammatory properties using the oxygen radical absorbance capacity (ORAC) and total phenolics assays and a TNF-α in vitro assay, respectively. All the medicinal plants and several LGV had antioxidant and anti-inflammatory activity. *A. dubius, V. unguiculata* (both LGV), *O. americanum*, and *Z. chalybeum* (medicinal plants) showed the greatest anti-inflammatory activity among the plants tested and were also widely consumed and used among the children in this study. There was a wide variety of LGV and wild fruits available in the study region, which may have contributed to the high mean food variety score (FVS) of 26 for the non-breastfed children. This study demonstrates a theoretical basis for investigating a link between health-promoting properties of biodiversity, dietary diversity, and the development of kwashiorkor for improved nutrition and health outcomes in children.
RÉSUMÉ

L’éthologie de kwashiorkor, une forme de malnutrition sévère et aigu chez les enfants, peut-être lié à l’inflammation et le stress oxydatif. La biodiversité peut contribuer des propriétés qui favorise la santé et qui adresse l’inflammation et le stress oxydatif qui se trouve dans le kwashiorkor. Six plantes médicinales, cinq légumes à feuilles vertes et deux fruits sauvages ont été recueillis par Kaiti Division, Makueni County, Eastern Province, Kenya et examiné pour des propriétés antioxydant et anti-inflammatoire en utilisant les dosages ORAC et des phénols totaux et un dosage TNF- α in vitro, respectivement. Tous les plantes médicinales et plusieurs LGV ont eu de l’activité antioxydant et anti-inflammatoire. A. dubius, V. unguiculata (LGV), O. americanum, et Z. chalybeum (des plantes médicinales) ont montré le plus grand activité anti-inflammatoire de toutes les plantes testées et ont été également largement consommé et utilisé chez les enfants dans cette étude. Il y avait une grande variété de LGV et de fruits sauvages disponibles dans la région étudiée, ce qui peut avoir contribué à des scores élevés moyennes alimentaires divers (FVS) de 26 pour les enfants non allaités au sein. Cette étude démontre une base théorique pour enquêter sur un lien entre promotion de la santé des propriétés de la biodiversité, la diversité alimentaire, et le développement du kwashiorkor pour améliorer la nutrition et la santé chez les enfants.
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CONTRIBUTION OF AUTHORS

The manuscript enclosed in this thesis was a collaboration of the co-authors. H. Tufts and T. Johns developed the research objectives and study design. H. Tufts developed the interview consent forms and questionnaires, chose the interview locations and households, trained the translators, conducted the interviews with the translators and collected the plant samples, transported the plant samples back to Canada, and performed all the laboratory analyses and data analyses of the interview and laboratory results. C. Harris provided significant guidance for which laboratory assays to use, training, laboratory and data analysis, and data presentation. H. Tufts wrote the manuscript and T. Johns provided editorial comments for improvement to the format and content. Colleagues from the Kenya Agricultural Research Institute (KARI) who are partners in the KARI-McGill food security project facilitated and participated in aspects of the fieldwork. Following further discussion among the project participants, one or more will be included as co-authors.
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<td>AAPH</td>
<td>2,2′-Azobis-2-methyl-propanimidamide, dihydrochloride</td>
</tr>
<tr>
<td>DDS</td>
<td>Dietary diversity score</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
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<tr>
<td>FFQ</td>
<td>Food frequency questionnaire</td>
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<tr>
<td>FVS</td>
<td>Food variety score</td>
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<tr>
<td>HAZ</td>
<td>Height-for-age z-score</td>
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<tr>
<td>LGV</td>
<td>Leafy green vegetables</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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1.0 INTRODUCTION

Over the past three decades, research on kwashiorkor, a severe acute malnutrition state in children, has been steadily declining, despite the fact that its etiology remains unknown. The clinical features (ie. edema, skin lesions, fatty liver, hair discolouration, hypoalbuminemia) of kwashiorkor are well defined. The bulk of research, occurring from the 1950’s through the 1980’s, widely debated etiology and treatment methods. Protein deficiency represented the early main stream of thought for the etiology of kwashiorkor, and in many cases still persists to this day as the etiology despite evidence that suggests otherwise (Golden, 2002). Notably, some of the strongest evidence against protein deficiency are the studies that show the diet between children who develop kwashiorkor and those that develop marasmus or remain healthy are not different (Gopalan, 1968; Lin et al., 2007).

Additional theories have been put forward, of which oxidative stress by Golden and Ramdath (1987) has been the most prominent. Markers of oxidative stress are consistently identified in children with kwashiorkor (Becker et al., 1994; Fechner et al., 2001; Lenhartz et al., 1998; Manary, 2000). Although this theory has been challenged by a study that showed daily antioxidant supplementation was not effective at preventing kwashiorkor (Ciliberto et al., 2005), the effectiveness of antioxidant supplements in preventing illnesses is debatable (Herrera et al., 2009) and additional evidence suggests that there may be another component, such as inflammation. Elevated markers of inflammation have been identified in children with kwashiorkor, whether they have an infection or not.
(Dülger et al., 2002; Sauerwein et al., 1997). Inflammation also induces oxidative stress, edema, and can potentiate fatty liver disease (Abdelmalek & Diehl, 2007; Fuhrman et al., 2004; Osorio, 2011). Therefore, kwashiorkor may be seen as malnutrition state linked with inflammation.

Anti-inflammatories and antioxidants in plants and in the diet should be assessed in relation to kwashiorkor. Although a few studies have looked at the diet of children who develop kwashiorkor, the focus has primarily been on protein, or on foods with antioxidant vitamins, such as tomatoes (Lin et al., 2007). However, polyphenols are consumed in greater quantities than antioxidant vitamins and can be more functionally active (Scalbert et al., 2005). Polyphenols also can have anti-inflammatory activity (Gautam & Jachak, 2009), which may have more importance for kwashiorkor than antioxidant activity. Biodiversity can contribute to improving health and perhaps reducing the development of kwashiorkor through providing both nutrients and health promoting properties (Johns & Eyzaguirre, 2006).

Traditional and wild plant species are elements of biodiversity that are rich in these qualities, are culturally relevant in developing countries, and are often underutilized in research. This study evaluated the potential role of plant diversity by investigating the antioxidant and anti-inflammatory properties of traditional and wild plant species that were widely consumed or used medicinally by children in a rural region of Kenya. The plants were identified through interviews with mothers who had at least one child under the age of five years and were assessed in the laboratory using the ORAC assay for antioxidant activity, Folin-Ciocalteu
method for total phenolics, and a TNF-α *in vitro* assay for anti-inflammatory activity. Dietary diversity and medicinal plant use were also explored to gain a snapshot of the contribution of plant diversity towards the diet and health of the children.

The identification of plants that are rich in antioxidant and anti-inflammatory properties allows for future research that could address in more detail the contribution of these plants to dietary diversity and prevention and treatment of kwashiorkor. Antioxidant and anti-inflammatory properties in traditional and wild plant species have not been assessed in research related to kwashiorkor before, but could be a rich resource to explore with potential to improve nutrition and health outcomes in children.

![Diagram of the theoretical basis for the thesis. Biodiversity is represented by LGV, wild fruits, and medicinal plants. These are rich sources of antioxidants and anti-inflammatories which may contribute both to improved health and reduced risk of kwashiorkor in children under five years.](image)

**Figure 1.1** Diagram of the theoretical basis for the thesis. Biodiversity is represented by LGV, wild fruits, and medicinal plants. These are rich sources of antioxidants and anti-inflammatories which may contribute both to improved health and reduced risk of kwashiorkor in children under five years.
2.0 LITERATURE REVIEW

2.1 Kwashiorkor origins

Kwashiorkor is a complex state of severe acute malnutrition seen in young children. Its etiology remains an enigma, despite many competing theories that have arisen since it was first described. Because of the uncertainty over the etiology, kwashiorkor cannot be as clearly defined as marasmus (a condition of severe protein and energy restriction characterized by loss of body muscle and fat mass (Waterlow, 1984)) but is distinguished by definitive clinical features that include edema, skin lesions, fatty liver, hair discolouration, and hypoalbuminemia (Golden & Ramdath, 1987).

Dr. Cicely Williams in Ghana first ascribed the term kwashiorkor, which corresponded to the child who was weaned after another was born, to the malnutrition state in 1935. She observed that the children who developed the malnutrition state she referred to as kwashiorkor were often weaned using a maize staple diet with very little protein (Williams, 1935). It was believed that a deficiency of protein was the primary cause of kwashiorkor due to similar observations in other regions and countries which showed that where children were fed a predominately low protein and carbohydrate rich diet there was a greater prevalence of kwashiorkor than regions where the children were fed milk and meat (Brock & Autret, 1952). However, the nutritive value of the foods was not well established, neither were quantitative values of food consumption, and no statistical analyses were performed to see if the diets were significantly different.
From these early observations though, it was postulated that kwashiorkor was caused by a severely low protein diet but with adequate energy intake, which can also be referred to as the classical theory of kwashiorkor (Waterlow, 1984). This has been the long-standing paradigm of kwashiorkor, despite strong evidence against it.

2.2 Protein deficiency as the etiology

The main argument used by the proponents of protein deficiency as the etiology of kwashiorkor is the theory that low protein intake causes low serum albumin, which leads to hypoalbuminemia resulting in edema, the hallmark clinical feature of kwashiorkor (Waterlow, 1984). The evidence put forward to support this theory is presented by studies associating hypoalbuminemia with kwashiorkor. Montgomery in 1963 measured serum albumin concentrations in the plasma and liver enlargement and function of 200 children admitted consecutively to a hospital in Jamaica for what was determined as protein malnutrition at the time. He classified edema into three groups (absent or slight edema in face and extremities; moderate or isolated severe edema in lower trunk only; severe edema all over) based on clinical assessment and the percentage of weight loss from edema on recovery (Montgomery, 1963). There was an inverse relationship between serum albumin and severity of edema. The group with the most severe degree of edema showed significantly (p<0.01) lower serum albumin concentrations than the group with the lowest degree of edema (Montgomery, 1963). However, only half those with low serum albumin had severe edema or significantly enlarged liver size. Correlation between individual serum albumin
concentrations and liver size was minimal, similarly there was no correlation between liver size and liver dysfunction as measured by serum pseudocholinesterase activity. This study could also not show causation between liver dysfunction and hypoproteinemia.

Those who support the protein deficiency theory believe that the fatty liver seen in kwashiorkor is caused by low amino acid concentrations due to low protein intake. The low amino acid concentrations are thought to cause a reduction in the synthesis of export proteins by the liver, such as apolipoprotein B-100, leading to reduced export of fatty acids and triglycerides and ultimately leading to fatty liver disease (Waterlow, 1984). James and Hay (1968) showed that the synthetic rate of albumin (absolute and fractional) in nine malnourished and nine recovered malnourished children decreased quickly in response to a low protein diet and rose quickly when the children returned to a high protein diet. Since the study by James and Hay in 1968 measured albumin synthesis, not apolipoprotein synthesis, the results cannot be linked to causing fatty liver. A decrease in apolipoproteins synthesis has not been shown to have a corresponding decrease in albumin synthesis (Charlton et al., 2002). Therefore, although albumin synthesis was decreased in malnourished children, this does not necessarily mean that there was a global decrease in hepatic export proteins as postulated. The main studies supporting protein deficiency were conducted more than 40 years ago and few recent studies support this as the etiology. Instead, there has been strong evidence put forward against this theory.
2.3 Evidence against protein deficiency

According to the protein deficiency theory, reduction in edema should result from the normalization of serum albumin upon treatment and recovery. If low dietary protein leads to amino acid deficiencies and reduced synthesis of albumin, then it would be expected that improved dietary protein intake would lead to an improvement of serum albumin and loss of edema. However, it does not appear to be such a simple case, as edema has been shown to recover without initiation of protein synthesis and a change in serum albumin concentration (Golden et al., 1980). The edema has also been shown to recover independent of dietary protein (Golden, 1982) and low serum albumin levels also correspond to a range of degrees of edema, suggesting it cannot be the sole perpetrator in causing edema (Montgomery, 1963). In addition, children with kwashiorkor who are fed a low protein treatment diet have been shown to improve significantly better and have lower mortality than children with kwashiorkor fed a high protein treatment diet (Golden, 2002). Therefore, like fatty liver, the occurrence and loss of edema does not seem to fit the pattern presented by the protein deficiency theory.

The evidence used by the proponents of the protein deficiency theory for the cause of fatty liver in kwashiorkor has been based on indirect measures. The theory is that the fatty liver is caused by the reduced synthesis of apolipoprotein B-100 and impaired VLDL export of triacylglycerols (TAGs) due to low protein intake, based on measures of plasma concentrations of TAGs, cholesterol, and or non-esterified fatty acids (Badaloo et al., 2005). However, these measures have produced inconsistent results, as the studies reported low plasma concentrations
upon admission that increased upon recovery but also normal and high plasma concentrations that either did not change or decreased upon recovery, respectively (Agbedana et al., 1979; Dhansay et al., 1991; Rao & Prasad, 1966). Direct measurement of VLDL apolipoprotein B-100 synthesis was done by Badaloo et al. in 2005, by the use of isotopically labeled leucine infused intravenously in thirteen children admitted to the hospital with severe malnutrition. The degree of fat content in the liver was also measured using a CT scanner to determine the ratio of liver attenuation to spleen attenuation (L:S), interpreted as low L:S ratios corresponding to greater fat content of the liver (Badaloo et al., 2005). Badaloo et al. (2005) found that as the fat content increased in the liver the synthesis of apolipoprotein B-100 also increased. In addition, livers with high fat content also had elevated plasma concentrations of TAGs and cholesterol. Therefore, it would appear that fatty livers show an increase in lipid movements from the liver (Badaloo et al., 2005). This study was limited in that fact that they used mean plasma volumes of VLDL-apo B-100 from children classified as kwashiorkor, marasmic kwashiorkor, or marasmus that were calculated previously from a different group of children with similar ages, fluid intake, and rehabilitation stage, as opposed to using the specific plasma volumes from each child in the study from 2005 (Badaloo et al., 2005). Despite this limitation, the previous assumption that changes in albumin synthesis in response to dietary protein would also translate to changes in apolipoprotein synthesis is not supported by this study. Low dietary protein, therefore, cannot seem to fully explain the clinical features of kwashiorkor.
Qualitative assessment of the protein content of the diet of children who develop kwashiorkor and those who develop marasmus or remain healthy also does not support the theory of protein deficiency. A prospective observational study by Lin et al. (2007), conducted in 8 rural villages in Malawi over 10 weeks with 1651 children found no associations between the dietary intake of protein or any individual food component and the development of kwashiorkor. In general, the diet of the children who developed kwashiorkor and those that did not was based mainly on maize and had very little variety of foods and low amount of energy and protein. Therefore, if all the children had a similarly poor diet, there must have been additional factors that led to only some of the children developing kwashiorkor. Although the diet was only assessed at the start of the 10 weeks and the HIV status of the children was not known, the study confirms the results of a previous study that found no qualitative difference in the diets of children in India who developed kwashiorkor and those developed marasmus (Gopalan, 1968). The evidence seems to point towards additional complexity in the etiology of kwashiorkor. Other theories have since been developed to try and explain the contradictory results.

2.4 Oxidative stress and kwashiorkor

The strongest unifying theory to challenge the classical theory of kwashiorkor was presented in 1987 by Golden and Ramdath who suggested that oxidative stress could explain the etiology of this disease. Under normal conditions, the oxidation and reduction reactions are tightly controlled. However, with oxidative stress, the control on the balance between oxidation and reduction
is disrupted which can result in an imbalance of antioxidants and pro-oxidants (Jones, 2006). Golden and Ramdath proposed that a radical inducing stimuli causes an increase in radical oxygen species (ROS), while inadequate dietary intake of protein and/or energy and micronutrients results in a reduction in the antioxidant protective mechanisms, leading to oxidative stress (Golden & Ramdath, 1987). ROS are produced by the immune system in response to infection, inflammation, inadequate disposal of toxin, or damage to the liver which could be caused by abnormal bacterial overgrowth of the small intestine, a feature seen in kwashiorkor (Golden & Ramdath, 1987). Small intestinal bacterial overgrowth greater than the bacterial growth seen in healthy controls is associated with cirrhosis of the liver and may increase the permeability of the small intestine to endotoxins, which act as hepatotoxins and stimulate the release nitric oxide, TNF-α, and ROS in the liver (Bauer et al., 2002; Madrid et al., 2001; Wigg et al., 2001). The ROS could overwhelm antioxidant defenses and lead to oxidative stress which could cause edema, fatty liver, hair discoloration and skin lesions through damage to the cell membranes leading to apoptosis and cellular dysfunction (Golden & Ramdath, 1987).

