Department for Farm Animals and Veterinary Public Health

University of Veterinary Medicine Vienna

Institute of Animal Nutrition and Functional Plant Compounds

"Functional Botanical Substances"

(Head: Prof. Dr. Johannes Novak)

Phytochemical investigation of an antimalarial plant *Zanthoxylum zanthoxyloides*

Diploma thesis

University of Veterinary Medicine Vienna

submitted by

Johannes Michael Klinger

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Dedicated To

My Loving Parents,

Who Supported Me My Entire Life

Thank You

1. Introduction:

1.1. Introduction and Research Question

In 1640 Agostino Salumbrino[1] introduced in Europe to the grounded bark of *Cinchona calisaya* from South America, blended it with sweetened water and produced what was later named "tonic water". *Cinchona calisaya* is the botanical name of the chinchona tree; its origins are the highlands of northern South America. With this composition he subsequently cured fever symptoms caused by malaria-related pathogens. This highly infectious disease was endemic in the city of Rome and in its surrounding swamps and marshes in the middle of the 17th century. The word Malaria derives from the Latin words *"mala"* and *"aria"* which literally translates to "bad" and "air". This Name was chosen because of the horrible air quality in and around the eternal city during the endemic period. Though it worked very well on numerous diseased victims, the European pharmacists' society did not avail them of this opportunity to incorporate this new remedy. Finally the English Pharmacist Robert Talbot (1642 – 1681) perceived the potential of this new plant. Furthermore he even cured the English King Charles II (1630 – 1685) and the French King Louis XIV (1638 – 1715) from a set of symptoms that were also caused by a mosquito-borne infection, which was almost certainly a malaria infection. Especially due to the case of the French king Louis XIV the European pharmacists acknowledged the root bark from *Cinchona calisaya* as the potential drug that it was and still is. Additionally the famous English doctor Thomas Sydenham (1624 - 1689) endorsed the positive effects on the human body, which was finally the inception of a striking success for the later named **quinine** in pharmacology use during the following two and a half centuries. In 1820 quinine was first isolated and named by the French scientists Pierre Joseph Pelletier and Joseph Bienaimé Caventou (Meyer 2012, Wikipedia 2014).

To this day the blood parasite *Plasmodium spp*, that causes malaria symptoms, has not completely grown resistant to the essence of *Cinchona Calisaya* although it has been in use for nearly 400 years.

[1] Agostino Salumbrino (1561-1642) was a Jesuit pharmacist, how lived in Lima, Peru and worked a lot with local medical plants

Furthermore it is however noteworthy that some resistances have been observed. But compared to the evolvement of growing resistance in the field of bacteriology this phenomenon is quite astounding. Furthermore this particular plant and its secondary product is not the only one in use. Many different species have been in use for decades. They just have not been given enough attention. Luckily this has changed in last couple of decades and there are numerous international programs to determine various plant species that hold the potential to not only treat but maybe even cure malaria. Through many new diseases such as the virus infection ebola, malaria has not been the center of attention in the research world, even though it still claims countless victims every day across the world. In the international research project MEAMP financed by the Austrian research program APPEAR, which is dedicated to contribute to fight this disease, five different local medicinal plants have been selected that may have this potential to be a strong weapon against this disease, which is still present in everyday life in many countries throughout the world, especially in third world countries. The selected plants were chosen according to their use in traditional medicine against Malaria in Burkina Faso. The phytochemical investigation and following isolation of compounds may truly draw more attention to this particularly promising field of research.

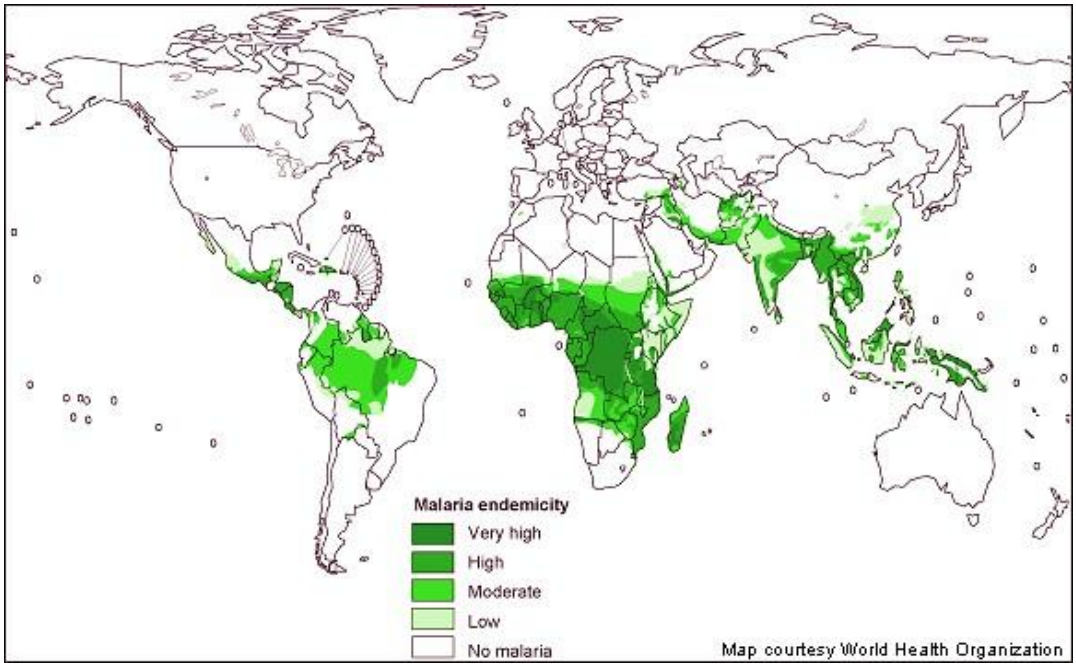

Figure 1 Malaria endemic areas (www.nathnac.org)

In detail, the primary materials were collected from the wild as reference and target samples purchased from the market. This added up to 25 samples of Z. zanthoxyloides in total, two leaf samples which were both acquired at the market. The rest were root samples. The use of reference is especially important for roots. Due to the fact that the precise distinction between roots in general can be very difficult, as there are few morphological characteristics that would make an identification possible. So, in order to confirm this, a specific pattern of a chromatographic analysis from a reference sample can be compared to a market sample and provide the necessary information and confirmation.

1.2. Malaria

1.2.1. The disease

Given how long we have been confronted with this disease we are just at the very beginning of fighting it in a more or less profound way. But what makes this malady so dangerous and tenacious, that humanity has not been able to contain it more effectively? To understand the disease malaria, the life cycle and the pathogen itself must be carefully examined.

The pathogens leading to malaria are members of the genus *Plasmodium* (*Plasmodiidae*). This family is a member of the Kingdom *Chromalveolata*, Superphylum *Alveolata*, Phylum *Apicomplexa*, Class *Aconoidasida* and the order of *Haemosporida* (Lucius and Loos-Frank, 2008). *Haemosporida* are blood parasites with a host shift, which means that they need the intestinal system of a blood-sucking insect within their development to sexually procreate. The various species of pathogens result in distinct forms of human malaria. The most important are (Lucius and Loos-Frank 2008):

- *Plasmodium falciparum*, which leads to malaria tropica,
- *Plasmodium vivax*, which leads to malaria tertiana,
- *Plasmodium ovale*, which leads to malaria tertiana,
- *Plasmodium malariae*, which leads to malaria quartana
- *Plasmodium knowlesi*

The most common infection is malaria tropica with almost 75 %, followed by malaria tertiana with about 20 % and malaria quartana with about 5 %. The classic symptoms are fever attack every other day, which can be ascribed with the accumulation of various metabolic products produced by the host. If the fever peak manifests every 3 days it is most likely to be caused by *Plasmodium vivax*. In analogy *Plasmodium malariae* will manifest with ague fever every 4 days (Lucius and Loos-Frank 2008, Mehlhorn 2012).

1.2.2. The *Plasmodium* life cycle

With the sting from infested *Anopheles maculipennis* the *Plasmodium* parasite is introduced into the system. *Plasmodium* is now in its transportation form: sporozoite. This sporozoite will then attach to a liver or endothelial cell and penetrate it. In these cells it will grow and go through multiple cell divisions. This phase is called schizogony or endohistocytic phase. The following cells – merozoites – will then erupt from the cell in which they have proliferated and reenter the blood system. They are now capable of attacking and penetrating erythrocytes. Apparently they prefer immature red blood cells. Therefore, they attack not more than 2 % of the erythrocytes. Subsequently they will attach and enter a red blood cell and start evolving into an amoeboid cell in order to produce yet again more merozoites. These afflicted cells can be detected easily, because they are much bigger than the other red blood cells and have the characteristic "Schüffner's stipplings" within them. This is a residual body within erythrocytes that correlate with a *Plasmodium* infection. Subsequently these newly produced merozoites will then destroy the erythrocyte in which they have underwent their development. This leads to residual body in the red blood cells that used to be hemoglobin, though its original molecular structure was damaged and destroyed. If all the parasites originate in one infection the cycle of schizogony will proceed in sync (Lucius and Loos-Frank 2008).

The overflow of metabolism products in the blood will eventually lead to spike of fever every three to four days depending on the pathogen. The amoeboid cell does not only produce merozoites. Some also produce micro- and macrogamonts which circulate the blood waiting for their departure which will verifiably occur when the *Anopheles maculipennis* strikes again. By sucking the blood this mosquito will reintroduce the gamont back to its original gastrointestinal system. On the one hand the one microgamont will produce eight microgametes through multiple cell division, while the one macrogamete will mature into one macrogamete. This phase in the cycle is called gametogony and it will only happen in the middle section of the mosquito's intestine. This is the place of fertilization. Further on the newly formed zygote will elongate and penetrate the intestinal lumen and the endothelial cells as an Oocyte. After passing the intestinal endothelial layer it will ensconce itself in the muscle layer of the gut.