In support of the theory by Golden and Ramdath (1987), markers of oxidative stress have been consistently identified in children with kwashiorkor but not in marasmic or healthy children. Direct products of oxidation, such as the oxidized amino acid o,o’-dityrosine, a marker of damage by tyrosyl radicals, was shown to be seven-fold greater (p<0.01) in twenty-five children with kwashiorkor, with or without an infection, compared to ten healthy well-nourished children
Markers of lipid peroxidation, such as low levels of highly polyunsaturated fatty acids (PUFA) in erythrocytes and plasma lipids, were lowest in twelve children with kwashiorkor compared to thirty-two children with marasmus and twenty-three healthy children (Leichsenring et al., 1995). Lipid peroxidation was also seen by low PUFA and elevated leukotrienes and malondialdehyde, which are radicals involved in lipid peroxidation, in a case study of a child who developed kwashiorkor while admitted to the hospital for malnutrition (Lenhartz et al., 1998). Reduced antioxidant defences are seen in children with kwashiorkor by low plasma levels of vitamin E, alpha- and beta-carotene, and a significantly lower tocopherol/lipid ratio than in children with marasmus or healthy controls (Becker et al., 1994). Glutathione, the main antioxidant of the body, has also been shown to be reduced in the erythrocytes of children with kwashiorkor compared to normal children (Becker et al., 1995; Jackson, 1986), although low levels have also been seen in children with marasmus (Becker et al., 1995). Levels of pro-oxidants have been shown to be elevated above normal levels in children with kwashiorkor, such as plasma free iron (Golden & Ramdath, 1987; Sive et al., 1997), nitric oxide (Fechner et al., 2001), leukotrienes (Mayatepek et al., 1993), and the pro-inflammatory cytokines interleukin-6 (IL-6), and TNF-α (Dülger et al., 2002; Kmiec, 2001; Sauerwein et al., 1997). Lastly, children with kwashiorkor show reduced mobilization of leukocytes and reduced transformation of lymphocytes (Geehuysen et al., 1971; Kulapongs et al., 1977) and therefore increased susceptibility to infections, which may be due to the low levels of vitamin A, zinc, and selenium (Golden & Ramdath, 1987).
Increasing evidence for the importance of oxidative stress suggests that targeting the elevated level of oxidative stress may be effective for preventing and treating kwashiorkor. One study supplemented the treatment diet of children with kwashiorkor with N-acetylcysteine while the control group received a placebo supplement of alanine in their treatment diet in order to see if N-acetylcysteine would lead to improved glutathione concentrations and synthesis (Badaloo et al., 2002). Erythrocyte cysteine concentrations were significantly increased in the N-acetylcysteine group, along with significant increases in erythrocyte glutathione concentration and synthesis (Badaloo et al., 2002). The children who received N-acetylcysteine also lost their edema sooner than the control group (Badaloo et al., 2002).

A second study randomly assigned children with kwashiorkor to four groups and had a control group of healthy children as a comparison. The placebo group received a standard treatment and three treatment groups received additional supplements of either glutathione, α-lipoic acid, or N-acetylcysteine (Becker et al., 2005). The glutathione concentrations were doubled within eight days in the group receiving glutathione supplements, but this quick increase was not seen in the other groups. Lethality was reduced the greatest, by 33%, in the glutathione group and this group also had significantly improved survival, compared to the standard treatment group, when glutathione full-blood concentration and height were controlled for (Becker et al., 2005). There was
also a positive association between baseline and follow-up glutathione concentrations with improved survival (Becker et al., 2005). The α-lipoic acid and N-acetylcysteine improved glutathione concentrations to greater than that of the standard treatment group over twenty days, but did not show the initial quick response or a significantly improved survival like the glutathione group. Together with the study by Badaloo et al. (2005), these findings suggest that improving glutathione concentrations during treatment of kwashiorkor improves survival of the children.

Ciliberto et al. (2005) looked at prevention of kwashiorkor by a daily supplementation of antioxidants (riboflavin, vitamin E, selenium, and N-acetylcysteine) in children one to four years of age in Malawi. They failed to show a statistically protective effect against kwashiorkor, although the effectiveness of antioxidant supplements in preventing illness is debatable (Briançon et al., 2011; Herrera et al., 2009). Health benefits from antioxidants are more likely through synergistic interactions between nutrients taken in the diet, specifically the phytochemicals in vegetables and fruits (Liu, 2003) which are usually not included in antioxidant supplements. Supplements are important for correcting nutritional deficiencies, however, long-term health benefits are more likely achieved through focusing on specific foods, nutrient interactions, and a diverse diet (Jacobs et al., 2009). The antioxidant supplement may also have been ineffective if there was an underlying state of inflammation. The oxidative stress seen in kwashiorkor may not be the primary cause, but actually a secondary
outcome of inflammation, which could also explain why the antioxidant supplement was ineffective at preventing kwashiorkor.

### 2.5 Inflammation as a unifying etiology for kwashiorkor

Inflammation is involved in many diseases and can induce oxidative stress (Osorio, 2011). Inflammatory markers, such as interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α), and oxidized amino acids, have been shown to be increased in children with kwashiorkor, whether they had an infection or not (Dülger et al., 2002; Manary, 2000; Sauerwein et al., 1997). This observation could explain the fatty liver infiltration in kwashiorkor, as TNF-α has been shown to stimulate the recruitment of additional inflammatory mediators to the liver and cause apoptosis of hepatocyte cells. TNF-α promotes the production of free radical species which can further potentiate fatty liver development (Abdelmalek & Diehl, 2007). TNF-α also causes capillary membrane leak, resulting in edema, as part of its inflammatory actions (Fuhrman et al., 2004). In addition, elevated bacterial overgrowth in the small intestine, as seen in kwashiorkor (Golden & Ramdath, 1987), can cause the liver to produce TNF-α, which may further potentiate its fatty liver infiltration and oxidative actions (Abdelmalek & Diehl, 2007; Li et al., 2003).

The reduction of negative acute-phase proteins in inflammation, such as albumin, prealbumin, and transferrin, is controlled by TNF-α (Fuhrman et al., 2004). It is possible, then, that the low concentrations of albumin in kwashiorkor could be attributed to inflammation and not to nutritional status (Fuhrman et al.,
2004). With recovery and a reduction in inflammation, there is a lessening in the actions of pro-inflammatory cytokines, which results in the restoration of albumin synthesis, hepatic protein synthesis in general, and capillary membrane permeability (Fuhrman et al., 2004). TNF-α also causes a net increase in protein catabolism, which can have an anorexic effect and increase the risk of malnutrition in a person (Fuhrman et al., 2004; Manary et al., 1997). However, although children with kwashiorkor are anorexic (Osorio, 2011) and have elevated TNF-α and IL-6 (Dülger et al., 2002; Sauerwein et al., 1997), they have reduced protein catabolism (Manary et al., 1998). These discrepancies have not been explained and require further investigation.

IL-6 increases positive acute-phase proteins (Gabay & Kushner, 1999), principally C-reactive protein, and can contribute to chronic inflammation (Burger & Dayer, 2002). C-reactive protein has also been found to be increased in children with kwashiorkor (Sauerwein et al., 1997). The response of acute-phase proteins is individually regulated by cytokine production, such that even among people with the same illness the acute-phase response may not be uniform (Gabay & Kushner, 1999). The individual regulation of cytokine production may explain some of the discrepancies seen in kwashiorkor in terms of clinical manifestations and biochemical marker inconsistencies.

A potential stimulus that could induce an inflammatory response is exposure to aflatoxin. The occurrence of aflatoxin contamination overlaps with tropical areas that experience kwashiorkor (Hendrickse, 1984). Aflatoxin is a
mycotoxin that has serious carcinogenic and hepatotoxic effects, depending on the exposure duration (Lewis et al., 2005). Aflatoxin has been associated with cases of kwashiorkor (de Vries et al., 1987) and long-term exposure to low levels could be a stimulus that induces elevated inflammation and oxidative stress (Hendrickse, 1984).

Evidence, as suggested by a recent review (Osorio, 2011), indicates that kwashiorkor should be seen within a framework of malnutrition and inflammation. Studies have consistently shown that inflammation and oxidative stress are elevated in kwashiorkor. Viewing kwashiorkor through the lens of malnutrition linked with inflammation and oxidative stress may prove more beneficial for developing effective prophylactic and therapeutic measures. The diet has an important role to play, not only by targeting malnutrition, but also for providing anti-inflammatory and antioxidant properties for prevention and treatment.

2.6 Health promoting properties in the diet

Additional factors that have largely been overlooked in kwashiorkor research are the roles that phytochemicals, such as polyphenols, play in maintaining and promoting good health. Although a few studies focused on kwashiorkor have looked at antioxidant vitamins and minerals, largely vitamins C and E, riboflavin, and/or selenium, (Ciliberto et al., 2005; Lin et al., 2007), no studies have considered polyphenols.
Polyphenols, classified as flavonoid or non-flavonoid compounds, are secondary metabolites produced by plants that have a characteristic chemical structure of a benzene ring with one or more hydroxyl groups attached (Fraga et al., 2010). There are over 8000 distinct polyphenols within dietary plants (Fraga et al., 2010) and it has been estimated that the daily intake of polyphenols exceeds that of antioxidant vitamins (Scalbert et al., 2005). Substantial research has focused on the various biological functions of polyphenols, especially on their antioxidant functions (Scalbert et al., 2005). Research has also shown that polyphenols can be more active than antioxidant vitamins in terms of antioxidant activity (Lu & Yeap Foo, 2000; Pulido et al., 2000). Along with their antioxidant activity, polyphenols have been shown to function as potent anti-inflammatories (Gautam & Jachak, 2009). Anti-inflammatory activity may have more relevance for kwashiorkor than antioxidant activity because inflammation is most likely the cause of the oxidative stress seen in kwashiorkor.

The health benefits attributed to polyphenols when they are consumed as part of the diet fit within the concept of food synergy, which is the idea that the complex of individual constituents within food has greater biological actions than the individual constituents on their own (Jacobs et al., 2009). Food synergy focuses on whole food components and the effects of the overall average intake in a variety of foods over a long period of time (Jacobs et al., 2009). Often the constituents within food interact with each other through digestion, in some cases by enhancing bioavailability or antioxidant activity, and after surviving digestion, the constituents must also be biologically active for food synergy to work (Jacobs
et al., 2009). The interaction among food constituents and biological activity after digestion supports having a diverse diet in order to increase the variety of these constituents, their interactions, and the biological activity as a result. Most likely it is these synergistic interactions among vitamins, minerals, and polyphenols within the diet which provides health benefits (Liu, 2003), although further research is needed on bioavailability and in vivo actions (Scalbert et al., 2005). A diverse diet in foods rich in anti-inflammatory and antioxidant polyphenols may be a research area requiring further investigation for effectiveness in the prevention and treatment of kwashiorkor.

2.7 Biodiversity for nutrition and health: relevance for kwashiorkor

Biodiversity provides a framework for healthy ecosystems, agrobiodiversity, and sustainable practices and livelihoods (Johns & Sthapit, 2004). Biodiversity, specifically plant diversity, has relevance for kwashiorkor as it contributes to both nutrition and health through diversifying the diet when promoted, thereby improving nutrient intakes, and through the additional health properties attributed to phytochemicals. This has important implications for malnutrition by contributing a more holistic approach to the interrelationships among health, diet, individual physiological dietary needs, and culture (Johns, 2003).

A variety in plants can equate to a more varied diet and consequently a more varied intake of nutrients and functional properties, as seen by greater dietary diversity associated with improved health outcomes in children (Onyango
et al., 1998; Steyn et al., 2006). The relevance of this for kwashiorkor is furthered by the fact that plant diversity is a rich and often underutilized source of both nutrition and health properties, such as antioxidants (Hassimotto et al., 2005; Ndhlala et al., 2006), anti-inflammatories (Fawole et al., 2009), antibacterial, and anti-parasitic properties (Alam Ripa et al., 2009; Irungu et al., 2007; Muregi et al., 2007). Low consumption of these properties of local plant diversity by children can distinguish those who develop severe malnutrition from others (Johns & Sthapit, 2004). Within many societies, traditional and wild plant species are an integral component of dietary diversity, yet they are often ignored in dietary surveys, policy, or food security initiatives (Flyman & Afolayan, 2006; Grivetti, 2000). Particularly, traditional LGV, wild fruits, and medicinal plants are elements of plant diversity that have both an impact on nutrition and health with implications for kwashiorkor.

2.7.1 Leafy green vegetables

In many developing countries traditional species are an important part of the culture and diet. Within the African context, traditional LGV often used in stews or soups and accompany starchy staples to add flavour (Chewya & Eyzaguirre, 1999). LGV can supplement the diet during famine to provide additional nutrition and are also more affordable than other exotic market vegetables, such as carrots or spinach (Oniang'o et al., 2008). Many traditional LGV are cultivated, while others may be collected from the wild, especially during seasons when cultivated species are unavailable (Chewya & Eyzaguirre, 1999). Not only are they an invaluable part of the diet, they often have a
medicinal attribute as well, such as treating various illnesses or promoting appetite (Chewya & Eyzaguirre, 1999; Oniang'o et al., 2008).

Traditional LGV are not only important culturally, but nutritionally as well, containing rich sources of nutrients such as vitamins A, B, and C, iron, and zinc (Chadha et al., 2007; Odhav et al., 2007) and of polyphenols (Subhasree et al., 2009; Uusiku et al., 2010; Yang et al., 2007). Traditional LGV are often richer in these properties than exotic species (ie. non-indigenous) such as cabbage and spinach (Uusiku et al., 2010). The nutrient and polyphenol content of traditional LGV can be reduced by cooking and compounds in the plants that inhibit nutrient absorption, however, there are some cooking techniques, such as blanching, which can reduce factors that inhibit nutrient absorption while minimizing reduction on the nutritional content (Flyman & Afolayan, 2006). Therefore, traditional LGV can still be a rich source of nutrients and polyphenols when combined with an appropriate cooking method. The cultural, nutritional, health promoting, and medicinal properties of traditional LGV highlight their potential to contribute to alleviating malnutrition. These properties, specifically the polyphenols, also highlight their potential for the prevention and treatment of kwashiorkor.

2.7.2 Wild fruits

Wild fruits often make supplementary contributions to diet, serve as snacks, and are consumed more often by children than adults (Johns & Kokwaro, 1991; Nyambo et al., 2005). They are also important emergency foods during
drought and famine (Nyambo et al., 2005). Wild fruits tend to be consumed by children and women during their daily tasks and activities which involve more exposure to the natural environment than the daily tasks of men, although consumption by adults, in general, is often less due to societal factors that devalue wild fruits (Fentahun, 2009). The tendency of wild fruits to be supplementary to the diet does not rule out their contribution towards improving dietary diversity and micronutrient status, which is especially important for children, who have higher growth-related dietary requirements. Many wild fruits are rich in vitamins and minerals, such as vitamins A and C, potassium, phosphorous, and calcium (Maundu et al., 1999a; Nyambo et al., 2005), and can have a greater nutrient content than exotic fruit species which are grown and cultivated (Fentahun, 2009). Wild fruits can also be rich in polyphenols and have shown antioxidant activity (Egea et al., 2010; Lamien-Meda et al., 2008). The contribution of wild fruits to the dietary diversity of children and their rich nutritional and phenolic content provides a conceptual framework for their potential use in prevention and treatment of kwashiorkor.

### 2.7.3 Medicinal plants

Many cultures use traditional medicine to treat illness or promote good health, often alongside of Western medicine (Dahlberg & Trygger, 2009). According to the World Health Organization (WHO), 80% of people in Asian and African countries have used traditional medicine (WHO, 2008). These uses could be within both the medicinal and dietary context, as multiple meanings are often ascribed to medicinal plants (Etkin, 1986). In many cases, medicinal plants and
food plants are seen to be overlapping, with “medicinal” plants being taken as food and “food” plants being taken as medicine (Iwu, 1986). The chemical constituents within the medicinal plants used for both food and medicine may have both nutritional and functional properties (Etkin, 1986), although the phytochemical properties are largely what are studied.

The widespread use of medicinal plants and their impact on diet and health can be partly due to their active chemical constituents. Vast research has been carried out on the multiple biological functions of medicinal plants, including antioxidant and anti-inflammatory properties (Fawole et al., 2009; Na, 2011). With particular relevance to this study and the possible pro-inflammatory role of TNF-α in kwashiorkor, a study by McCune in 1999 demonstrated inhibition of TNF-α and strong antioxidant properties by medicinal plants used by Indigenous peoples in North America. McCune (1999) used an in vitro assay that involved stimulating human macrophage cells to release TNF-α into the surrounding media. The cells were incubated with the plant extracts and the amount of TNF-α that was released was measured. Three of the plant extracts, at a concentration of 1μg/mL, significantly inhibited the release of TNF-α by around 30% compared to the negative control (McCune, 1999). Another study, using human peripheral blood mononuclear cells in a similar TNF-α in vitro assay, found that the Asian and African herb, Cardiospermum halicacabum, also potently inhibited TNF-α with an IC₅₀ (ie. concentration at which 50% of TNF-α production is inhibition) of 17μg/mL for the plant extract (Venkatesh, 2009). The relevance of medicinal plants for kwashiorkor could be seen from their often overlapping medicinal and
dietary uses and because of their potent antioxidant and anti-inflammatory properties. An evaluation of the role of biodiversity in the diet and health of children can apply to traditional LGV, wild fruits, and medicinal plants, whether they are used strictly as food or medicine or both.

2.7.4 Dietary diversity and biodiversity

Biodiversity, dietary diversity, malnutrition, and health are linked together, with each contributing towards the overall well being of children. Biodiversity contributes to the ability to have a diverse diet, as highlighted by Ekesa et al. in 2009. They employed a cross-sectional study to determine the influence of agricultural biodiversity (crops grown, animals kept, and food plants harvested from the wild) on the number of food items eaten over seven days (dietary diversity) in preschool aged children in a rural region of western Kenya (Ekesa et al., 2009). Despite a low dietary diversity (average of 13 food items eaten over seven days) by the children, agricultural biodiversity was positively correlated with dietary diversity and contributed to 48.5% of the dietary diversity of the children (Ekesa et al., 2009). Indigenous vegetables and wild fruits were only used by 11.8% and 6.3% of the households, respectively, even though they were available (Ekesa et al., 2009). Changing cultural perspectives in the study region that favour exotic species have discouraged the use of the indigenous and wild species, which has been seen in other regions and countries as well (Chewya & Eyzaguirre, 1999; Ekesa et al., 2009; Maundu et al., 1999a). If agricultural biodiversity accounted for approximately 50% of dietary diversity, an increase in
the variety of plant use in the diet could have substantial improvements in dietary diversity, at least for the region of study by Ekesa et al. (2009).

Greater diversity in the diet of young children, measured using a FVS or DDS, has shown a significant positive association with overall nutrient adequacy (MAR) in both urban and rural settings (Hatløy & Torheim, 1998; Steyn et al., 2006). Dietary diversity has also been significantly correlated with nutritional status indicators such as improved HAZ (r = 0.21 for FVS and r = 0.15 for DDS; p<0.0001) and WAZ (r = 0.14 for FVS and r = 0.10 for DDS; p<0.01) (Steyn et al., 2006). The link between dietary diversity and child nutrition is illustrated by Torlesse et al. in 2003, who showed that due to increasing rice prices in Bangladesh, the households that decreased their expenditure on rice and increased their expenditure on non-rice items improved their dietary diversity, which was correlated with a reduced percentage of underweight children (Torlesse et al., 2003). Dietary diversity can be low among children who are receiving complementary foods (Macharia et al., 2004; Rah et al., 2010), even though they have high macro- and micronutrient demands during this period. Onyango et al. (1998) looked at the dietary diversity of children between 12-36 months of age in rural western Kenya who were either fully weaned or partially breastfed. The children who were partially breastfed were not anthropometrically different from those who were fully weaned. However, dietary diversity intake was positively associated with the anthropometric outcomes WAZ (p=0.001), HAZ (p=0.008), WHZ (p=0.01), triceps skin fold (p=0.05), and mid-upper arm circumference (p=0.006) (Onyango et al., 1998). The methodologies and food groupings
between studies on dietary diversity have been different. However, the overall conclusion is that greater dietary diversity has a positive impact on the nutritional status of children.

The prospective observational study by Lin et al. in 2007, mentioned previously in terms of providing evidence against protein deficiency, also assessed whether dietary diversity was associated with the development of kwashiorkor in 1651 Malawian children between 12 to 36 months of age. They did not find that a more diverse diet or any specific food item or nutrient significantly reduced the risk of kwashiorkor (Lin et al., 2007). However, this study occurred in a region of Malawi where the diet consists mainly of a monotonous and corn-based staple (Lin et al., 2007). The dietary diversity was very low and was the same for children who developed kwashiorkor and those that did not (DDS = 2.9 out of 7 groups). Therefore, dietary diversity cannot be assessed as a contributing factor for kwashiorkor if there was not a wide range of dietary diversity intakes to begin with. Determining the association between dietary diversity and kwashiorkor development in a region with a wide range of dietary diversity intakes would be needed to determine if a more diverse diet has a role in the prevention of kwashiorkor.

The use of traditional vegetable and fruit species is an integral cultural component that contributes to child nutrition. These species are often ignored when determining dietary diversity. However, Ogle et al. in 2001 specifically looked at the use of wild vegetables in contributing to dietary diversity and
micronutrient intakes in 196 women in rural regions of Vietnam. Wild vegetables were eaten the most and with a greater variety in the group with the highest FVS (FVS ≥ 21) compared to the lowest FVS (FVS ≤ 15). Greater intakes of wild vegetables also significantly contributed to greater micronutrient intakes (Ogle et al., 2001). This study was able to show that dietary diversity tools are able to capture the use of wild foods and their relative contribution to nutritional adequacy.

Traditional LGV, fruits, and medicinal plants are consumable elements of plant diversity by children. These plants can also be rich sources of polyphenols, which have both anti-inflammatory and antioxidant properties. This is of importance for kwashiorkor, as it is most likely linked with inflammation. The knowledge of the importance of biodiversity, dietary diversity, and health promoting properties all contribute to the rationale of this study.

2.9 Study Rationale

The prevalence of kwashiorkor in rural regions of Kenya is not known, but prevalence rates of hospital admissions in two major centers of the country have been documented. In Kenyatta National Hospital in Nairobi, a referral centre for the country, 15% of hospital admissions over three months for 101 children ages 6-59 months had kwashiorkor (Nzioki et al., 2009). In Kilifi District Hospital on the coast of Kenya, a cohort study over three years found that 9.5% of 8190 children ages 12-59 months admitted to the hospital suffered from kwashiorkor (Berkley et al., 2005). The hospital admission rates are unlikely to be
representative of national or rural percentages. Nevertheless, it can be inferred that kwashiorkor is still a concern in the country in general.

Makueni County is a semi-arid region located in Eastern Province, which is between Nairobi and the coast. The feeding practices in regions of this county show a low diversity in complementary foods. The main food provided is a cereal-based porridge of poor nutritional and energy quality, while the addition of animal proteins, vegetables, or fruits to complementary foods is infrequent and not diverse (Macharia et al., 2004; Ndiku et al., 2010). A variety in complementary foods is important to ensure adequate micro and macronutrient intakes (WHO, 2000). Makueni County experiences drought, which can increase the susceptibility of grains for aflatoxin contamination (Diener et al., 1987). There is a high prevalence of aflatoxin in the staple grains (Mwihia et al., 2008) with one district that found 29% of grains were contaminated with aflatoxin (Okoth, 2008). This same distract also reported that the incidence of kwashiorkor appeared to be high (Okoth, 2008). Makueni County has seen severe aflatoxin outbreaks, particularly in 1982, 2004, and 2005 (Lewis et al., 2005; Mwihia et al., 2008; Ngindu et al., 1982). The combination of food insecurity, poor nutritional quality of complementary foods, droughts, and potential of aflatoxin to induce inflammation and oxidative stress may be conditions that contribute to the occurrence of kwashiorkor.

Kenya also has a vast plant diversity, with over 7100 plant species documented and over 220 traditional LGV (Maundu et al., 1999b). This diversity
could be a rich source of both nutritional and nutraceutical benefits if utilized. In addition, many species of traditional and wild plant species are not as affected by drought as exotic cultivars because they are more acclimatized to drought conditions and therefore are often important famine and drought foods (Mahapatra & Panda, 2012; Maundu et al., 1999a). However, traditional and wild plant species are on the decline in Kenya and also in other countries despite their important nutritional and health promoting properties and contribution towards household food security (Chewya & Eyzaguirre, 1999; Musinguzi, 2011; Oniang'o et al., 2008). Promotion of commercial, exotic species and loss of cultural knowledge on the use, nutrition, and health benefits of traditional plant species contribute to this decline (Musinguzi, 2011). Because many of the traditional plant species have not been extensively studied or documented, more research on their nutritional and phytochemical content can help increase the awareness of the health benefits of these species and provide a knowledgeable basis for their promotion in the diet to contribute to alleviating malnutrition and more broadly for food insecurity.

The rich source of nutrients and phytochemicals in plant diversity, specifically traditional LGV, wild fruits, and medicinal plants, is an important area of research for kwashiorkor and forms the rationale for this study, as the promotion of these components of biodiversity would assist in addressing food insecurity, malnutrition, micronutrient deficiencies (Uusiku et al., 2010), and potential protection from inflammatory and oxidative damage in kwashiorkor, assuming inflammation is part of the etiology.
2.10 Study Objectives

The overall objective for this study was to explore a theoretical link between (1) the etiology of kwashiorkor as a malnutrition state linked with inflammation and (2) the anti-inflammatory and antioxidant properties of components of biodiversity (ie. LGV, wild fruits, and medicinal plants). This study adopted an exploratory approach as opposed to a hypothesis testing approach.

The specific objectives for this study were:

- To conduct interviews with mothers who have at least one child under the age of five years to identify target species of LGV, wild fruits, and medicinal plants in Kaiti Division, Makueni County, Eastern Province, Kenya, that are consumed frequently or used for medicinal purposes by the children
- To gain a brief overall picture of the dietary diversity of the participants using a food variety score (FVS) and dietary diversity score (DDS)
- To understand medicinal plant use and its relationship to the pharmacological activity of the plants through an informant consensus analysis.
- To collect the identified target plant species and analyze them at McGill University for antioxidant and anti-inflammatory properties
The following chapter (chapter 3) highlights the dietary diversity of and the medicinal plant use by the children in the study. The manuscript that follows in chapter 4 comprises the main focus of this thesis, which is to investigate the anti-inflammatory and antioxidant properties of the LGV, wild fruits, and medicinal plants that were collected and their potential relevance for kwashiorkor.
3.0 FIELDWORK METHODOLOGY AND RESULTS

3.1 INTRODUCTION

To identify widely used LGV and wild fruits, a 7-day FFQ was applied. The FFQ allows for a quick assessment of the diet and can be used to develop FVS and DDS. These scores are useful tools for assessing dietary diversity. Both scores have demonstrated a significant positive association with overall nutritional adequacy in developing countries (Hatløy & Torheim, 1998; Torheim et al., 2003) and significant positive associations with anthropometric indicators of nutritional status, such as HAZ and WAZ, in young children (Onyango et al., 1998; Steyn et al., 2006). A traditional knowledge/ethnobotanical questionnaire was used to identify commonly used medicinal plants to treat illnesses in children. This type of survey can be used to determine the degree of consensus for plant use, which may be highly used either because it has been proven to be effective and/or it has a widely known reputation for treating specific illnesses. Carrying out analysis of FVS, DDS, and consensus of medicinal plants can provide a brief estimate of the adequacy of the diet, the diversity of foods that are eaten, and the knowledge and contribution of medicinal plants to health.

3.2 METHODS

3.2.1 Study Area and Site Selection

Kenya is organized regionally as follows: provinces > counties > districts > divisions > locations > sub-locations > villages. Kaiti Division, within Makueni County, Eastern Province, Kenya, was chosen for this study based on several
criteria. First, the division has a diversity of agroecological zones, which meant it could have a greater diversity of wild edible plants and medicinal plants. This was of importance as there would be a greater likelihood of finding plants that showed potential antioxidant and anti-inflammatory properties and of plants being available while the researcher was in Kenya. Second, the division was chosen based on recommendations from the District Agricultural Officer (DAO). According to him, the division struggles with malnutrition and aflatoxin contamination, a toxin that has been linked to kwashiorkor (Hendrickse, 1984). Third, the division was a less than two-hour drive to the Kenya Agricultural Research Institute (KARI) research station where the researcher stayed throughout the interview period and had access to KARI vehicles for transportation, which meant the researcher could commute daily to the research area. The researcher was assisted by KARI through affiliation with the KARI-McGill food security project, entitled Enhancing sustainable food and nutrition security in semi-arid Kenya through innovative and resilient farming systems and institutions.

After Kaiti Division was selected, the researcher met with the Divisional Agricultural Extension Officer (DAEO) of Kaiti Division to organize two focus groups with approximately ten women each from the division. The focus groups occurred in June 2012 in Kaiti Division. The purpose of the focus groups was to generate a list of all the foods eaten in the division, especially the traditional and wild LGV and wild fruits. The women were asked to list all the food items they ate for each food category (cereals/grains, legumes, vegetables, wild LGV, fruits,
wild fruits, roots and tubers, meat, and oils). The list of foods from these two focus groups was used in the creation of the FFQ for the interviews.

3.2.2 Sampling Procedure

Within Kenya, divisions are further broken down into several levels: locations, sub-locations, villages, and lastly the household level. The researcher worked in consultation with the Divisional Agricultural Extension Officer (DAEO) of the Ministry of Agriculture, in Kaiti Division, to choose five sub-locations that represented a diversity of agroecological zones. Meetings were then conducted between the researcher, DAEO, and Assistant Chiefs of each sub-location, where the researcher was introduced and the project was explained.

During these meetings, the Assistant Chiefs provided the researcher with a list of all the villages within their sub-location. Each village was numbered and randomly chosen by running a pen back and forth across the list of numbers (the starting point of the pen was changed for each selection) with closed eyes and another person, also with closed eyes, told the researcher when to stop. Whichever village corresponded to the number the tip of the pen landed on was chosen. The Assistant Chiefs also provided a list of all the households within the selected village and households were randomly selected in the same manner as the villages, by assigning numbers then randomly selecting a number. Whenever a household was selected, the Assistant Chief was able to tell the researcher whether or not the household fit the inclusion criteria, which was a mother with a child under the age of five years. The child could be breastfeeding or non-
breastfeeding. If a selected household did not fit the inclusion criteria during the initial selection, another household was randomly chosen. This continued until ten households were selected, along with three to four replacement households. If the Assistant Chief did not have the household list during the initial meeting, the Assistant Chief created a list with households that only fit the inclusion criteria and a second meeting was set up for the researcher to select the households. Each Assistant Chief also provided the researcher with a translator for their sub-location. Overall, five sub-locations were selected: one village was randomly selected within each sub-location (five villages total) and ten households were randomly selected for each village (fifty households total).

3.2.3 Interviews

Interviews were conducted during July-August, 2012, which corresponded to one of the two dry seasons during the year. The researcher initially met with each translator to go through the questionnaires, which were written in English but also translated into Kikamba (the local language) by a Kamba-speaking colleague (Patrick Maundu) at Bioversity International (Appendix 4). The date and time of interviews were based on the schedule of the translator, the researcher’s schedule, and the market schedule, as mothers would likely be away from home on market days. On scheduled interview days, the researcher and translator would meet at a central place in the village then they would walk to each home for the interview.
If the mother of one of the selected households was not available when the researcher visited the home, or the household did not meet the inclusion criteria, the researcher then went to the first of the replacement households that were chosen. In a few of the villages, all the replacements households either did not fit the inclusion criteria or the mother was not available. If this occurred, additional households were randomly selected from the household list and were interviewed the same day or at another time. Households with children who were breastfeeding either partially or exclusively were included, as children have been documented to develop kwashiorkor while they are still breastfeeding and occasionally while exclusively breastfeeding (Golden, 2002; Lin et al., 2007). Therefore, the diet of breastfeeding children and their mothers was of interest. However, exclusively breastfed children (< 6 months of age) and all the mothers were later excluded from the data analysis to further focus the results and interpretation only on the children most at risk for kwashiorkor. Partially breastfed and non-breastfed children were included in the analysis but were analyzed separately.

Before starting the interview, the consent form was read by the researcher, which was translated to the mother by the translator. If the mother gave consent, she signed the form, or the translator signed on her behalf if she was not able to write (Appendix 1). The interviews consisted of two questionnaires, the FFQ and the traditional knowledge/ethnobotanical questionnaire. Each interview lasted approximately one hour and was recorded. Three to five interviews were completed each day and a total of fifty interviews were conducted.
The FFQ had a total of 89 food items and was used to gain a brief picture of the diet and to identify the traditional LGV and wild fruits that were most commonly eaten by the children under the age of five years. The food item list was based on focus group data that the researcher collected and a food item list developed by Bioversity International of wild foods from Kitui County, approximately 100km from Makueni County. Wild LGV and fruits from the Kitui County wild food list that were considered to be eaten in Makueni County were included in the FFQ based on consultation with a Bioversity International colleague (Patrick Maundu) familiar with the area. Food intake was asked for the past seven days for all food items. For the vegetables and fruits, intake over seven days for each season during the past year was also asked. The FFQ also asked if the child or children were still being breastfed and if so, how many days per week they were breastfed (Appendix 2).

The traditional knowledge/ethnobotanical questionnaire (TKEQ) was used to provide a picture of medicinal plant use by the mothers for treating illnesses in children less than five years and to identify the most commonly used plants. The questionnaire included a list of questions about the mother’s knowledge of malnutrition, kwashiorkor, and the cause and treatment of kwashiorkor, plants used to treat malnutrition, commonly used medicinal plants, and plants used to treat common illnesses affecting children under five years (malaria, pneumonia, diarrhoea, swelling, flu/fever, skin diseases/rashes, inflammations/wounds, additional GI track symptoms/stomach aches), and any additional plants that were
used medicinally or in promotion of health (Appendix 3). The participants were also asked to explain what part of the plant was used, the preparation methods, dose amount, and how it was administered (Norscia & Borgognini-Tarli, 2006).

3.2.4 Ethics

Ethics approval was obtained from the Research Ethics Board of the Faculty of Agricultural and Environmental Sciences at McGill University, Montreal, Canada. The researcher had local approval through affiliation with the Kenya Agricultural Research Institute (KARI) and the KARI-McGill food security project. The food security project had ethical approval through KARI.

3.2.5 Food variety score

The FVS was based on the definition by Hatløy et al. (1998), which is the number of different food items eaten over the specified time period, in this case over the previous seven days. The maximum number of food items was equal to 86. The FFQ had a total of 89 food items after onion, passion fruit, and a wild fruit were later added during the interview process when they were discovered as missing. These three food items were excluded from the FVS calculation because three to six households were missing at least one of these food items from their FFQ data (the FVS only decreased by 1 food item when the three food items were excluded, largely due to onion). Weekly intake for the vegetables and fruits was asked about for each season and a FVS was created for the weekly intake of the vegetables and fruits for each of the four seasons (two rainy, nthwa and uua, and two dry, thano munini and thano munene) which had a maximum number of 55. When there was more than one child either breastfeeding or not breastfeeding in
the same household, the data for one child was randomly selected so that there was a maximum of one breastfed and/or one non-breastfed child per household. This was done to account for household bias, as children in the same household would likely have the same diet. FVS were calculated and analyzed separately for breastfed and non-breastfed children. The age of the children was also taken into account. The seasonal FVS were also calculated and analyzed separately for breastfed and non-breastfed children. Comparisons among the seasons were done within the breastfed and non-breastfed groups.

3.2.6 Dietary Diversity Score

The DDS in this study was defined as the number of food groups that were eaten in the past seven days. For a food group to be counted, at least one food item within the group had to be eaten at least once in the past seven days. The food groups represented in this study were modified from methods used by Steyn et al. (2005), who used an FAO recommended set of groupings. For the vegetables and fruits, Steyn et al. (2005) used three groups: (1) vitamin-A-rich vegetables and fruits (2) other fruits (3) other vegetables. However, because the researcher was specifically interested in the contribution of LGV and wild fruits, and the vitamin A content of some of the indigenous vegetables and fruits was not known, the vegetable and fruit groupings were modified to reflect the research interests and limitations. Therefore, the groupings used in this study were (1) cereals, roots, tubers, and plantains (2) legumes (3) leafy green vegetables (4) other vegetables (5) fruits (6) wild fruits (7) meat (8) eggs (9) milk (10) fats and oils. As with the FVS, when there were two children in a household who were
either non-breastfeeding or breastfeeding, the data for only one of the children was randomly selected and used. The DDS for breastfed and non-breastfed children were analyzed separately and age was also taken into account.