Encystation, meiosis and multiple cell division will then prolong into a poly-nuclear mass of plasma. From this construct many little sporozoites will pinch of and take their journey through the vesicular system. There they will eventually reach the mosquitos' salivary gland, where it will find its last resort within *Anopheles maculipennis*. Here they await the next sting victim to restart the cycle and evolve into their next stage inside the human body (Lucius and Loos-Frank 1997& 2008, Mehlhorn 2012)

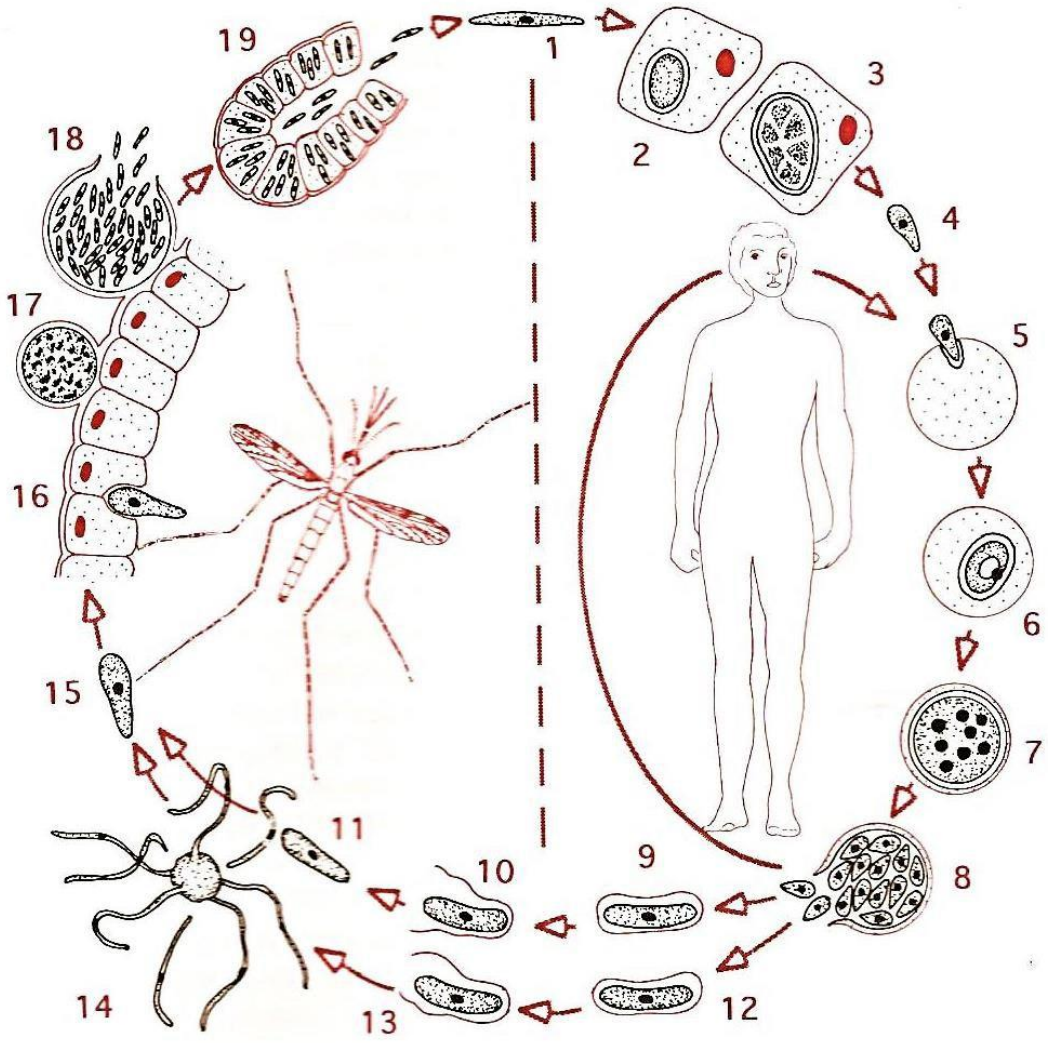

Figure 2 Life cycle of *P.falciparum* (Lucius & Loos-Frank, 1997)

Understanding this cycle there are necessary precautions to be made, even if a patient already suffers from this mosquito-borne infectious disease. For instance, if a patient is infected he still needs to make certain required arrangements to break the cycle. Otherwise

the number of mosquitos, who carry the *Plasmodium* parasite, will steadily increase. Consequently, this is one of the main complications when dealing with malaria patients. The big problem at hand is that after being stung and infected the patient is more likely to be careless during the time of day when the *Anopheles* female is out searching for a victim. This crucial mentioned time period is usually around nightfall, when the previously infected patient is possibly less anxious to prevent a sting by the mosquito. Unfortunately this crucially benefits the pathogen and may engender severe consequences for the uninfected.

1.3. Zanthoxylum zanthoxyloides

1.3.1. Origins and family tree

*Zanthoxylum z*anthoxyloides (Lam.) Zepern. & Timler, Rutaceae, is a member of the subfamily Toddalioideae of the Rutaceae family. A common synonym within this is *Fagara* spp.. *Z. z*anthoxyloides can be found in West Africa particularly in the south of the Sahara Desert from Senegal in the west to Cameroon in the east. The samples chosen for this study originate in Burkina Faso. In this particular country and in the surrounding *Z. zanthoxyloides* (homotypic synonym: *Fagara zanthoxyloides* Lam.*;* heterotopic synonym*: Zanthoxylum senegalense* DC*, Fagara senegalesis* (DC.) A. Chev.*, Fagara zanthoxyloides* Lam.*, Zanthoxylum polygamum* Schum. et Thonn.) is used in the traditional medicine to cure various diseases and maladies.

Figure 3: A *Z. zanthoxyloides* tree in the western region of Burkina Faso

In addition to the different *Zanthoxylum* species they are applied in numerous sectors of everyday life aside the pharmaceutical use. For instance *Zanthoxylum macrophylla* or *Fagara macrophylla* - which are evidently the same (Waterman 1975) - is principally used to poison the end of hunting tools such as spears and arrows. In East Africa, more precisely in Tanzania *Zanthoxylum chalybeum* is applied for similar use.

Conclusively the use of *Zanthoxylum* may occur contradictory considering the toxicity of this plant (Ngono Ngane et al. 2008), but bearing in mind Paracelsus' *"dosis sola venenum facit"* the difference might not be as conflicting as it primary appears.

1.3.2. Botanic description

Zanthoxylum is either a small tree or a shrub often elongated into a hedge. The maximal height differs from 4 m up to almost 11 m. The often very dense and bushy crown can hardly be penetrated. It is often planted around stables and houses and therefore utilized to work as a fence. The trunk of the tree or shrub is often quite short. The bark surrounding the tree differs in color, depending on the amount of sun exposure and the different nutrients found in the ambient soil. The spectrum of color varies from brown to yellow to grey or even to red. Additionally the outer layer features fine vertical rifts with occasional wood-based protuberances that are prickles (Figure 6). The cut surface appears yellow-colored and orange-spotted underneath, including a certain aromatic smell. The foliage alternately

Figure 4: Leafs of a *Z.zantoxyloides* tree, with the characteristic elliptic shape and the fruit

arranged and with the opposite leaflets here and there. The main leaves are glabrous and placed on a petiole with a length of 2-5 cm. The lamina can reach a length up to 20 cm but the average lies at about 13 cm. It also features ventrally buckled thorns on the side. Moreover, it is spiny on its ventral surface. The leaflets are quite smaller with a lamina height of 5-10 cm. It is furthermore attached to the branch by a petiole with about 5 mm of length. Base and apex appear obtuse or rounded. The general shape of the leaves can be considered elliptical as can be observed in Figure 4. The lamina is generally veined with eight to fourteen sets of lateral vesicles, which are barely noticeable. They eventually meet near the margin. The glandular vessels on the surface of the leaves hold the essential oils of the plant. The odors within these containers can be freed if the compartments are compromised. If penetrated the volatile matter will emerge of which the smell recalls

Figure 5: The trunk of the *Zanthoxylum. zanthoxyloides* tree

lemon or pepper. This appears to be logical since they are both members of the Rutaceae and the smell of lemon is often considered to be typical for that plant family. The panicle is often axillary and can reach a length of fifteen centimeters. The flower is unisexual, pentamerous and colored green or sometimes even white, whilst the corolla remains marginally open. The prior ovaries are within the female blossom and are regularly single-celled. The brown-colored fruit of this sex is an ovoid follicle with a radius of about two to three millimeters. Additionally glandular vessels can be further found on the fruit. The seeds within the green or red colored fruit are dark brown or even black and very persistent, whilst stored safely within the fruit. The average weight of the seeds differs (from 15.9 g – 18.1 g / 1000 seeds) (Schmelzer & Gurib-Fakim 2004). The male flowers with the stamen are a little excreted, which is very typical for the male sex in this botanic family.

Figure 6: The stem of *Z. zanthoxyloides* with the typical wooden-based protuberances

Zanthoxylum can bloom one or even two times a year. It usually flowers within the first half of the dry period. Additionally, it can also bloom during a rainy period. The main habitat of *Zanthoxylum* is in West Africa from Senegal in the west to Nigeria in the east. It does not grow in always dry parts of western Africa such as savanna regions. Throughout its natural habitat it shows gregarious behavior. It can be mainly found in fringing forests. Although due its hydrophilic attitude it additionally indwells areas that are in proximity to rivers and damp territories. Furthermore, its ability to withstand termite attacks can also be found in vicinity to their nests. This again makes it a very useful material whilst doing construction work (Arbonnier 2004 & Adesina 2005).

1.3.3.Common usage of *Zanthoxylum zanthoxyloides* in traditional medicine and cultural life

One of the main usages of ***Zanthoxylum*** in traditional African medicine lies on its antiseptic features. Nevertheless, various applications are considered to vigorously oppress pain and therefore have an analgesic effect. In addition, its impact on the sudoriparous glands is also mentionable, causing perspiration (Parmpeh & Mensah-Attipoe 2008, Queiros et al. 2006). The drugs made from the root and the seeds of the plant should always remember and consider this as a mentionable side effect.

1.3.2.1. Oral Application

In the countries mentioned above *Z.* is used to treat various kinds of diseases. Mainly the leaves, roots and the stem bark are used in different forms of extracts. For instance, roots and stem mark are used to produce macerations, decoctions and infusions. They are then applied to people tormented by numerous maladies and their symptoms such as malaria, general body weakness, paralysis, tuberculosis, edema and all sorts of fevers (Meyer 2012, Ogwal-Okeng et al. 2003). It is worth mentioning and rather curious that it is also applied to treat and cure sickle cell anemia. Because of its popular use as medicinal plant the stem bark and roots of the *Z.* are even exported from some West African countries such as Burkina Faso, Ghana, and Cote d'Ivoire. Furthermore, an infusion can be prepared to treat gastrointestinal problems such as dysentery and colic. In addition, it can also be brought into action if the patient is infected with intestinal worms. Prominent examples include hookworms. Additionally, it is also used against infection with *Schistosoma* species. Subsequently *Z.* concoctions are intended to ease pain during child birth. It can also be used to treat gonorrhea and urethritis. Moreover, it is a common treatment against migraines and other various forms of neuralgia (Neuwinger 1997).