3.2.7 Consensus analysis

The TKEQ resulted in a list of medicinal plants that were used in the study region by the mothers to treat a variety of symptoms. A few of the medicinal plants cited by the mothers were also considered food plants and were in the list of foods in the FFQ, such as *C. maximai*. However, the majority of medicinal plants cited by the mothers were separate from the food plant list. A consensus analysis was carried out for the plants that were collected and had been attributed a medicinal use in the TKEQ (whether they were on the medicinal or food plant list), and non-collected medicinal plants where the genus name was known for the Kikamba plant name. The consensus analysis was modified from methodology used by Leduc et al. (2006). Consensus takes into account 1) the number of different symptoms the plant is used to treat and 2) how frequently the plant is cited for any symptom by different correspondents, in this case the mothers (Leduc et al., 2006). The parameters for consensus are expressed in the following equation, modified from Leduc et al. (2006): 

\[ Consensus = \frac{(\sum s) + \left(\sum \frac{f}{F}\right)}{2s} \]

In the equation, \(s\) is the symptom contribution for the plant, which is calculated for each symptom as \(s = 0\) if the symptom is not treated and \(s = 1\) if the symptom is treated by the plant. For example, for eight symptoms, if the plant treats five of these symptoms, the sum of \(s\) would be \(1+1+1+1+0+0+0 = 5\).
Therefore, the sum of $s$ is equal to the total number of symptoms the plant treats. $F$ is the total number of interviews ($F = 46$). $S$ is the total number of symptoms asked about ($S = 8$) or asked about and additional symptoms cited ($S = 30$). $f$ is the citation frequency for the plant by all correspondents which is calculated for each informant as $f = 0$ if not cited and $f > 0$ if cited by the informant to treat one or more symptoms. The sum of $f$ would be the total citation frequency where the maximum total would be equal to $S \times F$ (Leduc et al., 2006). For example, if there were only 3 informants and 2 symptoms and the first informant used the plant to treat one symptom, the second did not use the plant, and the third used it to treat two symptoms, the sum of $f$ would be $1 + 0 + 2 = 3$. The maximum would be $2 + 2 + 2 = 6$ or $S \times F = 3 \times 2 = 6$. In this study, the maximum for the sum of $f$ was $46 \times 8 = 368$ or $46 \times 30 = 1,380$. Because both the symptom contribution and citation frequency are equally important, the average of the two values is calculated, as seen by the 2 in the denominator of the equation (Leduc et al., 2006). The consensus results for the plants were calculated in two different ways, either using only the eight symptoms specifically asked about or using the additional 22 symptoms that were cited by the mothers for a total of 30 symptoms. The consensus values ranged from 0.06 to 0.35 for the eight symptoms or from 0.02 to 0.09 for the 30 symptoms.

Consensus scores give an idea of the degree of use for a plant. These scores can be compared to laboratory results to see if there is a correlation between consensus over plant use and pharmacological activity. The plants collected in this study were analysed for antioxidant activity using the ORAC and
total phenolics assays and for anti-inflammatory activity using a TNF-α in vitro assay (see chapter 4). Correlation between consensus scores and laboratory results was assessed using the Spearman correlation test.

3.2.8 Statistics

A Student’s $t$-test was used to make comparisons between the FVS of breastfed and non-breastfed children, with exception of the FVS for the dry season, thano munene (TE), which was not normally distributed and therefore a Wilcoxon signed-rank test was used instead. The Wilcoxon signed-rank test was also used for comparisons between non-breastfed and breastfed children for DDS. A multiple regression model was used to control for age when comparing the FVS or DDS between the children. The dependent variable was the FVS or DDS and the independent variables were age and a dummy variable for breastfeeding. An ANOVA with a Dunnett post-hoc analysis was used to compare the FVS of the rainy season, $uua$, to the FVS of the other three seasons for the NBF and BF children. An ANOVA with a Scheffe post-hoc analysis, which allows for comparisons among groups, was used to compare FVS between the five different villages for the NBF and BF children. For the medicinal plant data, the Spearman correlation was used to compare the relationship between consensus scores and ORAC and total phenolics results for antioxidant activity, and between consensus and $IC_{50}$ results for anti-inflammatory activity. All statistical procedures were performed using SAS version 9.2.
3.3 RESULTS

3.3.1 Food variety scores

From the 50 households interviewed, 58 children under the age of five years were included in the data analysis. 41 were not breastfed (NBF) and 17 were partially breastfed (BF). The children who were BF were significantly younger (P<0.0001) than those who were not, as would be expected. For the time period of seven days, the NBF children had a mean FVS of 26 with a minimum of 13 and a maximum of 43 food items that were eaten (Table 3.1). For the BF children, the mean FVS was 18 with a minimum of 3 and maximum of 40 food items that were eaten over seven days. Both the NBF and BF children had wide ranges of FVS, showing that there is a wide diversity of food items being consumed in the area. Initial comparison (non-adjusted) of the FVS for NBF and BF children showed a significant difference (P=0.0004) between the two groups of children. However, when age was controlled for, the difference between the two groups was no longer significant (P=0.1432). Age on its own was a significant predictor of FVS (P=0.0002) but when breastfeeding was added to the model, age was no longer significant (P=0.0806).

Out of all the vegetables eaten among the NBF children for the previous seven days, 52% were LGV. Kale and cabbage were the most widely consumed by 95% and 88% of NBF children, respectively. Wild fruits comprised 65% of the fruits that were eaten among the NBF children. Two wild fruits, which were mainly available at the time of the study, were eaten by 47% and 63% of the NBF children. A total of 72 food items were eaten among all the NBF children over
seven days. Out of the 72 food items, 21 were vegetables (8 of these were traditional LGV) and 23 were fruits (15 of these were wild fruits). For the BF children, traditional LGV comprised 38% of the vegetables eaten in the past seven days and wild fruits comprised 43% of the fruits. Among the BF children, 60 food items were eaten overall and less vegetables and fruits were eaten in general than NBF children (18 vegetables and 14 fruits). Out of the vegetables, 6 were traditional LGV and out of the fruits, 6 were wild. When the traditional LGV and wild fruits were taken out of the FVS calculation, the mean FVS only decreased slightly from 26 to 23 and from 18 to 16 for the NBF and BF children, respectively, but the maximum scores decreased by 8 food items for both groups of children (Table 3.1).

The discrepancy between the high variety of LGV and wild fruits previously mentioned among all the food items eaten and the small decrease in the FVS when these foods were taken out is likely due to a few reasons: (1) the scores are calculated as means, therefore, only the most widely available or popular species eaten would have an impact on the FVS (2) there were only two to three traditional LGV and wild fruits sold in the market at the time of the study, making these the most widely consumed species (3) the remainder of the LGV and wild fruits that were eaten at the time of the study were unavailable in markets and only eaten by small percentage of children, either because the family was able irrigate their field and grow the LGV year-round or because the wild fruits might be available in one area but not another or because the species may not be as popular among the general population. The fact that several species were eaten
by a small percentage of children could explain why the maximum FVS decreased by a greater number of food items than the mean FVS.

Differences among the five villages for FVS were minimal due to small sample sizes and high variation within each village for both the BF and NBF children. There was an exception with one village for the NBF children, which had a mean FVS that was significantly greater (P<0.05) than the other villages, although the variation within this village was also great. Regional variations in FVS, therefore, most likely are not great enough to influence the variation in the overall mean FVS.

Seasonality for the vegetables and fruits greatly determined their availability throughout the year. In general, more varieties of LGV, particularly traditional and wild species, were more available during the rainy seasons compared to the dry seasons. The LGV were more widely consumed during the rainy seasons as well. For example, the traditional LGV, amaranth and cowpea leaves, were eaten by 93% and 81%, respectively, of the NBF children during the rainy season, *uua*. For the BF children, the traditional LGV, *ua* and amaranth, were eaten the most during the rainy season *uua* by 59% and 53%, respectively. Wild fruits were available during all seasons, with 11 to 20 different species being eaten throughout the seasons by NBF children and 5 to 14 wild fruit species eaten by the BF children. Within the seasons, the FVS for the rainy season, *uua*, was significantly greater than the other seasons for both the NBF and BF children (P<0.05; Table 3.1)
Table 3.1 The food variety scores (FVS), maximum score of 86, for 41 non-breastfed (NBF) and 17 breastfed (BF) Kenyan children under the age of five years.

<table>
<thead>
<tr>
<th>NBF and BF Children</th>
<th>FVS$^4$</th>
<th>Min</th>
<th>Max</th>
<th>P-value (non-adjusted)$^5$</th>
<th>P-value (adjusted)$^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Previous 7 days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBF children</td>
<td>26 ± 7</td>
<td>13</td>
<td>43</td>
<td>0.0004</td>
<td>0.1432</td>
</tr>
<tr>
<td>BF children</td>
<td>18 ± 8</td>
<td>3</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No wild fruits or traditional LGV$^2$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBF children</td>
<td>23 ± 6</td>
<td>12</td>
<td>36</td>
<td>0.0003</td>
<td>0.0977</td>
</tr>
<tr>
<td>BF children</td>
<td>16 ± 6</td>
<td>3</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Seasons$^3$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBF children Rainy N</td>
<td>13 ± 5</td>
<td>3</td>
<td>27</td>
<td>0.0033</td>
<td>0.1725</td>
</tr>
<tr>
<td>BF children Rainy N</td>
<td>8 ± 5</td>
<td>1</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBF children Rainy U</td>
<td>20 ± 6</td>
<td>1</td>
<td>21</td>
<td>0.0002</td>
<td>0.2475</td>
</tr>
<tr>
<td>BF children Rainy U</td>
<td>13 ± 7</td>
<td>1</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBF children Dry TI</td>
<td>9 ± 4</td>
<td>1</td>
<td>21</td>
<td>0.0181</td>
<td>0.3115</td>
</tr>
<tr>
<td>BF children Dry TI</td>
<td>6 ± 3</td>
<td>0</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBF children Dry TE</td>
<td>10</td>
<td>5</td>
<td>22</td>
<td>0.0006</td>
<td>0.0549</td>
</tr>
<tr>
<td>BF children Dry TE</td>
<td>7</td>
<td>0</td>
<td>13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 FVS calculated as a simple count of all the food items eaten in the previous week. 2 FVS were calculated for the previous seven days without wild fruits or traditional LGV to see their contribution to the overall score. 3 Seasonal FVS for vegetables and fruits only (maximum of 58) during the two rainy seasons, abbreviated N and U, and two dry seasons, abbreviated TI and TE. 4 Results expressed as mean ± SD or median. 5 The Student’s t-test or Wilcoxon signed-rank test was used to detect differences between non-breastfed and breastfed children. 6 Comparisons were adjusted for age of the children.
3.3.2 Dietary diversity scores

The median DDS, out of a maximum of 10 food groups, for the NBF children was 9.2, with a minimum of 7 and a maximum of 10 food groups (Table 3.2). The majority of children had a DDS of 8 and higher (93%). For the BF children, the median DDS was 7.5, with a minimum of 2 and maximum of 10 food groups. There was a significant difference between the NBF and BF children’s DDS upon initial comparison with a Wilcoxon signed-rank test (P=0.0007). When age was controlled for in a regression model, there was no longer a statistical difference between the DDS of NBF and BF children (P=0.2696). However, age remained statistically significant (P=0.0112). A larger percentage of BF children than NBF children had a median DDS below 8 food groups (47% versus 7%, respectively). Five food groups were eaten by 100% of the NBF children (cereals, roots, tubers, and plantains; LGV; other vegetables; other fruits; fats and oils) (Table 3.3). In comparison, only two food groups were eaten by 100% of the BF children (cereals, roots, tubers, and plantains; milk) (Table 3.3). In general, the BF children had a lower percentage of children in each food group than the NBF children, with exception of milk. This could be expected based on the lower mean FVS of the BF children than the NBF children.

LGV consumption was high among BF and NBF children. In the past week, 71% and 100% of BF and NBF children ate LGV, respectively. LGV may be an important source of vegetables for the children, as the percentage of consumption for LGV and other vegetables were similar. Wild fruits were eaten in the previous week by 78% of the NBF children and 53% of the BF children.
The fact that more than half the children ate at least one wild fruit in the previous week suggests that wild fruits are contributing in some way to the diet of the children.

**Table 3.2** The dietary diversity scores (DDS), out of a total of 10, for 41 non-breastfed (NBF) and 17 breastfed (BF) Kenyan children

<table>
<thead>
<tr>
<th>DDS range</th>
<th>0 - 2</th>
<th>3 - 5</th>
<th>6 - 7</th>
<th>8 - 9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBF</td>
<td>9.2</td>
<td>(0)</td>
<td>(0)</td>
<td>(7)</td>
<td>(42)</td>
</tr>
<tr>
<td>BF</td>
<td>7.5</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

1 DDS calculated as the number of food groups eaten in the previous seven days. 2 Results expressed as the median. 3 The number and percentage of NBF and BF children in each range of DDS represented as N (%).

**Table 3.3** Food groups consumed over the previous week by non-breastfed (NBF) and breastfed (BF) Kenyan children

<table>
<thead>
<tr>
<th>Food groups</th>
<th>NBF (n=41)</th>
<th>%</th>
<th>BF (n=17)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grains, roots, tubers, plantains</td>
<td>41</td>
<td>100</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>Legumes</td>
<td>40</td>
<td>98</td>
<td>12</td>
<td>71</td>
</tr>
<tr>
<td>LGV</td>
<td>41</td>
<td>100</td>
<td>12</td>
<td>71</td>
</tr>
<tr>
<td>Other vegetables</td>
<td>41</td>
<td>100</td>
<td>14</td>
<td>82</td>
</tr>
<tr>
<td>Wild fruits</td>
<td>32</td>
<td>78</td>
<td>9</td>
<td>53</td>
</tr>
<tr>
<td>Other fruits</td>
<td>41</td>
<td>100</td>
<td>15</td>
<td>88</td>
</tr>
<tr>
<td>Meat</td>
<td>28</td>
<td>68</td>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td>Eggs</td>
<td>32</td>
<td>78</td>
<td>11</td>
<td>65</td>
</tr>
<tr>
<td>Milk</td>
<td>39</td>
<td>95</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>41</td>
<td>100</td>
<td>16</td>
<td>94</td>
</tr>
</tbody>
</table>

1 At least one food item from the food group had to be eaten in the previous week for a food group to be counted
3.3.3 Kwashiorkor awareness

For general awareness, 98% mothers had heard of kwashiorkor when they were asked if they had heard of the name kwashiorkor. Of the mothers who knew of kwashiorkor, 82% described symptoms, the main one being a swollen stomach, 88% described causes and 80% described treatments. The main cause described was a monotonous/poor diet or lack of a balanced diet, followed by lack of food, particularly lack of fruit. In terms of treatment, providing a balanced diet, vegetables, and/or fruits were the top ones mentioned. Several mothers also mentioned LGV were given to treat malnutrition in terms of a medicinal context. Although no other medicinal plants were cited that treated kwashiorkor specifically, medicinal plants were cited to treat other illness affecting children.

3.3.4 Medicinal plant use and consensus

Out of the 50 mothers interviewed, 46 cited medicinal plant use or knew of specific medicinal plants and overall, 97 plants and plant combinations were cited for treating illnesses in children. Eight illnesses were specifically asked about. These eight illnesses were in the top ten illnesses cited as being treated in children, as would be expected, along with two additional illnesses, coughing and head rashes, which were ranked 6 and 9, respectively (Table 3.4). In general, for the top 10 illnesses, there were either one or two main plants that were used most commonly to treat each illness. All the plants that were collected, based on availability during convenience sampling and on seasonal availability, were either the main plant, or among the top five plants for treating each illness in the top ten, with exception of wound and head rashes.
Table 3.4 The top ten illnesses treated in children under five years of age using medicinal plants

<table>
<thead>
<tr>
<th>Illness</th>
<th>Number of plants used</th>
<th>Citation frequency of illness</th>
<th>Rank by citation frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach ache*</td>
<td>31</td>
<td>79</td>
<td>1</td>
</tr>
<tr>
<td>Flu/fever*</td>
<td>17</td>
<td>65</td>
<td>2</td>
</tr>
<tr>
<td>Malaria*</td>
<td>15</td>
<td>55</td>
<td>3</td>
</tr>
<tr>
<td>Wound*</td>
<td>22</td>
<td>47</td>
<td>4</td>
</tr>
<tr>
<td>Diarrhoea*</td>
<td>24</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td>Coughing</td>
<td>8</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>Pneumonia*</td>
<td>6</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Rashes/skin disease*</td>
<td>13</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>Mouth sores</td>
<td>4</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Swelling*</td>
<td>6</td>
<td>6</td>
<td>10</td>
</tr>
</tbody>
</table>

1 Medicinal plants were cited by interviewed mothers who had at least one child under the age of five years.

2 Citation frequency was calculated as the total number of times stomach ache was cited by the mothers as being treated by a plant. The total number is greater than the number of mothers because stomach ache could be cited multiple times by each mother. *Indicates the illnesses that were specifically asked about in the traditional knowledge/ethnobotanical questionnaire.

The degree to which a plant is widely used and known among informants is the consensus for the plant (Leduc et al., 2006). If the plant is cited frequently for the same illness or for many illnesses by the majority of informants, the consensus value will be high compared to the other medicinal plants cited. The top ranked plants for consensus will be the most widely used and well known plants. The plants in this study were ranked by consensus in two different ways, either by using only the 8 symptoms specifically asked about or by including all the symptoms cited for a total of 30 symptoms. Two of the plants included in the consensus calculation, Aloe spp. and E. divinorum, were not among the collected plants because not enough Aloe spp. was available and because E. divinorum was only cited by a few mothers, therefore, more commonly used plants were collected instead. These two plants were included in the consensus calculations.
because the genus species name was known for the kikamba name and they could be used as a comparison to the collected plant samples. A different rank order was generated when calculating the consensus as either the total number of symptoms equal to 8 or 30. However, the top 5 plants were the same among both rankings, with only the order differing (Figure 3.1). The consensus for only the 8 symptoms asked about provides a rank for the plants which were most relevant for the 8 symptoms of interest, whereas the consensus using all 30 symptoms also includes the relative importance of the plant for illnesses the mother saw as important or most commonly known by the mother. The top five plants are likely to be most relevant for the 8 illnesses asked about and for their importance to the mothers.

![Figure 3.1](image)

**Figure 3.1** The ranking of Kenyan medicinal plants by consensus. Consensus was calculated in two ways: total number of symptoms either was 8 (specific symptoms asked about in questionnaire) or 30 (all symptoms cited by the mothers).
The plants that were collected in this study were tested for antioxidant and anti-inflammatory properties (see chapter 4). When comparing the consensus values for the collected plants to the laboratory results, there was no relationship found between consensus, either with 8 symptoms or 30 symptoms total, and antioxidant activity or total phenolic content using the Spearman correlation test (Table 3.5). A significant negative correlation \((r^2 = -0.7845; \ P<0.05)\) was found between consensus using all 30 symptoms (but not 8 symptoms) and \(\text{IC}_{50}\) results for anti-inflammatory activity (Table 3.5). Lower \(\text{IC}_{50}\) results correspond to greater anti-inflammatory activity. Higher consensus values may indicate that the plant is commonly used due to its effectiveness in treating illnesses. It should be noted, however, that due to the small number of data points \((n=6-7)\), it would be difficult to determine a significant correlation, but it does appear that greater consensus values may correspond to greater anti-inflammatory activity.

**Table 3.5** The correlation coefficients of the relationship between the degree of consensus and the antioxidant activity, total phenolic content, or anti-inflammatory activities of the collected plants

<table>
<thead>
<tr>
<th>Consensus (8 symptoms)</th>
<th>(R^2) (^1)</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORAC (^2)</td>
<td>0.06</td>
<td>0.59</td>
</tr>
<tr>
<td>Phenolics (^3)</td>
<td>0.01</td>
<td>0.82</td>
</tr>
<tr>
<td>(\text{IC}_{50}) (^4)</td>
<td>-0.60</td>
<td>0.07</td>
</tr>
<tr>
<td>Consensus (30 symptoms)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORAC (^2)</td>
<td>0.05</td>
<td>0.64</td>
</tr>
<tr>
<td>Phenolics (^3)</td>
<td>0.02</td>
<td>0.76</td>
</tr>
<tr>
<td>(\text{IC}_{50}) (^4)</td>
<td>-0.78</td>
<td>0.02</td>
</tr>
</tbody>
</table>

\(^1\)The Spearman correlation test was used to determine correlation coefficients. \(^2\)ORAC represents antioxidant activity, as values increase the antioxidant activity increases. \(^3\)Phenolics represents total phenolic content and was measured using the Folin-Ciocalteu procedure. \(^4\)\(\text{IC}_{50}\) values represent anti-inflammatory activity. Low \(\text{IC}_{50}\) values correspond to greater anti-inflammatory activity.
3.4 DISCUSSION

The FVS and DDS, calculated from a FFQ, are useful tools to provide a snapshot of the diversity of the diet and can be a useful for assessing the diversity and nutritional quality of the diet for both adults and children in developing countries (Hatløy & Torheim, 1998; Steyn et al., 2006; Torheim et al., 2003). FVS and DDS also have shown significant positive correlation with growth indicators in children (Onyango et al., 1998; Steyn et al., 2006). The main use of the FFQ in this study was to identify traditional LGV and wild fruits that were widely eaten and available for collection in order to test them in the laboratory for antioxidant and anti-inflammatory properties (see chapter 4). Therefore, the diets were not thoroughly examined in the questionnaire format nor were food items quantified for nutrient adequacy as there was no intent to examine any indicators of nutritional status, whether biochemical or anthropometric, from the dietary information. However, FVS and DDS were able to be calculated from the FFQ and can still be useful in providing an overall picture of the dietary diversity and contribution of certain food items and groups.

The NBF children had a highly diverse diet, with a mean FVS of 26 and a median DDS of 9.2. Other scores that have been reported for children from rural areas in developing countries have been much lower, such as a mean FVS of 5.5 and a mean DDS of 3.6 out of 9 in South African children between the ages of 1-8 years (Steyn et al., 2006) and a mean FVS of 13 in pre-school aged children from western Kenya (Ekesa et al., 2009). Only a study by Hatløy et al. in 1998 in an urban area of Mali among children ages 13-58 months had a score that
approached the scores seen in this study, with a mean FVS of 20.5 and a mean DDS of 5.8 out of 8. The higher scores seen in the urban area may be because of a greater variety of food items available. The BF children in this study, when compared to other studies, also had a diverse diet, with a mean FVS of 18 and a median DDS of 7.5. However, the comparisons between studies need to take into account the fact that the FFQ, method of scoring, and food groups used are different among the previously mentioned studies and the results may not be equivalent to this study. When comparing the BF children to the NBF children in this study, their scores are less diverse (P<0.001), although the difference was no longer significant when age was controlled for. It may be that age is a stronger predictor for dietary diversity then breastfeeding and therefore the older children are more likely to have a more diverse diet.

When looking at the food groups that were consumed, only the cereals group and milk group were consumed by 100% of the BF children, compared to five food groups consumed by 100% the NBF children. If age is a stronger predictor, especially for DDS where it remained significant after being included with breastfeeding (P=0.0112), then it could be that the younger children are more likely to have a less diverse diet with the main staples being cereals, roots, tubers, or plantains, and milk. This could indicate the type of complementary foods given in the study region. A previous study found that the complementary foods in Makuenui County consisted mostly of a porridge that was not dense in energy or nutrients, while the introduction of vegetables and fruits was infrequent and not diverse (Macharia et al., 2004; Ndiku et al., 2010). Another study found that 70%
of the children who developed kwashiorkor were still breastfeeding non-
exclusively (Lin et al., 2007), which may logically suggest that the types of foods
given while weaning may have a role in kwashiorkor development. The NBF
children’s scores show that there is a high diversity of food items being eaten in
the study region and is available to improve the diversity of the BF children.

Among all the children, LGV were an important contribution to vegetable
intake, comprising around 50% of the vegetables eaten in the past seven days in
general. During the rainy seasons, the traditional and wild species were more
available and more widely eaten than the exotic species (kale, cabbage, and
spinach). Amaranth (*Amaranthus dubius*), kikowe (Kikamba name; species not
known), cowpea leaves (*Vigna unguiculata*), pumpkin leaves (*Curcubita
maximai*), and black nightshade (*Solanum scabrum*) were among the most popular
LGV for all participants. Some of these LGV (eg. pumpkin leaves, black
nightshade) were also cited in a medicinal context, such as for treating
malnutrition, stomach ache, or stomach ulcer. These LGV, with the exception of
kikowe (which was not available), were collected for laboratory analysis along
with kale, which was the most commonly eaten LGV at the time the study was
conducted and could provide a comparison of an exotic species to the traditional
species.

The wild fruits also comprised around 50% of the fruits that were eaten by
the children. Wild fruits can be important snack foods for children when they are
out foraging or walking to and from school (Maundu et al., 1999a). They can also
provide additional nutrition during periods of famine and drought (Mahapatra &
Panda, 2012; Maundu et al., 1999a). In this study, wild fruit varieties were available in all seasons, which can be crucial during the dry season when fewer varieties of cultivated fruits are available. Two wild fruits, loquat (*Eriobotrya japonica*) and chocolate berry (*Vitex payos*), were collected as they were available at the time of study and were widely eaten by the children.

When the FVS was calculated without the traditional LGV and wild fruits, the mean score decreased only by 2-3 food items, but the maximum FVS decreased by 8 food items. Only a few traditional LGV and wild fruits were available in the market at the time of the study. These species were primarily the ones eaten by the majority of the children. Because this study was conducted during a dry season, the contribution of traditional LGV and wild fruits may be greater in the rainy season. This is especially true for the LGV because they are more marketable than the majority of wild fruits. Many of the wild fruits, which were not sold in the market, were only eaten by a few children and therefore would not have impacted the mean FVS greatly. It would be beneficial to assess dietary diversity in each season to gain a better understanding of the contribution of traditional LGV and wild fruits for the majority of the children. The impact of these species, especially wild fruits, may be seen more at the individual level. At the same time, the diversity of wild fruits and traditional LGV available in the region shows potential for improving dietary diversity.

Medicinal plants are other elements of plant diversity that can contribute to the health of children. This study showed that medicinal plants were commonly known and used by the majority of the mothers who were interviewed.
Medicinal plants treated illnesses that commonly affect children under five years of age, such as diarrhoea, malaria, and flu/fever. Treatment of these illnesses could have relevance for kwashiorkor if inflammation is involved in the etiology. A study also found that the children who developed kwashiorkor experienced more days of illness in the month leading up to diagnosis (Lin et al., 2007).

Consensus values can identify the top plants that are used to treat several illnesses and are cited by many informants for treating those illnesses. Plants may become well known based on consistent results of efficacy for treating the illness, which would be expected to correlate with biological efficacy (Moerman, 2007). Six medicinal plants (Azadirachta indica, Mangifera indica, Ocimum americanum, Ocimum gratissimum, Psidium guajava, Zanthoxylum chalybeum) that among the top for treating the main illnesses affecting children were collected for further laboratory analysis. The consensus values for the collected plants were significantly correlated to the anti-inflammatory activity of the plants, but the small number of data points (n= 6-7) used to determine correlation may limit the significant finding. However, increased consensus may be associated with increased anti-inflammatory activity. The plants collected show anti-inflammatory and antioxidant activity (see chapter 4), although this study is not able to draw in vivo conclusions of the anti-inflammatory and antioxidant properties due to the laboratory assays used. Further investigation is needed to determine the in vivo biological activity.
Additional studies are needed in regions with more diverse diets and greater intakes of plants that contain anti-inflammatories and antioxidants, such as was shown to be the case in this study region. The LGV collected in this study that were commonly eaten by children were shown to have anti-inflammatory and antioxidant activities. Future studies should examine whether or not increased consumption of traditional LGV and wild fruits leads to improved dietary diversity, health outcomes, and reduced risk of kwashiorkor in children. In addition, the role of medicinal plants in the diet and health of children is another potential research area to investigate with implications for kwashiorkor and health outcomes in children.
BRIDGE TO CHAPTER 4

In chapter 3, the role of LGV, wild fruits, and medicinal plants in the diet and for treating illnesses in Kenyan children less than five years was highlighted. In chapter 4, the pharmacological activities of these components of biodiversity will be explored. Identifying plants that are rich in anti-inflammatories and antioxidants could not only contribute to improving dietary diversity but also may have relevance for the prevention and treatment of kwashiorkor if inflammation and oxidative stress are involved in the etiology. In the following manuscript, the LGV, wild fruits, and medicinal plants that were collected were analyzed for their antioxidant and anti-inflammatory properties.
Antioxidant and anti-inflammatory activities of Kenyan leafy green vegetables, fruits, and medicinal plants with potential relevance for kwashiorkor

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4.1 ABSTRACT

Background: Inflammation, which induces oxidative stress, could be linked with the etiology of kwashiorkor, a severe acute malnutrition state in children. A diet rich in anti-inflammatories and antioxidants could have potential for the prevention and treatment of kwashiorkor.

Objective: To analyze six medicinal plants, five leafy green vegetables (LGV), and two wild fruits that were collected from a rural region in Kenya, a country impacted by kwashiorkor, for their antioxidant and anti-inflammatory properties.

Methods: The oxygen radical absorbance capacity (ORAC) assay was used to determine antioxidant activity, the Folin-Ciocalteu procedure was used to determine total phenolic content, and a TNF-α in vitro assay was used to determine the anti-inflammatory activity. The results are expressed as mean ± SEM.

Results: All the medicinal plants were greater than the LGV and wild fruits in antioxidant activity and total phenolic content, with exception of the LGV, Solanum scabrum, which was ranked fourth overall. Mangifera indica, a medicinal plant, showed the greatest antioxidant activity (5940 ± 632μM TE/µg) and total phenolic content (337 ± 3mg GAE/) compared to the rest of the plants. For anti-inflammatory activity, both LGV and medicinal plants were equally active. Amaranthus dubius, a LGV, showed the greatest inhibition of TNF-α with an IC₅₀ of 9 ± 1μg/mL, followed by Ocimum americanum, a medicinal plant, with an IC₅₀ of 16 ±1μg/mL. The two wild fruits were largely inactive in all the assays.

Conclusion: This study identified plants commonly used in the diet or for medicinal purposes for children under five years of age that had active anti-inflammatory and antioxidant properties. These results indicate plant species that are relevant for children less than five years that could be evaluated further for their relevance for the prevention and treatment of kwashiorkor.
4.2 INTRODUCTION

Kwashiorkor is a form of severe acute malnutrition in children that is almost exclusively seen in tropical regions experiencing severe food insecurity. Although the etiology of kwashiorkor is not completely understood, many theories have been put forward including protein deficiency (Williams, 1935), aflatoxin contamination (Hendrickse, 1984), oxidative stress (Golden & Ramdath, 1987), and inflammation (Sauerwein et al., 1997). The evidence of biochemical markers, as supported by a recent review (Osorio, 2011), indicates that kwashiorkor is most likely a malnutrition state linked with inflammation.

Providing antioxidants to the treatment diet of children with kwashiorkor was shown to improve survival in children (Badaloo et al., 2002; Becker et al., 2005). On the other hand, the use of antioxidant supplements was not effective in prevention (Ciliberto et al., 2005). If inflammation was the root cause, though, antioxidants would not address the underlying inflammatory condition. Oxidative stress is also part of the inflammatory response, which could explain why antioxidants improved survival during treatment. Therefore, the use of anti-inflammatories and antioxidants may be more effective for prevention and treatment, which has not been assessed.

LGV and fruits are sources of polyphenols, which have both anti-inflammatory and antioxidant properties (Gautam & Jachak, 2009; Scalbert et al., 2005). Indigenous and local LGV have been shown to be richer in these compounds and in nutrients than exotic cultivars (Uusiku et al., 2010). In addition, they can be important components of the diet for children during famine
and drought (Maundu et al., 1999a). Medicinal plants are also rich in bioactive compounds that can exert antioxidant and anti-inflammatory effects and have been the source of natural products for drug development (Gautam & Jachak, 2009). In many cultures, the use of plants for both medicine and food is often overlapping (Chewya & Eyzaguirre, 1999; Oniang'o et al., 2008), which could suggest that these plants may also contribute to the diet of children. Therefore, this study sought to collect traditional LGV, wild fruits, and medicinal plants within a rural context in Kenya and analyze them for antioxidants and anti-inflammatories.

A prevalence of 15% of admissions of 101 children ages 6 to 59 months reported by Kenyatta National Hospital (Nzioki et al., 2009), a referral center for the country, demonstrates that kwashiorkor is a concern in Kenya. This study was conducted in semi-arid Kaiti Division, Makueni County, Eastern, Kenya. The county has characteristics conducive to the occurrence of kwashiorkor, with a poverty rate of 64.1% (KIHBS, 2005/6) and frequent experiences of famine, drought (Ifejika Speranza et al., 2008), and periodic episodes of aflatoxin contamination of food staples, a potential stimuli of inflammation (Lewis et al., 2005; Mwihia et al., 2008).

The aim of this study was to identify plants that could have relevance for kwashiorkor and to promote further research into assessing the use of anti-inflammatory and antioxidants in the diet for prevention and treatment of kwashiorkor. Interviews with mothers who had at least one child under the age of five years identified the leafy green vegetables and wild fruits that were most
commonly eaten and available at the time of the study and medicinal plants commonly used to treat illnesses in children less than five years of age. These plants were analyzed for antioxidant and anti-inflammatory activities. To the researcher’s knowledge, no studies have assessed these properties in plants based on their potential relevance for kwashiorkor.

4.3 MATERIALS AND METHODS

4.3.1 Chemicals

Trolox, gallic acid, Folin-Ciocalteu's phenol reagent, AAPH, fluorescein, LPS, and parthenolide were purchased from Sigma-Aldrich Corp., (St. Louis, MO, USA). The Human TNF-α DuoSet was purchased from R&D Systems, Inc., (Minneapolis, MN, USA). The CytoTox 96® Non-Radioactive Cytotoxicity Assay was purchased from Promega Corp., (Madison, WI, USA). The THP-1 monocyte cell line was obtained from the University of Ottawa, which were initially purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). All other chemicals used were of analytical grade and water used was from Barnstead Nanopure ultrapure water system (Thermo Fisher Scientific Inc., Waltham, MA, USA).

4.3.2 Plant Materials

Plant materials were collected through convenience sampling by the researcher and/or research assistants from five sub-locations (Iuani, Kyasini, Mukuyuni, Nthangu, Utaati) within Kaiti Division, Makuenei County, Eastern Province, Kenya from June-August, 2011. Plants for investigation were selected
based on either their frequency of use in the diet or traditional medicinal use by mothers and children under age five, as well as availability. Leaves of six medicinal plants (*Azadirachta indica, Mangifera indica, Ocimum americanum, Ocimum gratissimum, Psidium guajava, Zanthoxylum chalybeum*), five leafy green vegetables (*Amaranthus dubius, Brassica oleracea, Curcubita maxima, Solanum scabrum, Vigna unguiculata*), and two wild fruits (*Eriobotrya japonica, Vitex payos*) were collected. The medicinal plants were collected from the wild in Kaiti Division and the leafy green vegetables and two wild fruits were bought at local markets in Kaiti Division or Machakos town (a center nearby that is frequently visited by women from Kaiti Division). The two exceptions were *Solanum scabrum*, which was purchased from a grocery store in Nairobi, and *Vitex payos*, which was purchased in Kitui town, approximately 100-120km from Kaiti Division, as neither of these were available in Kaiti Division at the time the researcher was in Kenya. At least two samples were collected for each plant, either from similar areas or different areas depending on availability of the plant. If the plant was purchased in a market, a sample was bought from at least two different sellers, with exception of *Solanum scabrum* because it was not possible in the grocery store and only one sample was bought. Plant species were identified from samples and photographs by colleagues at the Kenya Agricultural Research Institute and by botanists at the National Museums of Kenya. Voucher specimens were deposited at the East African Herbarium in Nairobi and at McGill University. All plant samples were washed and stored in a regular freezer (for the fruits) or in a refrigerator (for the LGV and medicinal plants), the temperature was not measured but can be assumed to be within the normal range for a freezer.
(-6 to -18°C) and refrigerator (3 to 5°C), until they were transferred to the University of Nairobi to be freeze-dried, which occurred within two weeks after collection. The freeze-dried plant samples were brought back to Canada by the researcher in September 2011 and stored in the dark at -20°C at McGill University, Montreal, Canada. Additional freeze-dried plant material that was still being freeze-dried when the researcher left Kenya was brought back to Canada by Dr. Timothy Johns in November 2011 and stored in a similar manner.