It is widely documented that roots and stem bark of *Z.* have a numbing effect on skin and mucosa. Therefore, these parts of the plant are often in use to treat various forms of toothache. Given that common usage it is often colloquially called “Toothache-tree” among the numerous tribes and villages in West African countries. Furthermore, the description “Toothbrush-tree” is also commonly used in various cases. It is also mentionable that young roots of the tree seem to have a more promising effect on gum infections or even just sore gums. Subsequently a decoction of stem bark or roots also serves as a mouthwash and is even described to ease sore throats or an inflamed larynx. This reinforces the analgesic and antiseptic character of this species. Additionally, it is worth mentioning that the roots and young shoots even serve as a regular chewing device, without any background of a medical condition. It is just a way of strengthening the teeth and stimulating salivary. Because even though human salivary is full of various pathogens and inflammatory substances it also host a selection of antiseptic and anti-inflammatory substances, such as antibodies, immunoglobulins and mucins. Subsequently this again helps the body to overcome small infections and minor damage in the mucosa of the oral region (Schmid 2005).

In addition, it might even help to ease minor forms of digestive disturbances. It is also worth mentioning that minor conditions of nausea are treatable with this sprout. Hereinafter it fills the place of the common chewing-gum used in western civilization. (Iwu 2014, Adesina 2005)

1.3.2.2. External Application

Aside from the area of internal application *Z.* preparations are also used in external administration. For this application the roots are often chopped or even pulverized and blended with water resulting in a mass of muddy consistency which is then applied on the problematic area. This remedy finds application in cases of swellings or snake bites. Furthermore, it is also commonly used in the treatment of ulcers or abscesses. In addition many healers of traditional African medicine also treat hemorrhoids, leprosy wounds and various superficial symptoms of syphilis, although this medical disease is quite severe and the plant species might only reduce pain but not vanquish the virus (Neuwinger 1997). In case of hernia induced pains *Z.* also serves as a medication. It can also be used to ease the pain of rheumatic and arthritic pain. In Burkina Faso and in Cote d'Ivoire pulped stem bark can be in use to treat eye infections or even conjunctivitis. Furthermore, it is also in use against whooping cough. Whooping cough is a bacterial infection with *Bordetella pertussis* that mainly infects infants (Queros et al. 2006).

Many other usages are also likely and may even be acknowledged, due to the species analgesic and antiseptic features. Furthermore, this will yet again be applicable in many different cases of general inflammation. Positive consequential effects will subsequently be promising against both external and former mentioned internal maladies, making this a more encouraging research field.

1.3.2.3. Cultural Aspects

The *Z.* plant is often used in West Africa as a hedge. Because of its density is impenetrable. The bush itself is often browsed by all sorts of animals such as goats and sheep. Moreover the stem is often used in religious ceremonies. Because of its high concentration of resin especially its young twigs and branches are particularly favored. During fire a ceremony thinned dry twigs and spines are tossed into the flames to create strong and thick smoke. This fume then gives off a strong scent. Subsequently, the leaves are often found in the traditional cuisine in West African countries. They smell of citrus fruits and provide this taste for the food. The cinnamon flavored seeds are equally used to season food. Furthermore, the seeds are also used as jewelry. Many women make necklaces out of the little black seeds. Not only is the crown of the plant dense, also the wood of the tree has a high density. Therefore – provided that the plant itself is big and tall enough – the bole is commonly used to make poles and other primary structures in constructing work. Because of its given nature it is very termite resistant. This makes it the ideal base material for building houses and stables. Due to its general density and its visual cover it is also often planted used within the local religious customs. Thereinafter it is common use to shield houses and stables from evil spirits (Ngane et al. 2000, Neuwinger 1997).

But these examples are just a few from the various magical, religious and traditional usages that Z. provides in West African cultures.

1.3.3. Selected phytochemical analysis done in the past research papers

In various studies, done by laboratories in France, Cameroon, Senegal, Nigeria, Burkina Faso and Benin different *Z.* species were analysed. For instance in one of the research programs two Cameroonian Rutaceae (*Z. leprieuril* and *Z. zanthoxyloides*) were investigated. Conclusively it was shown that a 90% EtOH extract of these plants was able to inhibit the growth of *Candida albicans, Cryptococcus neoformans* and seven more filamentous fungi. The minimal inhibitory concentration was established from0.5 up to 1 mg/ml for root concoctions. As for those deriving from the stem of the plant the necessary concentration intervals were identified from 0.125 up to 1 mg/ml (Ngane et al. 2000). The essential oil of this Cameroonian *Z.* additionally had monoterpenes as their main components in following concentration*:* α-pinene (38.2%), trans-β-ocimene (5.4%), citronellol (3.3%), sabinene (3.2%), myrcene (3.1%), limonene (3.0%), citronellyl acetate (3.0%), α-terpinolene (2.7%), α-phellandrene (2.6%), geraniol (1.9%), terpinen-4-ol (1.5%), p-cymene (1.2%), methyl citronellate (1.2%) and β-pinene (1.2%) (Ngane et al. 2000). Another sample analyzed in that particular study showed very different concentrations regarding the main components as the subsequent values illustrate: β-citronellol (18.1%), geraniol (16.2%), 2,6-dimethyl-2,6-octadiene (9.3%), geranyl acetate (5.9%), isopulegol (5.4%), D-limonene (4.8%), β-citronellal (4.7%) and the sesquiterpene manoyl oxide (5.5%) (Ngane et al. 2000). Due to research work like this it is now considered scientific proof that *Z.zantoxyloides* extract does have an inhibitory effect on various microorganisms.

In a research paper done by a team of scientists from the *Laboratoire de Chimie Organique Physique* from the University of Montpellier *Z. zantoxyloides* leaves and fruits were phytochemically analyzed. The result regarding the composition of the plants leaves showed that the leaf oil composition presented only monoterpene hydrocarbons in a concentration of 98.2%, among them α-pinene (26.5%), myrcene (30.0%) and (E)-β-ocimene (31.9%) were the most predominant (Menut et al. 2000). Additionally, the fruits were also analyzed and the results show that the complexity of the fruit oil composition was considerable. The major compound were monoterpenoids with 85.5 %

also showing a lot of oxygen activity. The share of these compounds laid at 30.9 %, whilst linalool and geranial presented the largest share within this group. Interestingly

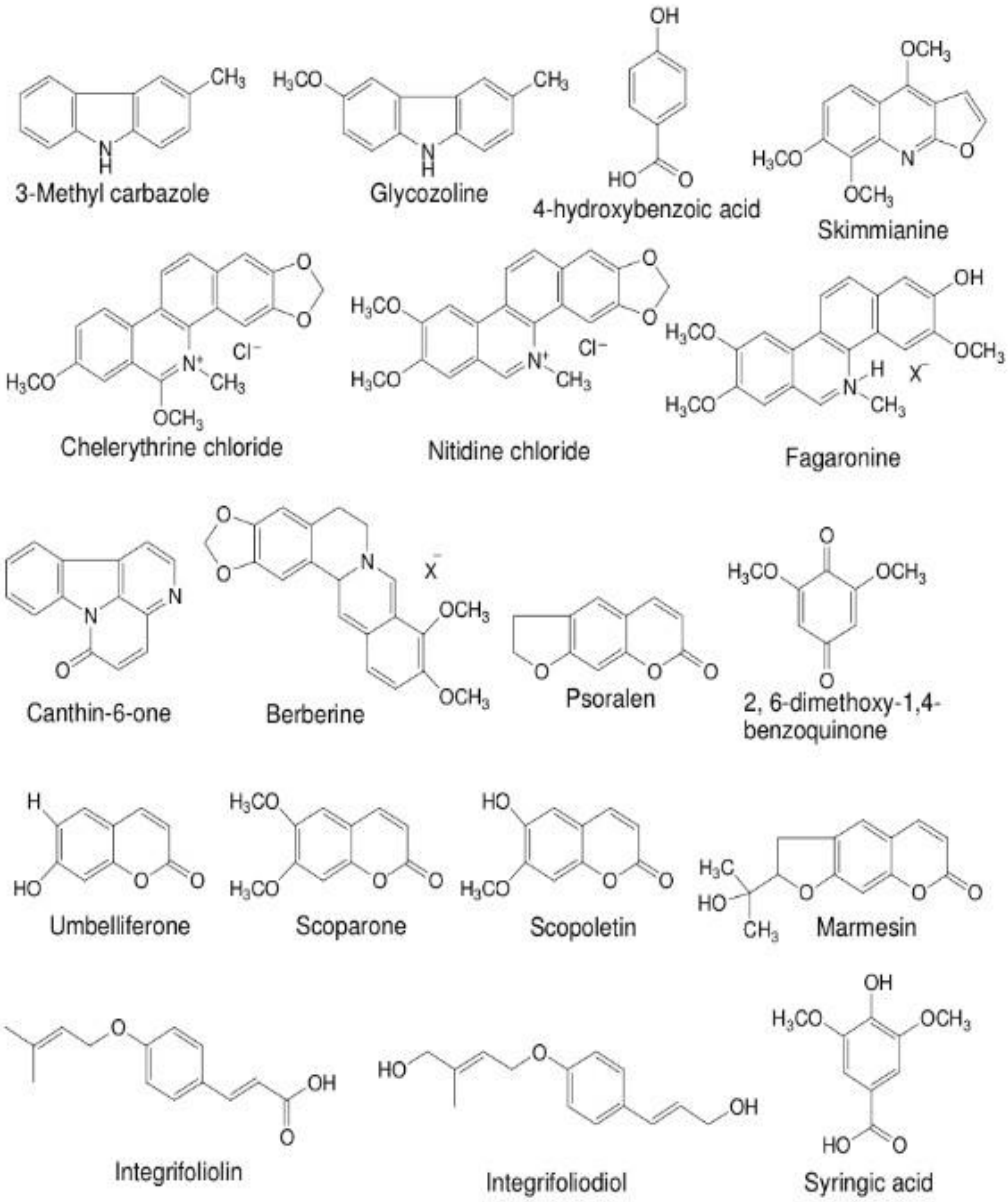

Figure 7: Structure of some isolated bioactive compounds found in *Zanthoxylum* (NEGI et al. 2011)

(E)-β-ocimene was both high in fruit oil with 41.5 % as well as leaf oil with 31.9 % (Menut et al 2000). Since the country of Benin evidently adjoins Burkina Faso in the north-west it is most likely that the phytochemical compositions are roughly similar and therefore comparable, as the botanical similarities are not affected by human frontiers.

Nevertheless it stands to reason to assume deviating results when *Zanthoxylum zantoxyloides* species are collected in different countries.