4.3.3 Plant Extractions

The freeze-dried leaves of the medicinal plants and LGV were ground using a Thomas Wiley® Mini-Mill (Thomas Scientific, Swedesboro, NJ, USA) and a sieve of 20-mesh. If a plant was collected from more than one area in the wild or purchased from different sellers, the leaves were either mixed together if there were enough from both locations, or if one location had healthier (non-infected) leaves than this location was used. The pulp and skins of the freeze-dried wild fruits were ground using a mortar and pestle until the particle size fit through the 20-mesh sieve. The ground plant samples were re-suspended in 80% ethanol at a volume of 10mL for 1g plant material, placed on a mechanical shaker for 24 hours and then filtered. The first filtrate was stored in a freezer at -20°C while the plant material was re-suspended a second time in 80% ethanol at 10mL for 1g plant material, shaken for another 24 hours on a mechanical shaker, then filtered a second time. The second filtrate was combined with the first filtrate and evaporated on a rotary evaporator. Nitrogen evaporation and freeze-drying were
subsequently used to further evaporate the extracts, which were then stored at -20°C in centrifuge tubes within a desiccator.

### 4.3.4 Total Phenolics

To assess total phenolics, the Folin-Ciocalteu procedure was used (Waterhouse, 2001). Phenolic compounds in the plant extracts reduce oxides present in Folin-Ciocalteu reagent, resulting in a blue colour with intensity approximately proportional to the amount of phenols. Gallic acid standards (0.5, 1, 2.5, and 5μg/L), ultrapure water (blank), and plant extracts diluted in 80% EtOH (20μg/mL for all extracts except Curcubita maximai (40μg/mL), E. japonica (80μg/mL), and V. payos (40, 160, and 585μg/mL)) were added to separate 2.0mL eppendorf tubes at a volume of 0.02mL along with 1.58mL of ultrapure water. Folin-Ciocalteu reagent (0.1mL) was then added and allowed to incubate at room temperature for 6 minutes. Addition of 0.3mL of sodium carbonate solution (1.887M) immediately followed and the reaction was incubated for 2 hours at room temperature. 0.2mL from each tube was then added to a 96-well black microplate (Costar® 96-Well Black Clear-Bottom Plate; Corning Incorporated, Corning, NY, USA) and absorbance was read at 765nm. The mean blank value was subtracted from all the standard and extract values. A standard curve was generated from the gallic acid readings, which was used to convert the plant extract values into milligrams gallic acid equivalents (GAE) per gram of dry extract. Final results are expressed as mean ± SEM (n = 9-15).
4.3.5 ORAC Assay

The ORAC assay measures antioxidant activity by the change in fluorescence from a fluorescent probe, fluorescein (Gillespie et al., 2007). Peroxyl radicals produced by the breakdown of AAPH reduce the fluorescence by the donation of a hydrogen atom from fluorescein to the peroxyl radical. If an antioxidant is present, it will donate a hydrogen atom to the peroxyl radical instead which will stabilize the loss of fluorescence (Cao et al., 1993). In brief, ten serial dilutions, using methanol, were made of the Trolox standard, (6.25-31.25μM) and the plant extracts (2.1875-43.75μg/mL) and added to a 96-well black microplate (Costar® 96-Well Black Clear-Bottom Plate; Corning Incorporated, Corning, NY, USA). Fluorescein (0.08μM) was then added to each dilution of Trolox, plant extracts, or to 75mM PBS (blank) and allowed to incubate for 10 minutes at 37 °C to allow for equilibration. 25μL of 150mM solution of AAPH was then added to each well and the loss in fluorescence was read every three minutes over a ninety minute period (Harris et al., 2011). The loss of fluorescence was measured at an excitation wavelength of 485nm and emission wavelength of 530nm. The area under the fluorescence decay curve (AUC) was used to measure the change in fluorescence intensity at the completion of the assay, which is compared to the AUC of the blank control (Gillespie et al., 2007). The final ORAC results were calculated as μM Trolox equivalents (TE) per microgram of dry extract. All results are expressed as mean ± SEM (n = 6-10) and had at least two replicates using three different stock solutions of extracts dissolved in methanol.
4.3.6 Cell viability and Cytotoxicity

Two markers of cell viability and cytotoxicity, trypan blue and lactate dehydrogenase (LDH), were used in order to ensure that plant extract concentrations were not toxic towards the THP-1 monocyte cells used in the TNF-α in vitro assay.

Trypan blue is a dye that stains dead cells blue and is not able to pass through the membrane of live cells. Plant extracts were tested initially at 100µg/mL, as this was the highest concentration tested in the TNF-α in vitro assay. If reduced cell viability was demonstrated (less than 80% viability), further tests were performed at 25, 50, and/or 75µg/mL. The plant extracts, appropriately diluted in 80% EtOH, were added to THP-1 monocyte cells (cell density range from 2.0-5.0x10^5 cells/mL) in 12-well or 24-well cell culture plates. A vehicle control, 80% EtOH, and a cell blank were also included in separate wells. The plate was then incubated at 37°C with 5% CO₂ for 22 hours, which was equivalent to the total incubation period in the TNF-α in vitro assay. Following incubation, a Vi-CELL Cell Viability Analyzer (Beckman Coulter Inc., USA) was used to determine cell viability using the trypan blue exclusion assay. Total cell number and total viable cells for the extracts were also compared to those of the vehicle control. However, the trypan blue assay cannot distinguish the difference between healthy live cells and live cells that are not functioning properly, which is why LDH was also used for the plant extracts that showed toxicity at 100µg/mL, as this is a more sensitive marker to low levels of cytotoxicity.
The CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega Corp., USA) was used to measure LDH release. LDH is normally contained within live cells but is released during cell death while maintaining function, which makes it a reliable marker. The amount of LDH released is quantified by the formation of red formazan products from the conversion of tetrazolium salts. The intensity of absorbance is approximately proportional to the quantity of LDH released. Plant extracts (only the extracts that showed toxicity with the trypan blue assay) were diluted with 80% EtOH and tested at 1, 10, 25, 30, 50, 75, and 100μg/mL. THP-1 monocyte cells (2.0x10^5 cells/mL) were added to a 96-well cell culture plate and either plant extracts or 80% EtOH, or 50M H_2O_2 (positive control for maximal LDH release) were added to the cells, for a total volume of 200μL. A column of wells contained media only as a blank. The plate was incubated at 37°C with 5% CO_2 for 22 hours, in a similar manner as the trypan blue assay. Following incubation, the plate was centrifuged at 2000rpm for 10 minutes and 50μL of the supernatant was transferred to a 96-well black microplate, while 100μL of the supernatant was discarded and 150μL of fresh media was added to re-suspend the cells. Assay buffer (50μL) was then added to the supernatant, covered, and incubated for 30 minutes at room temperature, after an acetic acid solution (ie. stop solution) was added (50μL) to stop the reaction and the absorbance was read at 490nm. The first supernatant was used to assess cytotoxicity, as any LDH in the supernatant would have come from dead cells. Absorbance values were blanked from the media control and percent cytotoxicity was determined compared to the positive control (H_2O_2).
To assess cell viability, the re-suspended cells were freeze-thaw lysed, to release LDH within the live cells, by 30 minutes at -80°C followed by 15-30 minutes thaw at 37°C. The plate was then centrifuged at 300g for 4 minutes and 50µL of the supernatant was transferred to a 96-well black microplate. Assay buffer (50µL) was added and the plate was covered and incubated for 30 minutes at room temperature, after which stop solution (50µL) was added and the absorbance was read at 490nm. Absorbance values were blanked from the media control and percent viability was determined compared to the negative control (EtOH).

4.3.7 TNF-α in vitro Assay

A modified in vitro model of TNF-α inhibition was used to assess anti-inflammatory activity through the measurement of the level of inhibition by the plant extract of TNF-α expression in human monocyte cells (THP-1) stimulated by LPS (Zhao et al., 2005). A water control, 80% EtOH vehicle control, and two different concentrations (10µg/mL and 1µg/mL) of the positive control, parthenolide, a proven anti-inflammatory sesquiterpene lactone, were used. The plant extracts were diluted with 80% EtOH to 10µg/mL and 50µg/mL or 100µg/mL (based on cytotoxicity at 100µg/mL) for initial screening of the active plants. Plant extracts that showed at least 20% inhibition of TNF-α at the highest concentration compared to the negative control were subsequently tested in dose response concentrations of 1, 10, 25, 50, 75, and/or 100µg/mL.

In brief, THP-1 monocyte cells, at a density of 1.01x10^5 cells/mL, were transferred to wells of a 96-well cell culture plate. The plant extracts and controls
were added at a volume equivalent to 160X dilution, each in replicates of four, and incubated for 2 hours. LPS (1µg/mL) was then added and the plate was incubated for 20 hours. Following incubation, the 96-well cell culture plate was centrifuged at 2000rpm for 10 minutes. The supernatant was transferred to new 96-well cell culture plates (duplicates) and stored at -80°C until further analysis with a Human TNF-α DuoSet ELISA kit to quantify the amount of TNF-α released. The final TNF-α results were calculated as percent inhibition of TNF-α expression relative to the LPS-stimulated EtOH vehicle control (0% TNF-α inhibition) and expressed as mean ± SEM (n = 11-32). To calculate the IC$_{50}$ results for the extracts that showed a dose response, three proportional groups of data for the concentrations tested were created for each extract, as there were an uneven number of results for each test concentration for each plant (due to initially screening the plants at two concentrations then testing the active plants at four dose concentrations). For each group, the means (or log of the means if a transformation was required) of the replicates for each concentration were plotted. The linear regression equation generated from the slope of the line was used to calculate the concentration at which inhibition (or ‘y’ in the equation) was equal to 50% (i.e. IC$_{50}$). Therefore, each plant extract had three separate IC$_{50}$ values created from three proportional groups of data for the concentrations tested. The average of the three IC$_{50}$ values was then taken to determine the overall IC$_{50}$ value in µg/mL. The IC$_{50}$ results are expressed as mean ± SEM (n = 3 proportional groups of data for each test concentration).
4.3.8 Statistics

An ANOVA with a Scheffe post-hoc (alpha = 0.05) analysis, which tests multiple comparisons among groups, was performed to assess statistical differences between plant extracts for results of the ORAC assay and between plant extracts for TNF-α IC<sub>50</sub> values. This was performed to make comparisons among the plants to see which were significantly different from each other. An ANOVA with a Dunnett post-hoc (alpha=0.05) analysis, which compares a specified group to all other groups, was performed to assess significant differences between TNF-α inhibition by the plants compared to the negative control. This was performed to see which test concentrations showed significant inhibition in comparison to the control. Pearson correlation and Spearman rank correlation analyses were both used to determine the relationship between mean ORAC and total phenolics results and between mean ORAC results and TNF-α IC50’s. SAS version 9.2 was used for all statistical procedures.

4.4 RESULTS

4.4.1 Total Phenolics

In general, the medicinal plants all showed higher phenolic content than the LGV and wild fruits, with exception of S. scabrum, which was ranked fifth overall (Figure 4.1). The plants with the highest phenolic content were three medicinal plants, M. indica, P. guajava, and O. americanum, which were all significantly greater than the rest of the plants and from each other (P <0.05) (Table 4.1). M. indica showed the highest phenolic content of 337 ± 3mg GAE/g compared to V. payos, a wild fruit, which showed the lowest phenolic content of
7±1mg GAE/g. Two other plants, *C. maximai* (LGV) and *E. japonica* (wild fruit), also were among the lowest in phenolic content, which were significant (P <0.05). *S. scabrum*, as mentioned previously, was the leafy green vegetable with the highest phenolic content of 92 ± 3mg GAE/g. The range of the other leafy green vegetables was between 24 ± 1 to 54 ± 1mg GAE/g (Table 4.1).

![Figure 4.1](image-url)

**Figure 4.1** Total phenolics of Kenyan medicinal plants (blue), leafy green vegetables (green), and wild fruits (purple) calculated as milligrams of Gallic acid equivalents (GAE) per gram of dry extract. Results shown as the mean and SEM (n= 9-15) for the error bars.

### 4.4.2 Antioxidant activity

As with total phenolics, the medicinal plants showed higher antioxidant activity in the ORAC assay than the LGV and wild fruits, again with the exception of the LGV, *S. scabrum*, which was ranked fourth overall (Figure 4.2). *M. indica* showed the highest activity at 5940 ± 632µM TE/µg, followed by *P.*
guajava at 3929 ± 411µM TE/µg and O. americanum at 3190 ± 163µM TE/µg. M. indica was significantly greater (P <0.05) than the rest of the plants (Table 4.1). The LGV, C. maximai, and the two wild fruits, E. japonica and V. payos, showed the lowest antioxidant activity, as seen with total phenolic content, with values of 447 ± 71, 412 ± 15, and 179 ± 8µM TE/µg, respectively. Although the LGV were lower in activity than the medicinal plants, they still showed high antioxidant capacity compared to the Trolox standard (1000µM TE/µg), with a range of 928 ± 43 to 1233 ± 116µM TE/µg, with exception of C. maximai, which showed much lower activity, and S. scabrum, which showed much higher activity than the rest of the LGV (Table 4.1).

Total phenolic content and antioxidant activity were directly related (Figure 4.3), with Pearson correlation and Spearman correlation analyses both showing a significant positive correlation (P<0.0001) with R-squared values equal to 0.938 and 0.978, respectively.
Figure 4.2 Antioxidant activity of Kenyan medicinal plants (blue), leafy green vegetables (green), and wild fruits (purple) calculated as μM Trolox equivalents (TE) at 1 μg/mL dry extract. Results shown as the mean and SEM (n= 6-10) as the error bars.

Figure 4.3 The correlation between the mean total phenolic content (mg GAE/g) and mean antioxidant activity (μM TE/μg) in Kenyan medicinal plants, leafy green vegetables, and wild fruits.
Values for total phenolics were calculated as milligrams gallic acid equivalents (GAE) per gram dry extract.

Values for ORAC were calculated as $\mu$M Trolox equivalents (TE) per microgram dry extract.

Superscripts represent statistical differences between plant species at $P<0.05$ using an ANOVA with Scheffe post-hoc analysis.

Table 4.1 Final calculated results and rankings for total phenolics$^1$ and ORAC$^2$

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>ORAC</th>
<th>Total Phenolics</th>
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<tr>
<td></td>
<td>µM TE/µg</td>
<td>SEM (n= 6-10)</td>
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<td>---------------------</td>
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<td>----------------</td>
</tr>
<tr>
<td>M. indica$^A$</td>
<td>5940</td>
<td>632</td>
</tr>
<tr>
<td>P. guajava$^B$</td>
<td>3929</td>
<td>411</td>
</tr>
<tr>
<td>O. americanum$^{BC}$</td>
<td>3190</td>
<td>163</td>
</tr>
<tr>
<td>S. scabrum$^{BCD}$</td>
<td>2675</td>
<td>115</td>
</tr>
<tr>
<td>Z. chalybeum$^{CDE}$</td>
<td>2414</td>
<td>117</td>
</tr>
<tr>
<td>A. indica$^{CDEF}$</td>
<td>1761</td>
<td>243</td>
</tr>
<tr>
<td>O. gratissimum$^{CDEF}$</td>
<td>1594</td>
<td>168</td>
</tr>
<tr>
<td>V. unguiculata$^{DEF}$</td>
<td>1233</td>
<td>116</td>
</tr>
<tr>
<td>B. oleracea$^{DEF}$</td>
<td>1184</td>
<td>78</td>
</tr>
<tr>
<td>A. dubius$^{EF}$</td>
<td>928</td>
<td>43</td>
</tr>
<tr>
<td>C. maximai$^F$</td>
<td>447</td>
<td>71</td>
</tr>
<tr>
<td>E. japonica$^F$</td>
<td>411</td>
<td>15</td>
</tr>
<tr>
<td>V. payos$^I$</td>
<td>179</td>
<td>8</td>
</tr>
</tbody>
</table>

$^1$Values for total phenolics were calculated as milligrams gallic acid equivalents (GAE) per gram dry extract. $^2$Values for ORAC were calculated as $\mu$M Trolox equivalents (TE) per microgram dry extract. $^A$-$I$Superscripts represent statistical differences between plant species at $P<0.05$ using an ANOVA with Scheffe post-hoc analysis.
4.4.3 Cell viability

Six plants (A. dubius, A. indica, C. maximai, O. americanum, S. scabrum, and V. unguiculata) showed toxicity (less than 80% viability) at 100μg/mL with the trypan blue and LDH assays. A. dubius also showed reduced viability at 50μg/mL with both assays. C. maximai and S. scabrum were shown to reduce viability at 100μg/mL with the LDH assay and with trypan blue when compared to the negative control (EtOH). However, both extracts decreased the total cell number at 100μg/mL and 50μg/mL by 38% and 22% for C. maximai and 43% and 46% for S. scabrum, respectively. This suggests that the extracts may be interfering with cell division in some manner, especially in regards to S. scabrum.