Furthermore, the root and stem bark of *Z. zantoxyloides* were analyzed as it can also often be found in the traditional medicine throughout West Africa. In these studies it was proven that the lignan **sesamine** is a component in both parts, whereas the root bark also contains seasmine's epimer asarinin.

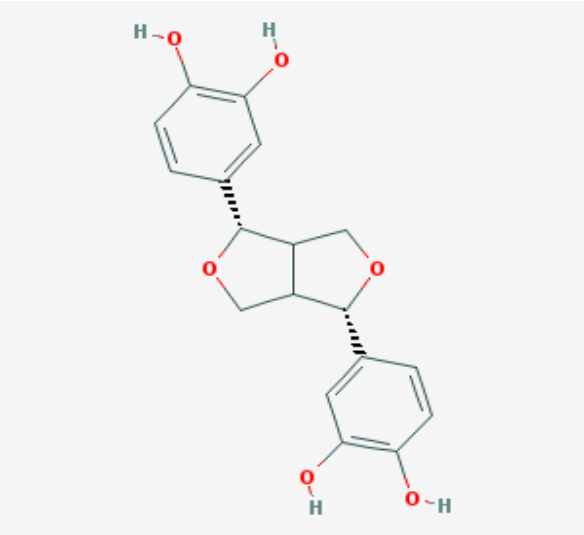

Figure 8 Sesamin ($C_{20}H_{18}O_6$)

Further on various alkaloids were identified in stem and root bark such as aporphine alkaloids. Moreover, the stem bark contains many coumarins such as xanthotoxin, scopoletin, imperatorin, scoparone, bergapten, umbelliferone, mermesin and pimpinellin which here in after featured antifungal characteristics. In the root the aporphines tembetarine and magnoflorine were identified as well as N-methyl-corydine and **berberine**. In addition, a group of furoquinoline and benzophenanthridine alkaloids were also identified within these parts of the species. To be more precisely the benzophenanthridines actually found in the stem of the tree were chelerythrine and **fagaronine**. Additionally, berberine and cheleterythrine both possess antimicrobial activities, which were reasonably encouraging. Fagaronine proved to be a useful medicine against leukemic diseases. The discovery of the auspicious alkaloid and its ability to attack either P-388 or L-1210 leukemic cells is considered a great scientific achievement. Additionally, the root bark covered an even wider spectrum of benzophenanthridine alkaloids. In addition to the two also detected in the stem two more where eventually

found. These are oxychelerythrine and dihydroavicine and they are only common in the root not the stem bark. Furthermore, the furoquinoline alkaloids in the root were identified as 8-methoxydictamine and skimmianine.

Several different groups of amides were further isolated from root bark extract, such as aliphatic and aromatic amides. The aromatic amides are mainly **fagaramide**, piperlonguminine, rubemamin, arnottianamide and N-isopentyl-cinnamamide. One of the most interesting amides found in the *Z.'* root bark is **pellitorine**. Pellitorine was earlier described by Bowden and Ross in 1963 and even before that by Paris and Magnon in 1947.

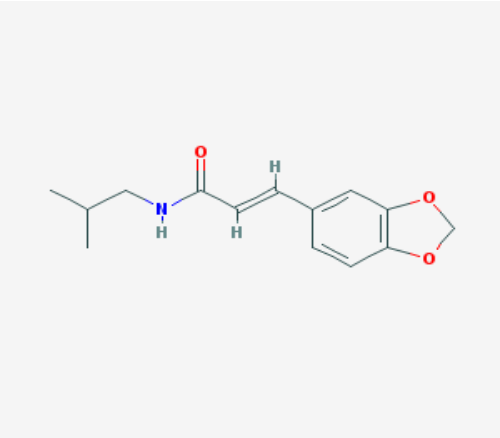

Figure 9 Fagaramide ($C_{14}H_{17}NO_3$)

The main quality of pellitorine is its local anesthetic character, for which the root bark extracts are used in traditional medicine. . This quite unique ingredient can interestingly enough even be isolated in the fruits and the stem bark. Due to the isolation of known anti-inflammatory and analgesic compounds, such as pellitorine, the positive effect on various maladies is yet again ensured. Moreover pellitorine showed promising antibacterial and anti-sickling effects in in vitro studies. Unfortunately, it is also very expensive, which was the main reason it was not considered a reference in this particular thesis. Nevertheless it is indeed a promising compound, especially considering its impact and potential as pharmacological agent.

The phenolic acids identified in the root bark are the vanillic acid and the hydroxymethyl-benzoic acid. Furthermore, a series of benzoic acids were detected and isolated such as the parahydroxy-, parafluoro- and 2-hydroxy-benzoic acid. Additionally, the divanilloylquinic acids burkinabin A, B, and C where found among the spectrum of discovered acids in crude root bark extracts.

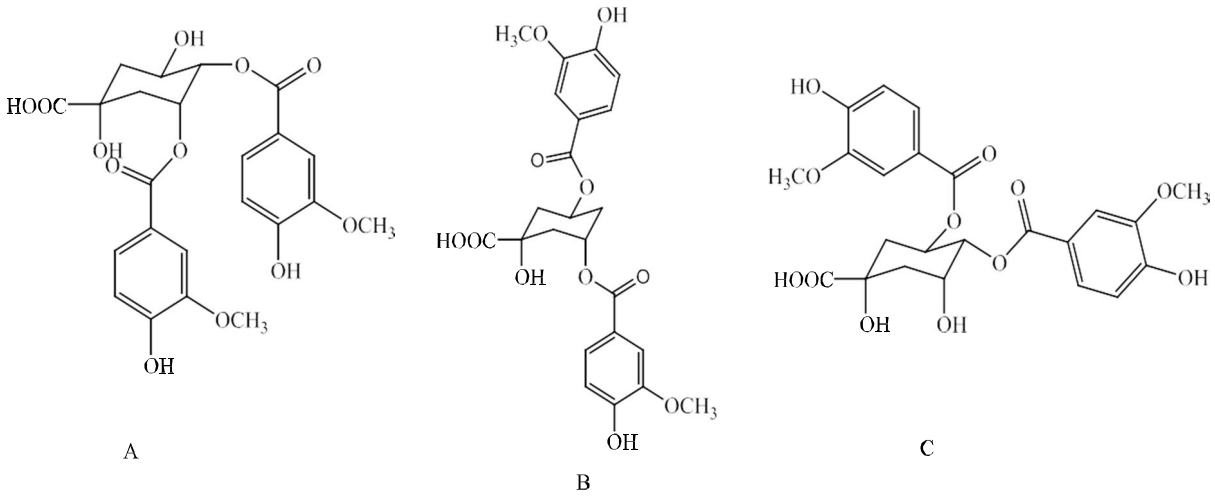

Figure 10: Burkinabin A, B, C (Ouattara et al. 2004)

In conclusion it can be acknowledged that crude bark MeOH or aqueous extract can evidently be administered to cure various maladies caused by general or local inflammation. Subsequently its usage against a series of pathogens was also well established (Prempeh & Mensah-Attipoe 2009, Prempeh & Mensah-Attipoe 2008, Queiros et al. 2006, Adesina 2005, Ngassoum 2003, Ossoba Et Al. 1989).

Although the positive and encouraging features of various concoctions are reasonably promising the shown toxicity in numerous animal trials must not be left out of consideration.

The LD50 of the methanolic extract was found at 5.1 g/kg body weight in mice within a confidence limit of 95 %. Clinically

the animals presented certain neurological symptoms. These cerebral irritations often occurred just before the mice died. Under the microscope focal necrosis herds were identifiable in renal tubules and liver samples (Ogwal-Okeng 2003, Arbonnier 2004, Ouattara et al. 2004).

Therefore it is necessary and recommended to prolong the investigation on how these bioactive compounds truly interact with the human body. The focus of this research should evidently be the activity that *Z.* extracts have on the cerebral mechanisms. Additionally, it is also worth mentioning that the former documented liver and kidney cell destruction should also be considered in future research work.

2. Material and Methods

2.1. The Samples

The eight different reference samples were collected in the west of Burkina Faso in the regions Cascades, Sud-Ouest and Hauts Bassins. The seventeen market samples were acquired on herbal markets in the capital Ouagadougou (10) and the city of Bobo-Dioulasso (7). Mainly all the samples were root samples except for two leaf samples from Bobo-Dioulasso (sample 14 EM_Bbo_189 and samples 15 EM_Bbo_190.

2.2. General Preparation

All the plant material was ground and furthermore weighted in portions of 500 mg. Five extracts were prepared for each sample with different solvents of different polarity (methanol (MeOH), ethanol (EtOH), acetone (DMK), dichloromethane (DCM) and water (H_2O)). These extract samples were generally stored at -20 ° C and defrosted at room temperature for at least 30 minutes before usage in various analysis.

2.3. Spectrophotometric Analysis

2.3.1. Total Phenolics

The reagent used in this phytochemical method was the Folin-Ciocalteu-Reagent (FCR). The *Z.* extract (10 µl) was mixed with 100 µl of H_20 and 5 µl of FCR, in a microplate well. The mixture was incubated at room temperature for 3 min, and 10 µl of sodium carbonate (35 g in 100 ml H_2O) was added. The final volume per well was filled to 250 µl with H_20. Subsequently the plate was covered with Parafilm® (Pechiney Plastic Packaging, Chicago IL, USA) and incubated in the dark for 60 min. The absorbance was measured at 750 nm using a microplate reader. Caffeic acid was used as standard at the concentration range of

0 -2.5 µg and the results were expressed as mg of caffeic acid equivalent (CAE) per gram of plant dry weight (DW). The results represent the means of quadruplet measurement.

In the microplate the phenolic ingredient interacted in an alkali environment with the Folin-Ciocalteu-Reagent (MERCK, Vienna, Austria), which was indicated in a change of color. This distinguish shade of blue corresponds with the amount of phenolic compounds in the samples used. After incubation the plate was put in a microplate reader (iMark Microplate Absorbance Reader®, Bio-Rad, California USA) for photometric analysis. It was measured at 750 nm with a mixing time of 20 seconds. The software Microplate-Manager 6.0 was used to determine the absorption of each compartment on the plate. The calibration curve was made using different quantities of caffeic acid – starting at 0µl and ending at 25 µl – and H_20 adding up to 110 µl in each compartment on the plate. Afterwards FCR was added in a volume of 5 µl. After a short incubation time of 3 min, whilst the plate was lightly agitated, 10 µl of sodium carbonate was added as well as 125 µl of H_20. After a defined time the plate was measured in mentioned device. The results were furthermore evaluated in a specially designed Excel-sheet.

The *Z. zanthoxyloides* samples were treated equally, only instead of caffeic acid in numerous concentrations the samples were used in a volume of 10 µl with 100 µl of H_20 adding up to the above-quoted 110 µl. Each sample was repeated 4 times in a plate and the average of the 4 results minus the zero value was taken as an estimate within the calibration.

Due to different concentration of phenolic compounds in some of the samples the absorption was not within the boundaries of the calibration curve and therefore not evaluable. These samples were diluted to fit into the linear range.

2.3.2. Antioxidant Activity

The stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH ®, Sigma-Aldrich, Missouri USA) is reduced in the presence of an antioxidant substance. The absorption was measured with a photometer (iMark Microplate Absorbance Reader®, Bio-Rad, California USA). An amount of 3.8 mg pure DPPH® powder was mixed with 25 ml of MeOH conc. to achieve the necessary ratio of DPPH® solution. The resulting mixture was permanently stirred to prevent precipitation. The calibration curve was generated by adding a known concentration of an antioxidant to DPPH®:
0.0063 g 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®, Sigma-Aldrich, Missouri USA) were mixed with 10 ml of EtOH (99 %) and then put into an ultrasonic bath (Bandelin Sonorex RK 156 BH® Germany) for a period of 3 minutes. In order to calculate a curve, varying quantity of trolox®-EtOH solution was pipetted in a microplate in following concentrations: 0 %, 1 %, 2 %, 4 %, 6 %, 8 % and
50 %.

The DCM samples were not analyzed in this method. The wells were first filled with 85 µl of MeOH conc and then afterwards filled up to 100 µl with 15 µl of extract. The exact ratio of the two ingredients was adjusted if the shade of color was to extreme. Immediately after the first two solutions 100 µl of DPPH® was added. Henceforth the plate was sealed with a layer of Parafilm® (Pechiney Plastic Packaging, Chicago IL, USA). After this step the microplate covered with the Parafilm® was stored in the dark for 30 minutes before undergoing photometric analysis in the microplate reader. This measurement was performed at a wavelength of 490 nm for a time period of exactly
20 seconds. The results were stored and processed in an Excel-sheet for further calculation and evaluation.

2.3.3. Fe $^{3+}$-Reduction

This method yet again detects antioxidant activity within plant-extracts, though a different reactant is being reduced. In this particular case Fe^{3+} is being reduced in a special buffered solution. As standard 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®, Sigma-Aldrich, Missouri USA) is used. The applicable solution of trolox® was obtained by dissolving 6.2 mg in MeOH. Afterwards the solution must spend 5 min. in an ultrasonic bath (Bandelin Sonorex RK 156 BH® Germany).

30 ml of the special buffered Fe^{3+}reduction-reagent consist of:

- **25 ml of an ethanoic acid buffer solution: (300 mM)**; 1.55 g of Na-CH_3COOH x 3 H_2O in 8 ml of ethanoic acid are added to 500 ml of H_20 with a pH of 3.6),
- **2.5 ml of 2,4,6-Tris(2-pyridyl)-s-triazin short TPTZ:** 0.0312 g of TPTZ (TPTZ 98%, Sigma-Aldrich, Missouri USA) in 10 ml of HCL with 40 mM were made and stirred in an ultrasonic bath for 5 min. Finally, the glass container with TPTZ was coated with aluminum foil to shield the solution from light.
- **and 2.5 ml of a $FeCl_3$-solution:** 0.032 g $FeCl_3$ x 6 H_2O mixed with 10 ml of H_20 and the glass container surrounded yet again by aluminum foil to prevent degradation.

This reagent was freshly prepared each day in order to be properly reduced by the antioxidant substances or by the standard Trolox®.

In order to calculate a calibration curve, varying amounts of Trolox®-MeOH solution were prepared in test tubes in following concentrations: 0 %, 1.5 %, 4 %, 8 %, 16 %, 32 %, 45 % and 64 %. Afterwards 6 µl of each concentration was pipetted on a Microplate (ProteOn Standard Microplates® Bio-Rad, California USA). Thereinafter 15 µl of H_20 and 180 µl of the reduction-reagent are added to each concentration.

Subsequently 9 µl of plant-extract are pipetted onto the microplate. Additionally, 15 µl of H_20 and another 180 µl of the reduction-reagent are added to the plate. After this step a Parafilm layer envelopes the plate to conceal the micro-environment. It was then stored in the dark for a period of 5 minutes.

The absorbances were read at a wave length of 593 nm with 20 seconds mixing time. The results were stored and processed in an Excel-sheet for further calculation and evaluation.

2.4. Chromatographic Analysis

2.4.1. Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is a chromatography method to separate compounds of a mixture. The method is performed on a glass sheet coated with an absorbent (in our case silica gel). This absorbent is the stationary phase. After applying the sample, the plate is transferred into a chamber and a solvent mixture (= mobile phase) is migrating through the plate driven by capillary forces.

The samples were applied on 20 cm x 10 cm silica gel plates (TLC Glass Plate SG 60 F_{254}, EMD/Merck KGaA, Darmstadt) by using a Linomat (Linomat IV Sample Applicator, Camag Scientific Inc, Muttenz Switzerland) in 5 mm wide lines of extract with a space of 5 mm between the samples. 15 µl of extracts and 5 µl of the references were used. Due to the fact that the usual mobile phase was not effectively separating the compounds, different solutions were tested in a series trials. After optimization the mobile phase consisted of seven parts of hexane and three parts of ethyl acetate. The distance of the base line and the solvent front was approximately 8 cm long.

In addition to the extracts a group of selected references were pipetted onto the silica gel plate.

1. Caffeic acid (Sigma-Aldrich, Missouri USA)
2. Vanillic acid (Carl Roth GmbH, Karlsruhe)
3. Lupeol (Carl Roth GmbH, Karlsruhe)
4. Sanguinarin (Sigma-Aldrich, Missouri USA)
5. Fagaramide (Sigma-Aldrich, Missouri USA)
6. Sesamine (Sigma-Aldrich, Missouri USA)
7. Quercetin (Carl Roth GmbH, Karlsruhe)
8. Gallic Acid (Carl Roth GmbH, Karlsruhe)

After the procedure of separating in a closed environment the silica gel plate was carefully dried. The plates were observed under natural white light, 254 nm and 366 nm and polarized light with 650 nm (Reprostar 3, CAMAG, Muttenz). The penta-cyclic terpene **lupeol** was not visible under these conditions. In order to assess the qualification and semi-quantification of this compound the silica gel plates were sprayed with anisaldehyde reagent (4-Anisaldehyde > 97.5 %, Carl Roth GmbH, Karlsruhe). The applied reagent made the evaluation of lupeol possible and furthermore simplified the **sesamine** analysis considerably. The documentation was achieved by taking pictures with a common digital camera (Samsung PL120, Samsung Electronics, Seoul South Korea). For further investigation the pictures were transferred to a computer and organized in a PowerPoint file. A complete collection of the slides within this file can be found in the Appendix.

2.4.2. High-performance liquid Chromatography (HPLC)

500µl of the extracts were mixed with 500 µl of concentrated MeOH in a syringe, followed by pressing the mixture through a filter (Rotilabo® syringe filter Nylon unsterilized, Carl Roth GmbH, Karlsruhe) into a small flat-bottomed test tube. These test tubes were especially designed to work in the autosampler of the HPLC-system. 10µl of the sample were injected into the HPLC-system (600 S Controller, 717plus Autosampler, 996 Photodiode Array Detector, Waters GmbH, Wien, Austria). The HPLC was equipped with a Luna (Torrance, CA, USA) C18 column (150 × 4.6 mm i.d.; 5 µm) and operated at a flow rate of 1 ml/min with a standard running solution for this machine. Whilst the samples pass the column, their compounds separate due to different interactions with the adsorbent material and the mobile phase within the column.

Detection was performed at 254 nm. Identification of the substances was achieved by original pure reference substances and comparison of spectral data. To determine the concentration of the compounds, a calibration curve for the reference compounds was established in the concentration range from 1 to 500 µg/ml.

2.5. Statistical Evaluation:

The statistical program used to calculate the results from each individual method within this thesis was IBM SPSS Statistics 23, IBM GmbH, Wien). Differences between leaf and root samples were analyzed using a Students t-Test. Comparison between origin and extract were performed using a 2-factorial ANOVA. Mean values were compared in assistance to a Tukey-B statistical evaluation.

3. Results

First it was considered a matter of great importance to determine causal links between origin of a sample and the correlating various solvents that were used to prepare the different extracts, as well as the possible differences between leaf and root samples from different origins. In latter there was incidentally a significant difference in total phenolic and ferric reduction from the photometric analysis. Furthermore it also showed significant differences in the case of fagaramide in the HPLC. While the results from the DPPH and sesamin from the HPLC showed no such phenomenon.

3.1. Results of the photometric analysis

The collected data of all the different results from each of the three photometric methods can be found in the appendix under the chapter "**6.3. Selected results from the photometric analysis**".

3.1.1. Total Phenolic (mg caffeic acid/g DW)

The results from the total phenolic photometric analysis showed a wide range of results, especially in the various solutions. The highest concentration of caffeic acid was found in a MeOH solution; market sample EM_Oua_122 with 64.29 mg/g DW. This was a market sample acquired in Ouagadougou, the capital of Burkina Faso. Given the fact that all the other results were considerably lower, the possibility of a measurement error cannot be ruled out. In this case, the maximum concentration is found in the market sample EM_Bbo_189 with 13.17 mg caffeic acid/g DW, which is also a MeOH solution.

This sample was however purchased at a market in Bobo-Dioulasso. The lowest trace was detected in field sample RC_ZXR_1 with 0.94 mg caffeic acid/g DW. This happened to be a DMK solution, which was cropped in the region Cascades. The homogeneous cohorting between the various solvents was preformed to determine comparability.

Hereinafter the results from the photometric total phenolic performance provided two groups could be significantly differentiated among the extracts:

1. GROUP 1: H_20 and EtOH
2. GROUP 2: DMK and MeOH

Regarding the three origins (Ouagadougou, Bobo-Dioulasso and References), they were not distinguishable from each other

Recapitulatory the results show that the phenolic compound was averaged higher in the market samples than in the collected reference samples. This phenomenon is made clear in the following table, that show the mean values of the total phenolic compounds in market samples from Ouagadougou and Bobo-Dioulasso, as well as the field samples from the west regions of Burkina Faso.