When the number of viable cells was compared to the total cell number for the extracts, viability was over 85%: therefore, the results at 50μg/mL are included, but because viability was also seen to be reduced with the LDH assay at 100μg/mL, results for 100μg/mL were excluded for both extracts. The median lethal dose (LD₅₀) was calculated for each plant tested with the LDH assay (Table 4.2).
Table 4.2 Highest test concentration (µg/mL) of plant extracts that showed cell viability greater than 80% with both the trypan blue and LDH assays

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Concentration (µg/mL)</th>
<th>LD50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medicinal Plants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. indica</td>
<td>50</td>
<td>133</td>
</tr>
<tr>
<td>M. indica</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>O. americanum</td>
<td>25</td>
<td>129</td>
</tr>
<tr>
<td>O. gratissimum</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>P. guajava</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Z. chalybeum</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Leafy Green Vegetables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. dubius</td>
<td>25</td>
<td>85</td>
</tr>
<tr>
<td>B. olereacea</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>C. maximai</td>
<td>50</td>
<td>184</td>
</tr>
<tr>
<td>S. scabrum</td>
<td>50</td>
<td>96</td>
</tr>
<tr>
<td>V. unguiculata</td>
<td>50</td>
<td>108</td>
</tr>
<tr>
<td>Wild Fruits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. japonica</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>V. payos</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

4.4.4 TNF-α Inhibition

Several plants showed significant inhibition of TNF-α. The plant with the greatest inhibition of TNF-α was A. dubius, which showed significant inhibition (P<0.0001) at 10µg/mL with 67% inhibition compared to the LPS-stimulated EtOH control (0% inhibition). O. americanum, V. unguiculata, and Z. chalybeum also showed significant inhibition (P<0.0001) at 10µg/mL with 42%, 17%, and 12% inhibition, respectively. At the highest test concentration for these plants, A. dubius and O. americanum showed 97% and 82% inhibition at 25µg/mL, respectively. V. unguiculata demonstrated 78% inhibition at 50µg/mL, and Z. Chalybeum demonstrated 92% inhibition at 100µg/mL. All were significant at the
It was also demonstrated that these plants responded in a dose response manner at the achievable (viable) test concentrations (Figure 4.4).

*B. oleracea, O. gratissimum, A. indica, and M. indica* also responded in a dose response manner and showed significant inhibition at their highest test concentrations, with 59%, 47%, and 22% inhibition at 100μg/mL for *B. oleracea O. gratissimum*, and *M. indica*, and 42% inhibition at 50μg/mL for *A. indica* (Figure 4.5). However, at lower test concentrations they did not show significant inhibition, although a few plants did show a significant increase in TNF-α expression. *O. gratissimum* significantly increased production at 10μg/mL (P<0.05) and *M. indica* at 25μg/mL (P<0.0001). *C. maximai* was also included in this figure because at 100μg/mL it demonstrated 34% inhibition. This result is not shown in the graph, however, because the viability, as mentioned previously, may be reduced at this concentration. However, this extract does appear to respond in a dose response manner, as at 10μg/mL, *C. maximai* significantly increased TNF-α expression (P<0.0001), but as its concentration increased, TNF-α inhibition also increased.

The wild fruits, *E. japonica* and *V. payos*, and the medicinal plant, *P. guajava*, did not show significant inhibition of TNF-α at 10μg/mL or 100μg/mL (P<0.05) nor do they seem to respond in a dose response manner (Figure 4.6). *S. scabrum* may actually be pro-inflammatory, as it significantly increased TNF-α expression at all test concentrations (10, 25, and 50μg/mL; P<0.05), as seen in Figure 4.6.
The median inhibitory concentration (IC$_{50}$) was calculated for the plant extracts that demonstrated a dose response (Table 4.3). Five plants had IC$_{50}$ values of less than 100µg/mL. *A. dubius* had the lowest IC$_{50}$ with 9 ± 1µg/mL, followed by 16 ± 1, 27 ± 5, 47 ± 1, and 58 ± 2µg/mL for *O. americanum*, *V. unguiculata*, *Z. chalybeum*, and *A. indica*, respectively. Unlike with the ORAC and total phenolics results, both medicinal plants and LGV were among the top plants, particularly *A. dubius*, a LGV which showed the greatest anti-inflammatory activity. The only similarity between the antioxidant and anti-inflammatory results was that the two wild fruits did not show any significant activity in either assay. This lack of an apparent relationship between ORAC and anti-inflammatory activity was supported by no significant correlation between the IC$_{50}$ values and ORAC results, for the corresponding plants, with either the Pearson correlation coefficient (P=0.3482) or the Spearman correlation coefficient (P=0.8312).
Figure 4.4  Dose response inhibition of TNF-α expression in THP-1 monocyte cells by the four most active extracts of Kenyan medicinal plants and leafy green vegetables compared to the negative ethanol control (0% inhibition). Parthenolide, a potent anti-inflammatory compound, was the positive control. Results shown as the mean and the error bars as the SEM (n=11-32).

Figure 4.5  Inhibition of TNF-α expression in THP-1 monocyte cells by the fifth to ninth ranked extracts of Kenyan medicinal plants and leafy green vegetables which showed a significant inhibition at the highest test concentrations and a dose response inhibition when compared to the negative ethanol control (0% inhibition). Parthenolide, a potent anti-inflammatory compound, was the positive control. Results shown as the mean and the error bars as the SEM (n=11-32).
Figure 4.6 Plant extracts which did not show significant inhibition of TNF-α except *S. scabrum*, which showed a significant increase in TNF-α expression at all test concentrations. Parthenolide, a potent anti-inflammatory compound, was the positive control. Results shown as the mean and the error bars as the SEM (n=11-32).

Table 4.3 The mean IC\textsubscript{50} (µg/mL) for Kenyan medicinal plants and leafy green vegetables which showed a dose response inhibition of TNF-α in THP-1 monocyte cells stimulated with lipopolysaccharide.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>IC\textsubscript{50} (µg/mL)</th>
<th>SEM\textsuperscript{1}</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. dubius</em></td>
<td>A</td>
<td>9</td>
</tr>
<tr>
<td><em>O. americanum</em></td>
<td>AB</td>
<td>16</td>
</tr>
<tr>
<td><em>V. unguiculata</em></td>
<td>ABC</td>
<td>27</td>
</tr>
<tr>
<td><em>Z. chalybeum</em></td>
<td>BC</td>
<td>47</td>
</tr>
<tr>
<td><em>A. indica</em></td>
<td>C</td>
<td>58</td>
</tr>
<tr>
<td><em>B. olerecea</em></td>
<td>D</td>
<td>111</td>
</tr>
<tr>
<td><em>O. gratissimum</em></td>
<td>D</td>
<td>112</td>
</tr>
<tr>
<td><em>C. maximai</em></td>
<td>D</td>
<td>131</td>
</tr>
<tr>
<td><em>M. indica</em></td>
<td>E</td>
<td>169</td>
</tr>
</tbody>
</table>

\textsuperscript{1} n =3 proportional groups comprised of the results for each test concentration

\textsuperscript{A-D} Superscripts represent statistical differences between plant species at P<0.05 using an ANOVA with Scheffe post-hoc analysis.
4.5 DISCUSSION

The medicinal plants in general, especially *M. indica*, *P. guajava*, and *O. americanum*, showed the highest antioxidant activity and total phenolic content. In previous studies, the leaves of *M. indica* have been shown to have high antioxidant activity using the DPPH assay and to protect cultured cells from death (Barreto et al., 2008; Ling et al., 2009). Of the numerous studies on the leaves of *P. guajava*, a few have identified additional biological functions, such as anti-inflammatory (acute, sub-acute, and chronic in mouse models), antimicrobial, anti-diarrhoeal, hepatoprotective, and antibacterial activities (Dutta & Das, 2010; Jaiarj et al., 1999; Lin et al., 2002; Roy, 2006; Sanches et al., 2005). The essential oil of *O. americanum* has been shown to be antibacterial, mosquito repellent against *Anopheles gambiae* and *Aedes aegypti* species, and larvicidal against *Aedes aegypti* (Carović-Stanko et al., 2010; Chokechaijaroenporn et al., 1994; Seyoum et al., 2002), in addition to antioxidant activity (Hakkim et al., 2008). Therefore, these plants show functional activity beyond their antioxidant capacity which could be important for the health of mothers and children.

*O. americanum* was the second most potent inhibitor of TNF-α among the medicinal plants with an IC$_{50}$ of approximately 16µg/mL, while *Z. chalybeum*, and *A. indica* also showed significant inhibition with IC$_{50}$ values of less than 100µg/mL. *O. gratissimum*, at the lowest test concentration, significantly increased TNF-α expression; however, at high concentrations it showed significant inhibition of TNF-α and an IC$_{50}$ value just above 100µg/mL.
Therefore, the medicinal plants had potent activity in both antioxidant and anti-inflammatory assays.

The leafy green vegetables, with exception of C. maximai, also showed high antioxidant activity and total phenolic content. The most active LGV was S. scabrum, followed by V. unguiculata, B. oleracea, and A. dubius. S. scabrum, one of the African nightshade species of Solanum, is often used as a LGV and medicinal plant in Kenya and in other African countries (Mwai et al., 2007). S. scabrum has been shown to contain phenols and flavonoids (Anokwuru et al., 2011; Yang et al., 2008) which could account for the pharmacological activity seen.

Several of the LGV including A. dubius, the most potent inhibitor of TNF-α with a mean IC₅₀ of approximately 9µg/mL, were equally as potent or more potent inhibitors of TNF-α than the medicinal plants. V. unguiculata leaves also showed potent inhibition, with a mean IC₅₀ of approximately 27µg/mL, while B. oleracea and C. maximai demonstrated significant inhibition in a dose response manner. A. dubius is not only nutritionally rich (Odhav et al., 2007), but it also contains flavonoids, phenols, flavonols, and has shown antioxidant and anti-inflammatory activities (Akula & Odhav, 2008; Ibrahim et al., 2011; Yang et al., 2008). The seeds of V. unguiculata have known anti-inflammatory and antioxidant activities, although little research has been done on the activity of the leaves (Gautam & Jachak, 2009; Sidduraju & Becker, 2007). A. dubius and V. unguiculata leaves are particularly important as these were the most commonly eaten LGV by the mothers and children interviewed by the researcher (see chapter
3) and are widely eaten across the country as well (Maundu et al., 1999b). Therefore, these results could have implications beyond the region of this study.

The two wild fruits, *E. japonica* and *V. payos*, did not show high activity in the antioxidant, total phenolic, or anti-inflammatory assays. However, wild fruits in general are an important contribution to the diet of children in the region of study, as wild fruits comprised about half of the fruit intake by the children in the study (see chapter 3). In addition, they are likely to contain vitamins and minerals important to the health of children; *V. payos* and *E. japonica*, for example, contain the antioxidant vitamins C and/or beta-carotene (Maundu et al., 1999a; Nyambo et al., 2005), suggesting that another extraction method or solvent may be more effective at isolating active antioxidant and/or anti-inflammatory compounds other than phenolics. Similarly, an antioxidant and anti-inflammatory assay with a different mechanism of action, such as the DPPH assay or LDL oxidation, may produce different results. This could explain how a previous study found high antioxidant activity in *V. payos*, as different extraction methods and antioxidant assays were used (Ndhlala et al., 2006). Therefore, wild fruits are still likely to have an important contribution to the overall health of the children.

Inflammation, including oxidative stress, seems to best explain the etiology of kwashiorkor when assessing biochemical markers, symptoms, serum albumin and edema, and can explain why antioxidant supplements were only effective in treatment but not prevention of kwashiorkor. The assays used in this study were specifically chosen to reflect biochemical markers that relate to this etiology. The ORAC assay was selected because it identifies hydrophilic chain-
breaking antioxidants. The assay mechanism involves sequestering peroxyl radicals through hydrogen donation. Peroxyl radicals oxidize PUFA and are intermediaries in lipid peroxidation (Chamulitrat & Mason, 1989). Markers of lipid peroxidation, such as low erythrocyte and plasma lipid levels of PUFAs and elevated leukotrienes and malondialdehyde, radicals involved in lipid peroxidation, have been found in children with kwashiorkor (Leichsenring et al., 1995; Lenhartz et al., 1998). Therefore, the mechanism of this assay could be relevant for the oxidative stress in kwashiorkor. The total phenolic content can also be a good indicator of antioxidant activity, as seen in this study. Although several of the plants selected for investigation have been previously assessed for antioxidant activity, different methods were used and the plants in this study were specifically targeted for their potential relevance for kwashiorkor.

TNF-α inhibition was used to assess anti-inflammatory activity because of the potential pro-inflammatory role it may play in kwashiorkor, as children with kwashiorkor have elevated levels of TNF-α whether they have an infection or not (Sauerwein et al., 1997). This is the first study, to the researcher’s knowledge, to assess TNF-α inhibition in the selected plants and specifically within the framework of kwashiorkor. Therefore, the results from these assays could identify plants with potential relevance for the prevention and treatment of kwashiorkor.

Medicinal plants are an important part of the local culture in Makueni County and were used by 92% of the mothers interviewed by the researcher for treating illnesses in children (see chapter 3). The use of these plants to treat
illnesses in children could be important, as one study found that children who
developed kwashiorkor experienced more fever, cough, and diarrhoea in the 28
days prior to diagnosis; this is consistent with inflammation being involved in the
etiology of kwashiorkor (Lin et al., 2007). The consensus values, as previously
described in chapter 3, identify plants that are used by the majority of informants
to treat many illnesses. A significant negative correlation ($r^2 = -0.7845; P<0.05$)
was found between consensus and IC$_{50}$ results for anti-inflammatory activity,
although this result may not be as strong due to the small number of data points
(n=6-7) used to determine correlation. However, it may still demonstrate that as
the degree of consensus increased, the IC$_{50}$ values decreased, or greater anti-
inflammatory activity was associated with the plants (see chapter 3). Among
these top plants for both consensus and anti-inflammatory activity were $O.$
americanum and $Z.$ chalybeum. These plants were commonly known by the
mothers to treat primarily stomach ache and cough in children under five years.
Several plants, including $O.$ americanum and $P.$ guajava, were also used not only
when the child was sick, but were given as a general digestion aid. $Z.$ chalybeum
is also added to tea for flavour and can be taken to improve appetite or provide
energy. The use of the medicinal plants, therefore, may not be limited to strictly
treating illness. The medicinal plants may have a more broad use in the diet of
children and could be particularly important for improving the overall health of
children, especially if plants that are commonly used are more likely to have
pharmacological activity.
The LGV already contribute to the diversity of the children’s diet and have shown high antioxidant and anti-inflammatory activity. Whether or not a more diverse diet in vegetables and fruits with anti-inflammatory and antioxidant properties leads to improved health outcomes and reduced risk of kwashiorkor still needs to be assessed epidemiologically. Medicinal plants and their use by children have also not been included in any studies addressing kwashiorkor.

Several plants identified in this study as having highly active anti-inflammatory and antioxidant properties could have potential relevance for the prevention and treatment of kwashiorkor and the overall health of children and warrant further investigation. In particular, further analysis should be done on the cooked/blanched LGV and medicinal plants, as the anti-inflammatory and antioxidant activity could be significantly reduced (Oboh, 2005). However, this study has shown the potential health promoting properties of traditional edible and medicinal plants which could be an aid in promoting their conservation and use, and as a new way of addressing kwashiorkor research.
CONCLUSION

This study explored a theoretical basis for assessing the health-promoting properties of elements of plant diversity and their relation to the etiology of kwashiorkor as a malnutrition state linked with inflammation. It was shown that there was a wide variety of plant diversity that was available in the study region and was utilized in the diet and for medicine. Approximately half of the vegetables and fruits that were eaten among the children were traditional LGV and wild fruits, respectively. The non-breastfed and breastfed children also had high dietary diversity scores (mean FVS = 26 and 18, respectively; median DDS = 9.2 and 7.5, respectively) when compared to other studies that have assessed dietary diversity in children in rural areas of developing countries (mean FVS = 5.5 and mean DDS = 3.6 by Steyn et al. 2006; mean FVS = 13 by Ekesa et al. 2009). The use of traditional and wild plant species were not included in the dietary assessment in these other studies that examined the dietary diversity of children in developing countries. One study by Ogle et al. (2001) included wild LGV in the FFQ for assessing the dietary diversity of 196 women in Vietnam and found that wild vegetables were eaten the greatest and in more variety in the highest FVS group (FVS ≥ 21). Traditional and wild plants are often an important part of the culture in many developing countries (Johns & Eyzaguirre, 2006). By including these in the dietary assessment, it provides a more holistic view of the diet and a closer representation of the dietary diversity and biodiversity available for utilization in the diet. The knowledge of plant use for medicinal treatment of illnesses in children was widespread among the participant mothers (46 out of 50
mothers). The fact that the higher the degree of use for the collected plants, which were among the top for treating the main illnesses in children, also significantly correlated with lower IC50 values ($r^2 = -0.7845$; $P < 0.05$) or greater anti-inflammatory activity, suggests that there may be a pharmacological basis behind why certain plants are selected and used to treated specific illnesses. Some of these medicinal plants also had a dietary context, in aiding digestion or flavouring tea. Therefore, looking at the medicinal and dietary uses of biodiversity may identify plants which could have anti-inflammatory and antioxidant properties.