Table 1: Averaged Total Phenolic values including standard deviation (mg caffeic acid/g DW)

Average (TP)	MEOH	ETOH	DMK	H_2O
Ouagadougou	**10.41 ± 1.14**	**3.01 ± 1.16**	**1.48 ± 0.33**	**7.52 ± 2.59**
Bobo-Dioulasso	**5.45 ± 4.86**	**2.47 ± 1.63**	**1.31 ± 0.20**	**6.04 ± 0.71**
Field Samples	**3.34 ± 0.95**	**2.32 ± 0.44**	**1.34 ± 0.33**	**6.11 ± 1.99**

When looking at the statistical analysis it appears that the total phenolic compounds are more cumulative in leaf than in root samples. This fact can especially be observed in MeOH extracts.

3.1.2. Fe^{3+}-Reduction (mg Trolox/g DW)

The outcome of the Fe^{3+}-Reduction photometric analysis showed a well-balanced range of results among the various solutions. The only exceptions are the solutions made with H_20. Which showed a wide range of numbers among the results from 1.74 mg Trolox/g DW in market sample (Ouagadougou) EM_Oua_118 to 38.77 mg Trolox/g DW in the field sample RH_ZXR_2. However, this sample also showed the highest concentration of trolox found in all used solution. This field sample RH_ZXR_2 was cropped in the region Hauts-Bassins. The results from the ferric reduction were statistically treated in accordance with the results of total phenolic content present similar pathway to a significant grouping. The first aspect was once again the extract and was partied as followed:

1. GROUP 1: H_20
2. GROUP 2: DMK, EtOH and MeOH

Secondly the origin was evaluated in light of significant comparability:

1. GROUP 1: Ouagadougou and Bobo-Dioulasso
2. GROUP 2: Collected references from the in the field

After consulting Dr. Lamien-Meda the decision to exclude these samples in this particular matter was generally agreed upon.
Given this, the assumption, that the H_20 solutions will have to be totally taken out of consideration, whilst evaluating this particular method, might not be overall conclusive. Furthermore, smallest trace of trolox was detected in field sample EM_Oua_114 with 0.89 mg trolox/g DW in an EtOH solution. This market sample was acquired in Ouagadougou. The following table shows that the mean values are considerably higher in the field samples than in the market samples.

Table 2: Averaged Fe^{3+}-Reduction (mg Trolox/g DW)

Fe^{3+}-Reduction	MeOH	EtOH	Acetone	H2O
Ouagadougou	3.22 ± 0.96	2.30 ± 1.13	3.26 ± 0.63	20.32 ± 11.47
Bobo-Dioulasso	4.01 ± 2.12	2.48 ± 0.68	2.30 ± 0.75	16.10 ± 2.40
Field Samples	5.90 ± 2.00	3.97 ± 0.61	3.16 ± 0.61	27.45 ± 7.12

3.1.3. Activity DPPH (mg Trolox/g DW)

The results of this final photometric analysis show a more narrow range of the averaged results among all the used solutions. On one side the maximum, 8.15 mg Trolox/g DW, was found in the market sample EM_Oua_122 from Ouagadougou, on the other side the minimum, 0.01 mg Trolox/g DW was detected in the market sample EM_Bbo_189 from Bobo-Dioulasso.

In the case of DPPH, the applied statistical procedure was done likewise as with the results from the Total Phenolic. The outcome concerning the extract comparability grouping presented two categories:

1. GROUP 1: H_20 and Acetone
2. GROUP 2: EtOH and MeOH

The grouping regarding origin was also noteworthy. Hereinafter all three origins presented individual cohorts, that were statistically significant:

1. GROUP 1: Ouagadougou
2. GROUP 2: Bobo-Dioulasso
3. GROUP 3: Collected references from the in the field

The following table shows, that the depending on the solution used and the origin of the sample strongly influenced on the result. For instance, the field samples showed the

highest values in the methanol solutions. The market samples from Ouagadougou were able to achieve the maximum in the EtOH solutions, whereas the market samples from Bobo- Dioulasso obtain the highest data of antioxidant activity in the H_20 category.

Table 3: Antioxidant Activity DPPH (mg trolox/g DW)

DPPH	MeOH	EtOH	Acetone	H2O
Ouagadougou	4.07 ± 1.36	5.40 ± 1.55	1.87 ± 0.54	2.37 ± 1.50
Bobo-Dioulasso	3.56 ± 1.75	4.32 ± 2.31	098 ± 0.62	2.74 ± 1.52
Field Samples	5.91 ± 0.94	5.36 ± 2.27	4.76 ± 2.02	2.56 ± 0.97

3.2. Results from the chromatographic analysis

3.2.1. TLC (Thin Layer Chromatography)

The plate was analyzed under common white light and additionally each plate was observed under light with a wavelength of 254 nm and a wavelength of 366 nm. The additional step of spraying the plate with an anisaldehyde reagent made the evaluation of lupeol easier. To each spot on the plate a value from zero to five was appointed due to the brightness.The sprayed plate was then again viewed under mentioned circumstances for more detailed and distinctive evaluation.

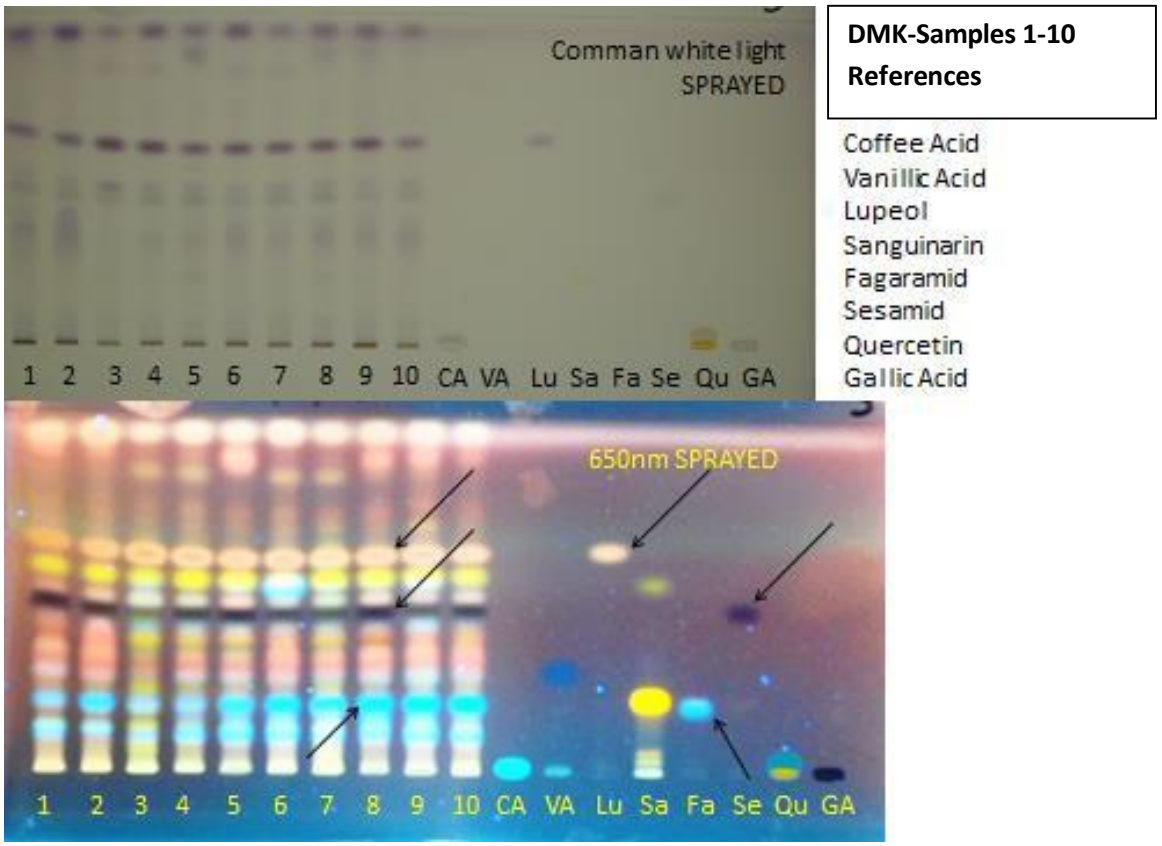

Figure 11: Slide 17 from the collection of the TLC pictures done in this project. The arrows show the compounds within the samples as well as the reference compounds.

As observable in Figure 11, the bars from the TLC under common white light represent only lilac to purple bars amongst the samples. The darkest and best visible bars appear at the same level on which the reference lupeol also appears. After spraying the plate with anis-aldehyde reagent the plate was again observed, but under polarized light with a wavelength of 650 nm. Hereby, lupeol was again visible but in a different color, namely peach to beige. Fagaramide was also detectable in the samples in a light blue color. The brightness in sample six to ten was especially noteworthy. Sesamine was also observable in a dark purple to black color in common white light, but only after being sprayed. When the plate was not sprayed, hardly any component was visible on the plate. This phenomenon can be well observed in Figure 12. Only the chromatographic footprint of sanguinarine is observable, which clearly indicates that it does not show up in the field samples. The samples in general and under polarized light presented a lot more bars. Although they did not include caffeic acid, vanillic acid, sanguinarine, quercetin or gallic acid, as these references did not produce similar data. None of the references mentioned was detectable in the samples. Concluding each sample presented about 20 very similar bars, of which the three formerly mentioned references were identified.

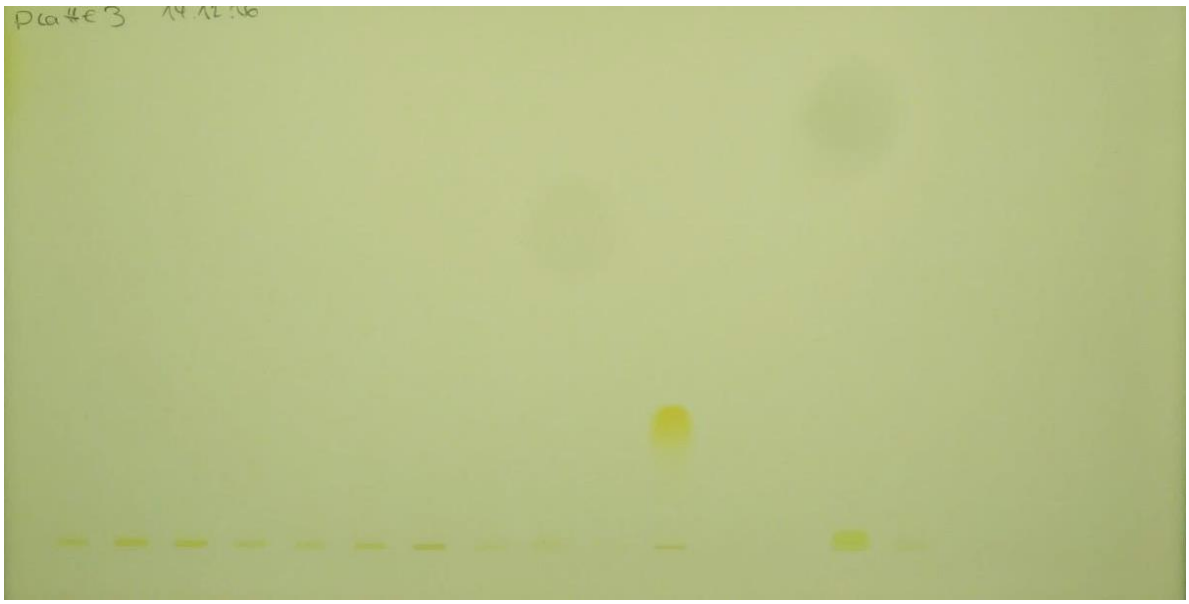

Figure 12: DMK-Plate with field samples A-H and then additionally the reference samples in following order: caffeic acid, vanillic acid, sanguinarine, fagaramide, sesamin, quercetin and gallic acid (without anis-aldehyde reagent and observed und common white light)

The assigned values (one through five) are viewed in the following table. Especially the samples 6, 8, 9 and 10 contained a lot of fagaramide. This goes for all solutions except the one with H_20. This is observable in Table 4 for the market samples.

Table 4: Market samples: collected TLC data of lupeol (L), fagaramide (F) and sesamin (S) inn different solvents established after assigning numbers to the bar brightness

	Ethanol			Methanol			Acetone			DCM			H2O		
Sample	L	F	S	L	F	S	L	F	S	L	F	S	L	F	S
1	3	2	3	2	2	2	3	3	4	3	2	4	**0**	1	1
2	2	4	1	2	4	2	3	4	3	3	4	3	**0**	2	1
3	4	1	2	3	1	1	**5**	2	1	**5**	1	1	**0**	1	1
4	4	1	3	4	3	4	**5**	2	3	**5**	2	3	**0**	2	1
5	2	**5**	3	2	4	4	3	4	4	4	4	4	**0**	3	1
6	2	**5**	2	4	**5**	3	4	**5**	2	4	**5**	3	3	3	2
7	2	**5**	3	3	**5**	3	4	4	2	4	4	2	1	3	2
8	3	**5**	3	3	**5**	4	4	**5**	4	4	**5**	4	2	4	3
9	3	**5**	3	3	**5**	2	**5**	**5**	2	**5**	**5**	2	1	3	1
10	1	**5**	3	2	**5**	2	3	**5**	3	3	**5**	3	**0**	1	2
11	2	2	1	3	3	1	3	2	1	3	3	1	**0**	2	1
12	1	4	3	3	4	2	3	4	2	2	4	3	**0**	3	3
13	1	3	3	3	4	2	3	3	3	2	3	4	**0**	2	2
16	2	4	1	4	**5**	1	4	4	2	3	4	2	3	2	2
17	1	1	2	4	3	2	3	1	4	2	1	3	**0**	1	2

The shortage of reference compound and the difficulties obtaining an appropriate mobile phase limited the amount of samples to be considered in this method. Nevertheless the MeOH, EtOH, DMK and DCM field samples were run with a selection of reference samples later on to obtain more knowledge in terms of comparability. The H_2O samples were not considered anymore. The RF-values were established and compared between the market samples and the field samples.

It is noteworthy to mention that lupeol was not run in with the field samples. Following up the data from field samples were collected and are observable in Table 5.

Table 5: Field samples, collected TLC data established after assigning numbers to the bar brightness

	Methanol		Ethanol		Acetone		DCM	
Sample	F	S	F	S	F	S	F	S
A	1	1	1	1	2	2	3	4
B	4	2	3	2	4	4	4	2
C	3	2	2	2	4	3	3	3
D	2	2	2	2	4	3	3	1
E	4	2	3	2	3	4	4	3
F	2	1	2	2	2	2	3	4
G	2	1	3	2	2	2	2	2
H	1	3	2	1	2	2	2	3

Table 6: RF-Values of the market and the field samples, lupeol RF- value from the field samples were estimated

AVERAGE SAMPLES (RF-Values)					
MARKET			FIELD		
Lu	Fa	Se	Lu (estimated)	Fa	Se
0.69	0.28	0.52	0.73	0.29	0.55

The Table 6 shows that the average Rf-values only differ from each other slightly. Since the plates both looked very alike and the reference lupeol was not available anymore the Rf-Value for lupeol from the field samples was estimated and calculated against mean values from all the other samples. This comparison led to the calculation of a coefficient which was then multiplied with the lupeol Rf-value from the market samples. This resulted in the Rf-value lupeol from the field samples.

A collected TLC Plate data can be found in the appendix.

3.2.2. HPLC (High Performance Liquid Chromatography)

All results of the HPLC analysis regarding the compounds that were actually identified were listed as mean values with standard deviation. For the samples on through seventeen, this is observable in Table 7. Only fagaramide and sesamine were established, as lupeol was not detectable.

Table 7: Established values (mean and standard deviation) without the results from the different water solutions

Mean Values and Standard deviations (µg/ml)					
Samples	Region	Fagaramid mean value	Fagaramid SD	Sesamin mean value	Sesamin SD
1	Ouaga	79,84	7,54	260,73	54,56
2	Ouaga	127,42	42,71	132,03	24,92
3	Ouaga	44,56	7,32	78,94	53,07
4	Ouaga	49,67	5,21	153,08	10,05
5	Ouaga	208,13	85,68	192,96	22,71
6	Ouaga	332,89	35,75	174,80	14,58
7	Ouaga	360,09	52,41	273,46	69,36
8	Ouaga	363,37	32,28	271,31	69,03
9	Ouaga	401,04	38,95	100,72	59,98
10	Ouaga	391,97	14,52	208,66	23,75
11	Bobo	82,54	16,88	125,22	34,22
12	Bobo	197,26	14,03	248,90	147,74
13	Bobo	145,10	15,78	134,73	135,43
14	Bobo	n.R	n.R	n.R	n.R
15	Bobo	n.R	n.R	n.R	n.R
16	Bobo	220,70	5,95	153,08	2,28
17	Bobo	44,49	1,14	158,74	28,72

3.3. Results in terms of comparability amongst the extract solutions

Each sample was prepped the same way, whether it was a market sample (numbered) or a field sample (categorized alphabetically). A MeOH, an EtOH, a DMK, a DCM and a H_2O extract solution was made for each and every sample.

3.3.1. Root samples

3.3.1.1. HPLC in root samples (High Performance Liquid Chromatography)

3.3.1.1.1. Fagaramide

Statistical evaluation of the HPLC results showed that there is a significant difference between the various solvents to concentrate fagaramide out of the roots. This was statistically affirmed with a Tukey-B Test and is observable in Figure 13. Fagaramide acts, as described above, as a very considerable antiplasmodial agent. In Figure 13 the subgrouping of extracts according to their potential to concentrate fagaramide is shown. The various extract solutions were able to educe fagaramide in concentrations from 28.34 µg/ml in H_2O up to 163.03 µg/ml in DCM extracts. The other samples were quantified as following EtOH (137.47 µg/ml), DMK (147.75 µg/ml) and MeOH (1610.92 µg/ml).

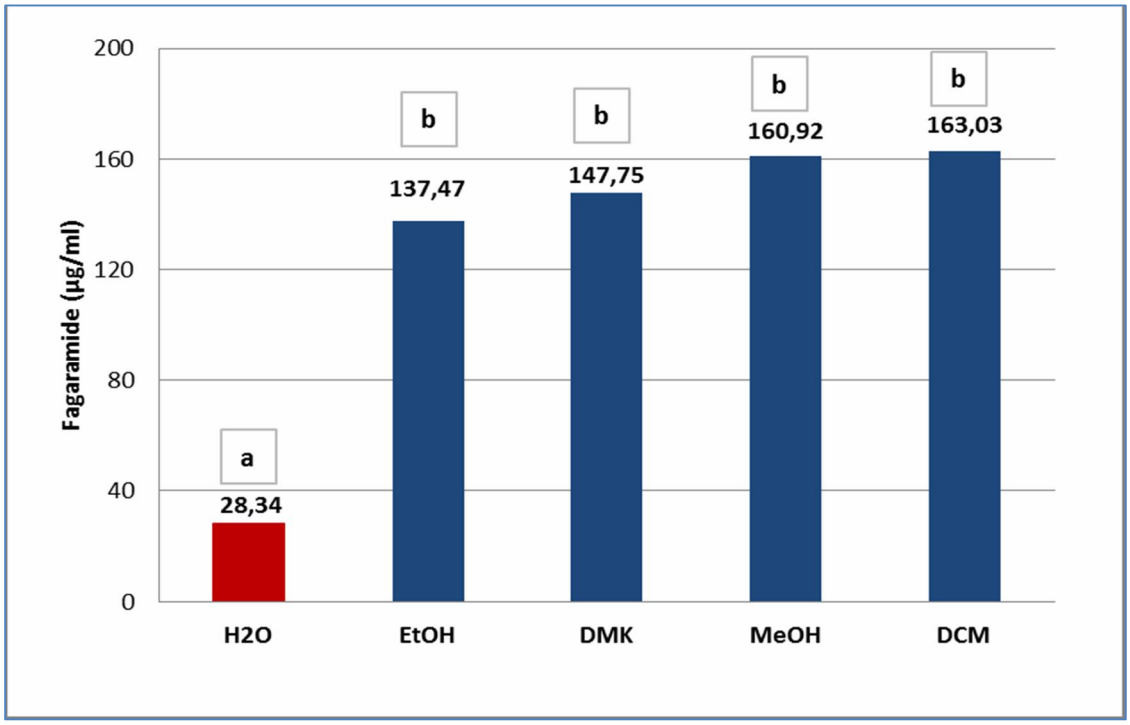

Figure 13: Subgrouping the fagaramide content in different extracts of the roots (different letters indicate significant differences).

H2O was not able to procure a great amount of fagaramide out of the plant material and significantly differed from the other extracts, whilst those other extracts (MeOH, EtOH, DMK and DCM) showed no significant difference amongst each other in the terms of extracting fagaramide from the plant. So forth two groups were established: group a with

only H2O and group b containing EtOH, DMK, MeOH and DCM..

3.3.1.1.2. Sesamin

Sesamin is known to have anti-inflammatory and antimalarial activity as discussed previously. Therefore it was also quantified by HPLC and showed a wide spread of results from H_2O extracts with 9.6 µg/ml up to 184 µg/ml in DCM extracts. The other results were quantified as following: EtOH (103 µg/ml), MeOH (130 µg/ml) and DMK (135 µg/ml).