The identification of plants that showed antioxidant and anti-inflammatory properties shows the potential for the wide variety of plant diversity that was available and used in the study region to contribute to the health of children. The general trend for antioxidant activity was medicinal plants $>$ LGV $>$ wild fruits. However, for anti-inflammatory activity, both the medicinal plants and LGV were ranked highest for activity. It was surprising that the LGV were ranked highly for activity while the wild fruits showed very little activity, especially as they are both known to contain antioxidant vitamins (Maundu et al., 1999a; Nyambo et al., 2005). If there are active constituents in the wild fruits, it may just mean that the active mechanism is different than the mechanisms of the assays that were tested, which were appropriate for this study. Plants which were of interest from the laboratory assays were the LGV *A. dubius* and *V. unguiculata*, because these two LGV are widely eaten across Kenya (Maundu et al., 1999b) and showed potent anti-inflammatory activity. The anti-inflammatory activity may be more relevant for kwashiorkor than antioxidant activity.
The results of this research should be looked at with the overall study objectives and limitations in mind. The field research was conducted during one season, which limited the plants that were available to be collected, especially because it was a dry season. Therefore, the dietary information is mainly a picture of the diversity available during the July-August time period. Although the lab assays are effective at determining potential biological functions, they are not able to determine \emph{in vivo} activity. Therefore, conclusions cannot be drawn on the effectiveness of the antioxidant and anti-inflammatory actions in the body, especially because interactions between phenolics and other compounds and cells \emph{in vivo} are complex and not completely understood. Also, the bioavailability of the active constituents needs to be assessed as well before any \emph{in vivo} conclusions can be drawn.

Nevertheless, this study showed several strengths including, collection of plants and qualitative data to gain an understanding of the plant diversity, dietary diversity, and medicinal plant use in a region in Kenya where kwashiorkor is a concern. As well, the study contained unique aspects, such as the rarely considered inclusion of wild plant species in the food frequency questionnaire for developing FVS and DDS. In addition, medicinal plant use in ethnobotanical research usually focuses on healers’ or elders’ knowledge of traditional medicine. This study was unique in that it assessed medicinal plant use by mothers, specifically for treating illnesses in children under the age of five years. Lastly, to the researcher’s knowledge, the assessment of TNF-\( \alpha \) inhibition has not been done in any of these plant species before. The results from this study can contribute
new information to the body of knowledge on the health-promoting properties of these species and for elements of biodiversity in general.

Overall, the main strength of this study was exploring the unique theoretical basis that plant diversity and its specific functional properties may have relevance to kwashiorkor if inflammation is involved in the etiology. As a proof of concept it demonstrates the feasibility of investigating the potential link between elements of biodiversity and kwashiorkor by using a combination of both fieldwork and laboratory work and shows the potential for further investigation in this manner. This study could also be relevant for the promotion of wild plant species in dietary assessment and for biodiversity conservation for food security and malnutrition.

Recommendations for future research:

- Assessment of the diet throughout the year to gain a better understanding of the year-round diet and also to be able to collect a greater variety of plant species for laboratory analysis.
- Collecting additional samples of the plants in this study from different regions to determine the regional variability in pharmacological activity
- Conducting the laboratory analysis on the cooked leaves to determine the loss of activity
• Further investigation of the antioxidant and anti-inflammatory mechanisms using additional assays would deepen the knowledge of the pharmacological activities of the plants in this study.

• Determining whether increasing traditional and wild species use leads to improved dietary diversity and reduced risk of kwashiorkor
6.0 REFERENCES


7.0 APPENDICES
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Appendix 1

Consent form for participants
CONSENT FORM OF PARTICIPATION

We invite you to participate in this study which is titled: **Investigation of the health promoting properties of local vegetables and fruits in Kenya in relation to childhood malnutrition (kwashiorkor).**

This study is being conducted by **Heather Tufts,** an MSc student in the department of Dietetics and Human Nutrition at **McGill University, Canada,** under the supervision of **Dr. Timothy Johns.** This study is part of a collaboration among Kenya Agriculture Research Institute (**KARI**), Kenya Medical Research Institute (**KEMRI**), and McGill University, and is funded by the Canadian International Food Security Research Fund (**CIFSRF**).

The purpose of this study is to conduct lab tests on local vegetables and fruits commonly eaten or used medicinally in Kaiti Division, Kenya, for their health promoting properties. This study will benefit the community by identifying potential local sources of dietary diversity that could contribute to the treatment and/or prevention of malnutrition in children (kwashiorkor) and to maintain the health of children. It will also help to promote local species of vegetables and fruits and biodiversity conservation.

Your participation in this study will involve answering two questionnaires through an interview format. The first will be about the frequency of intake, by yourself and your child, of specified local vegetables and fruits, and other foods. The second questionnaire will ask about your traditional knowledge of plants used for medicinal purposes. The questions will ask about specific plants used to treat malnutrition or any other illness in children. The method, time, and length of the interview will be at your convenience.

Your participation is voluntary and you are not required to participate. You have the right to refuse to answer any questions and to withdraw from the interview at any time. Your name will not be revealed and your questionnaires will not be shown to anyone else. Your answers will not be shown in any publications that may result from this study. Your name will not be available to any other person or organization and will be converted to a coding system to be used for data analysis and storage (E.g. Numbers will be used in replacement of names).

With your permission, a photo may be taken of you for personal reference of the researcher and potential use in future presentation of the results. Your name and location will not be identified with your photograph in the presentation and photos will not be used in publications. Also with your permission, this interview may
be recorded, for assistance to the researcher to fill in questionnaire answers or details. The recording will be deleted at the end of the study and will not be published or released to anyone.

If you have any questions or concerns, you may contact Heather Tufts at 0787-386390 or heather.tufts@mail.mcgill.ca, or Zipporah Bukania (KEMRI associate and Kenyan contact) at 0722-336292 or 020-2729891, zbukania@gmail.com or rbukania@kemri.org, or Dr. Timothy Johns at tim.johns@mcgill.ca.

If you have any questions or concerns about your rights or welfare as a participant in this research study, please contact the McGill Ethics Officer, Lynda McNeil at +1-514-398-6831 Email: Lynda.mcneil@mcgill.ca

**Consent**

I agree to be photographed: _____ YES _____ NO

I agree that my photograph may be used as described above: _____ YES _____ NO

I agree to be recorded: _____ YES _____ NO

I agree that the recording may be used as described above: _____ YES _____ NO

Participant’s name: ____________________________

Participant’s signature: ____________________________

Researcher’s signature: ____________________________

In lieu of participant signature, I verify that the objectives and procedures of the study have been explained to the participant and they have orally confirmed their consent to participate.

Translator’s signature: ____________________________
Appendix 2

Food Frequency Questionnaire
Food Frequency Questionnaire

Location: ____________________________ Date: ____________________________
Code for Interviewee: ____________________________ Mother’s Age: ____________________________ Child/Children’s Age: ____________________________

Q1. How many days in the past seven days did you eat (insert food item from list)? On those days, how many times did you eat (insert food item from list) per day?
Q2. How many days in the past seven days did your child under the age of 5 eat (insert food item from list)? On those days, how many times did your child eat (insert food item from list) per day?
Q3. How often do you consume (insert vegetable or fruit item) per week or overall during (insert season name)?
Q4. Did you consume any additional vegetables or fruits not in the food list within the past year?
Q5. How often did your child consume (insert vegetable or fruit item from list) per week or overall during (insert season name)?
Q6. Did your child consume any additional vegetables or fruits not in the food list within the past year?
Q7. How many times in the past 7 days did you consume (insert animal food item)? How many times per day? How many times in the past month?
Q8. How many times in the past 7 days did your child consume (insert animal food item)? How many times per day? How many times in the past month?
Q9. Did you use any additional plant not in the food list item as a spice in cooking?
Q10. How many days in the past 7 days did you breastfeed your child under the age of 5? On those days, how many times did you breastfeed?
<table>
<thead>
<tr>
<th>Food Item List</th>
<th>Frequency of Consumption</th>
<th>Mother</th>
<th>Child under 5 years of age</th>
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<td>Source (e.g. Market, forest)</td>
<td># days in past 7 days</td>
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<tr>
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<td>Sorghum</td>
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<td><strong>Legumes</strong></td>
<td><strong>Source</strong></td>
<td># days in past 7 days</td>
<td># times per day</td>
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<td>Mbooso</td>
<td>Beans</td>
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<tr>
<td>Nzooko/Nthooko</td>
<td>Cowpea</td>
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<tr>
<td>Nzuu</td>
<td>Pigeon pea</td>
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<td>Dengu</td>
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<tr>
<td>Matu ma Malenge</td>
<td>Pumpkin leaves</td>
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<tr>
<td>Matu ma Nthooko</td>
<td>Cowpea leaves</td>
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<td>Muchicha</td>
<td>Amaranth (big leaves; cultivated)</td>
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<td>Telele</td>
<td>Amaranth (small leaves; not cultivated)</td>
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<td>Vilinganya</td>
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**Additional Vegetables**
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<th>Uua</th>
<th>Thano munene</th>
<th># times past 7 days/per day</th>
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<th>Thano munini</th>
<th>Uua</th>
<th>Thano munene</th>
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**Additional Fruits**
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<td><strong>Animal Foods and Products</strong></td>
<td># days in past 7 days</td>
<td># times per day</td>
<td># times in past month</td>
<td># days in past 7 days</td>
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<td>Nyama ya Ng’ombe</td>
<td>Cow</td>
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<td>Nyama ya Mbui</td>
<td>Goat</td>
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<td>Nyama ya Ilunga</td>
<td>Sheep</td>
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<td>Chicken</td>
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<td>Duck</td>
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<td>Nyama ya Mbuku</td>
<td>Rabbit</td>
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<tr>
<td>Nyama ya Ngulue</td>
<td>Pig</td>
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<td>Nyama ya Nguue</td>
<td>Wild pig</td>
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<td>Nyama ya Mbii, kavii</td>
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<tr>
<td>Matumbi</td>
<td>Eggs</td>
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<td>Yiia (ithumo or ikatu)</td>
<td>Milk (fresh or fermented)</td>
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<tr>
<td>Mauta ma ng’ombe</td>
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<th>Uua</th>
<th>Thano munene</th>
<th># times past 7 days/per day</th>
<th>Nthwa</th>
<th>Thano munini</th>
<th>Uua</th>
<th>Thano munene</th>
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<td>Breastfeeding</td>
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Appendix 3

Traditional Knowledge/Ethnobotanical Questionnaire
Traditional Knowledge/Ethnobotanical Questionnaire

Q1. Have you heard of malnutrition? Have you heard of Kwashiorkor? What do you know about Kwashiorkor? If you have seen it in your own children, what did you do to treat it?

Q2. Do you know of any plants that have been used to treat kwashiorkor or malnutrition, either alone or in addition to treatment diet recommended by the health clinic?

Q3. Do you know of any plants that were used in the past (10-20 years ago) for treating malnutrition but may not be used now?

Q4. What plant species do you use to treat any illness in children or given to children in a tea or tonic for promoting good health in children?

Q5. Have you used any part of the plant mukenea (Zanthoxylum chalybeum) and/or mukinyai (Euclea divinorum) for medicinal use or heard of these plants being used? If so, what illness(es) were they used to treat?
Table for Q1-Q5

<table>
<thead>
<tr>
<th>Plant</th>
<th>Illness or health promotion</th>
<th>Part of plant used</th>
<th>Preparation/combination with other plants</th>
<th>Dosage</th>
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Q6. What plant species do you use or have heard of being used either in the present or past to treat the following illnesses (if not mentioned in the above table), in children and/or adults?

a. Malaria
b. Diarrhoea
c. Pneumonia
d. Swelling
e. Flu/fever
f. Skin diseases/rashes
g. Inflammations
h. Additional GI tract symptoms
<table>
<thead>
<tr>
<th>Illness</th>
<th>Plant</th>
<th>Part of plant used</th>
<th>Preparation</th>
<th>Dosage</th>
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Q7. From the plant species listed in the two tables above, which are the three most important ones for medicinal use in children? Which are the three most commonly given to children under the age of 5?

Q8. Are there any additional medicinal plants that you know of that are commonly used, either now or in the past, that have not been mentioned?
Appendix 4

English-Kikamba translation of questions
Food Frequency Questionnaire: Translation

Q1. (Kikamba): Mithenya Muonza mithelu, Uite maliu aa matuku meana ata?............ kila muthenya uisaa mavinda meana ata

(English): How many days in the past seven days did you eat (insert food item from list)? On those days, how many times did you eat (insert food item from list) per day?

Q2. (Kikamba): Mithenya Muonza mithelu, kana kaku kaitheo wa miaka itano kaithe maliu aa matuku meana ata..................., kila muthenya kaisaa mavinda meana ata?...........................

(English): How many days in the past seven days did your child under the age of 5 eat (insert food item from list)? On those days, how many times did your child eat (insert food item from list) per day?

Q3. (Kikamba): Ni ivinda yiva kwithiawa mboka ii kana matunda aa…………….? Kwa kila kyumwa, uisaa mboka ii kana matunda aa mala meana ata ..................? na ivinda yii .....................

(English): What season is (insert vegetable or fruit item) available? How often do you consume (insert vegetable or fruit item) per week or overall during (insert season name)?

Q4. (Kikamba): Mwaka ula unathelile, waaya mboka kana matunda angi eka aa maandikitwe vaa ......................

(English): Did you consume any additional vegetables or fruits not in the food list within the past year?

Q5. (Kikamba): Kana kaku kaisaa mboka ii kana matunda aa mala meana ata kwa kyumwa...............? kana ni ivinda yii yinenganitwe vaa............................

(English): How often did your child consume (insert vegetable or fruit item from list) per week or overall during (insert season name)?
Q6. (Kikamba): Kana kaku kaaya mboka ingi kana matunda angi mwakani usu unathelile eka ila inenganitwe vaa ……………………………
(English): Did your child consume any additional vegetables or fruits not in the food list within the past year?

Q7. (Kikama): Vandu va mithenya muonza mithelu, uite maliu aa maumite indoni mala meana ata..........................? mala meana ata kwa kila muthenya umwe .......................? Mala meana ata kwa mwai ula unathelile ..........?
(Enlgish): How many times in the past 7 days did you consume (insert animal food item)? How many times per day? How many times in the past month?

Q8. (Kikamba) Vandu va mithenya muonza mithelu, kana kaku kaite maliu aa maumite indoni mavinda meana ata............................?
Mala meana ata kwa kila muthenya umwe.......................?
Mala meana ata mwaini usu unathelile..............................?
(English): How many times in the past 7 days did your child consume (insert animal food item)? How many times per day? How many times in the past month?

Q9. (Kikamba): Waatumia maliu angi ma mimea eka aa manenganitwe vaa makwongeleela musamo liuni .....................?
(English): Did you use any additional plant not in the food list item as a spice in cooking?

Q10. (Kikamba): Vandu va mithenya muonza mithelu, kana kaku ka itheo wa miaka itano kongete matuku meana ata............................?
Mala meana ata kwa muthenya umwe
(English): How many days in the past 7 days did you breastfeed your child under the age of 5? On those days, how many times did you breastfeed?
TK/Ethnobotanical Questionnaire: Translation

Q1. (Kikamba): Niwisi ila mii ya syana ikosete maliu……………?. Waaiwa uwai wa syana witawa Kwashiorkor kana kumosa kwa syana………………….?. Uwisi ata……………………?. Eembwa nuwonete syanani syaku, usitaa ata……………?.
(English): Do you know what malnutrition in children is? Have you heard of Kwashiorkor and what do you know about it? If you have seen it in your own children, what did you do to treat it?

Q2. (Kikamba): Niwimbwa wisi miti yaatumika kuita Kwashiorkor kana kukosa kwa maliu ya mii ya syana, yiyoka kana yongeleewa ndawani syumanite na mandakitali ma sivitali ………………….?.
(English): Do you know of any plants that have been used to treat kwashiorkor or malnutrition, either alone or in addition to treatment diet recommended by the health clinic?

Q3. (Kikamba): Niwimbwa wisi miti yatumikie ivinda ivituku ta miaka ikumi kuvika miongo ili kuita uwau wa unyivu wa liu miini ya syana lakini yu nditumikaa………?
(English): Do you know of any plants that were used in the past (10-20 years ago) for treating malnutrition but may not be used now?

Q4. (Kikamba): Utumiaa miti yiva kuita Kuwaa kwa syana kana kusyikiiya kyaini kana kwiluila inywe nundu wa kwongeleela vinya
(English): What plant species do you use to treat any illness in children or given to children in a tea or tonic for promoting good health in children?

Q5. (Kikamba): Waatumia Mukenea vamwe na Mukinyai kana kilo imwe yiyoka ta ndawa kana waiwa andu angi maitumia miti isu……………………….? Eembwa miti isu nitumikaa ta ndawa, ni mawau mau iitaa …………………………..?
(English): Have you used the plant Mukenea (Zanthoxylum chalybeum) and/or Mukinyai (Euclea divinorum) for medicinal use or heard of these plants being used? If so, what illness were they used to treat?

Q6. (Kikamba): Ni miti yiva itawetetwe vaa utumia kana wiwaa itumikaa umunthi kana ivinda ithelu kuita mowau aa syanani vamwe na andu aima kana syana sisyoka na andu aima memoka
  a. Malalia
  b. Kwituua
  c. Kyambo
  d. Kwimba mwii
  e. Ikua
  f. Uwau wa kithuma
  g. Itau
  h. Mowau ma ivu

(English): What plant species do you use or have heard of being used either in the present or past to treat the following illnesses (if not mentioned in the above table), in children and/or adults?
  a. Malaria
  b. Diarrhoea
  c. Pneumonia
  d. Swelling
  e. Flu/fever
  f. Skin diseases/rashes
  g. Inflammations
  h. Additional GI tract symptoms

Q7. (Kikamba): Nthini wa miti ii inenganitwe vaa, nyuva itatu ila yimaana muno kwa kuita syana…………………………….? Niyiva itatu ila inengawa syana sya miaka itheo wa itano………………?

(English): From the plant species listed in the two tables above, which are the three most important ones for medicinal use in children? Which are the three most commonly given to children under the age of 5?

Q8. (Kikamba): Ve miti ingi wisi ya ndawa itumikaa ni andu aingi yu kana ivinda ithelu na ndiwetetwe vaa………………………………………?
Are there any additional medicinal plants that you know of that are commonly used, either now or in the past, that have not been mentioned?