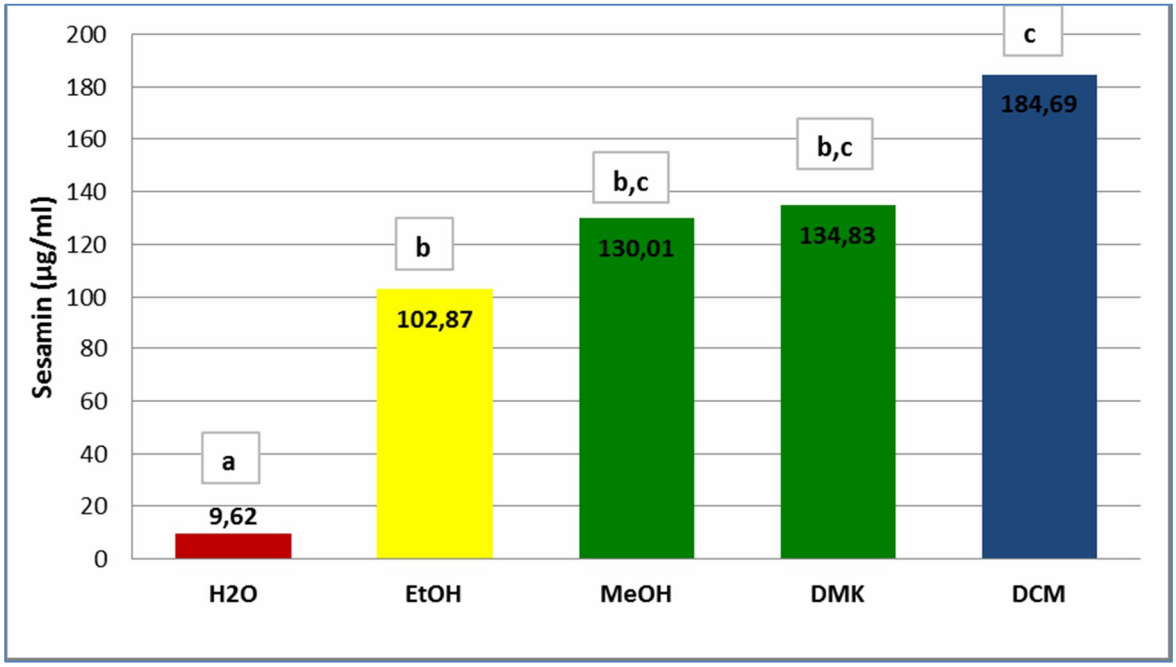

Figure 14: Sesamin subgrouping, three groups were established, namely group a, group b and group c; additionally MeOH and DMK samples were assigned to two groups: group b and group c (values in µg/ml)

The results showed that there were three groups that differed significantly from each other, although two extract solutions were allocated to two groups. This can all be observed in Figure 13. Group a, similar to the fagaramide grouping, only housed the H_2O extracts. In group b there are the EtOH extracts and group c contains DCM extracts. MeOH and DMK were assigned to both groups b and c

3.3.1.2. Results from the Photometric Analysis

The anti-oxidative activity of a concoction is always valued in case of an infection, no matter what sort of pathogen the organism is confronted with. In the first step only the root samples were statistically investigated by a Tukey-B Test. The demanding question hereby was if the results differed from each other in light of their extraction solvent. To enlighten this matter the samples anti-oxidative characteristics were statistically assessed amongst each method. The result was that their activity did indeed significantly differ from each other, regardless which of the formerly mentioned methods was applied. Then again also the subgrouping did present different results for each used method. It is however noticeable that especially the leaf samples did sometimes not produce any data within the method.

3.3.1.2.1. Total Phenolics

In this method the total phenolic contents of the DMK extracts (1.40 mg caffeic acid/g DW) and the EtOH extracts (2.44 mg caffeic acid/g DW) did not differ significantly from each other, they were placed in group a. The MeOH extracts (3.63 mg caffeic acid/g DW) were put into group b and the H_2O extracts (6.71 mg caffeic acid/g DW) into group c. All three groups differed from each other statistically significant, which is observable in Figure 15.

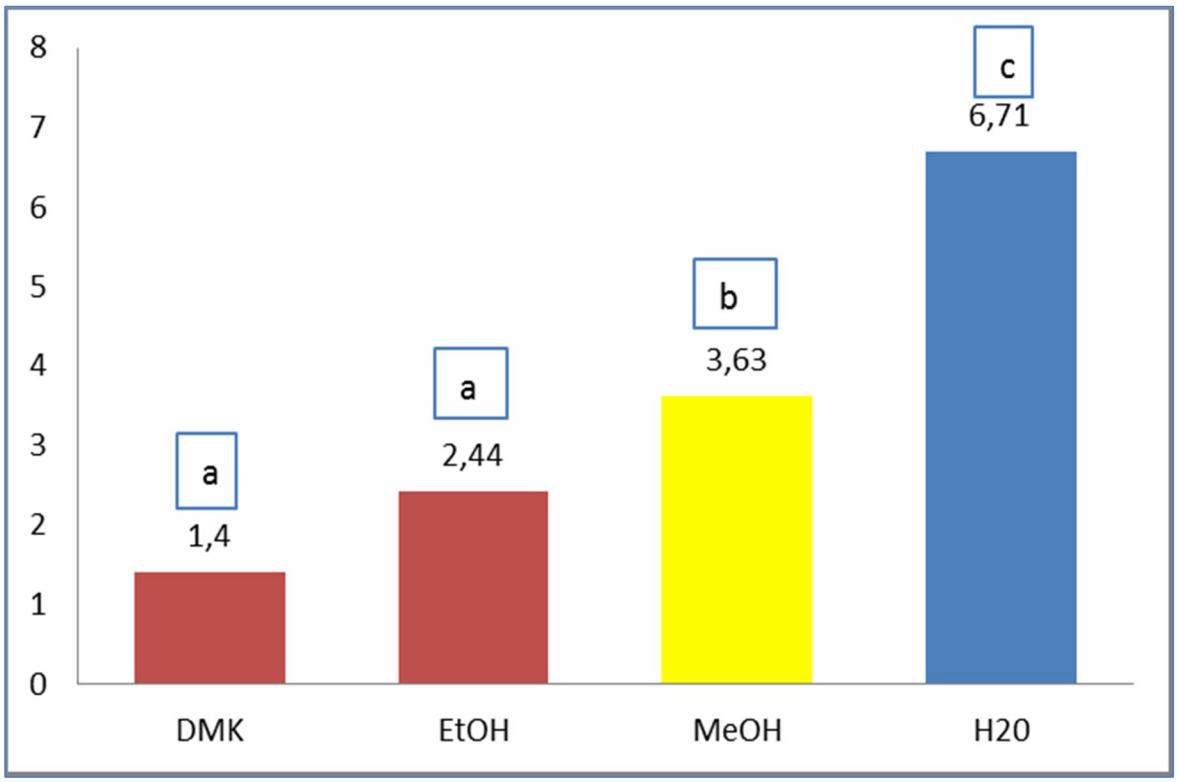

Figure 15: Total Phenolics content subgrouping, three groups were established, namely group a, group b and group c (mg caffeic acid/g DW)

3.3.1.2.2. DPPH

In case of the DPPH the antioxidant activity of the DMK extracts (2.75 mg Trolox/g DW) and the H_2O extracts (2.52 mg Trolox/g DW) did not differ significantly from each other and were labeled group a. MeOH extracts (4.39 mg Trolox/g DW) and EtOH extracts (4.86 mg Trolox/g DW), which both did not vary from each other significantly were addressed as group b. Both groups did nevertheless differ from each other statistically significant (Figure 16).

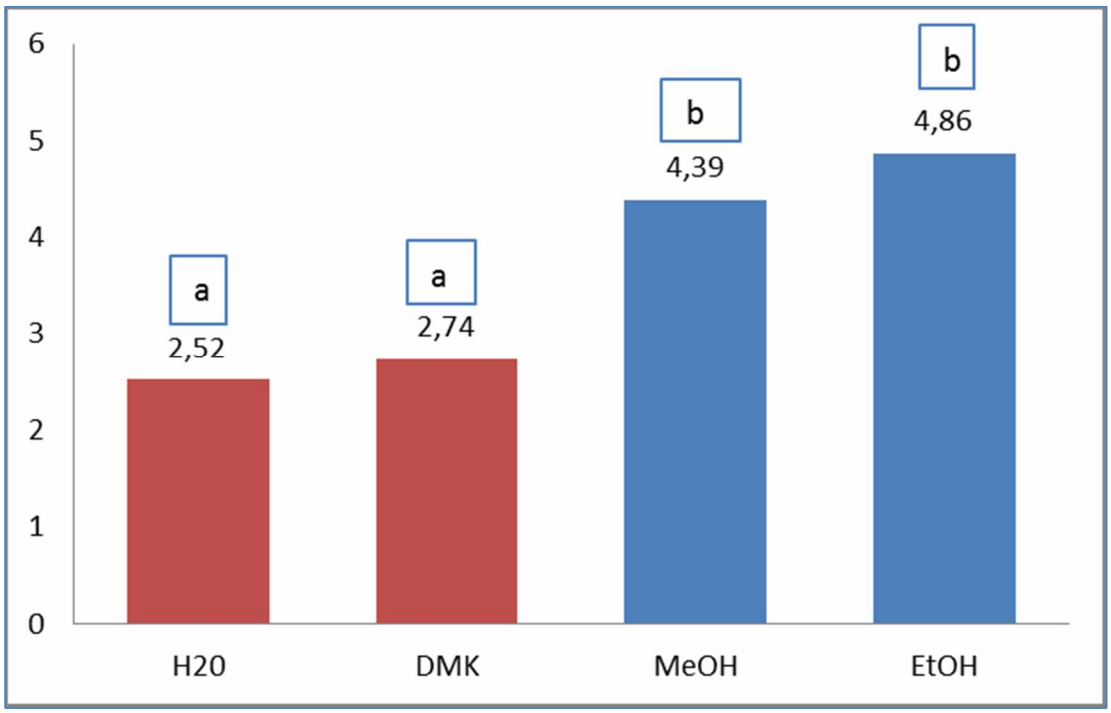

Figure 16: DPPH subgrouping, two groups were established namely group a and group b (values in mg Trolox/g DW)

3.3.1.2.3. FRAP

The ferric reduction showed that there are two groups (group a and b) of extracts that differ from each other statistically. Group a is made up by the antioxidant activity of EtOH extracts (2.99 mg caffeic acid/g DW), DMK extracts (3.09 mg caffeic acid/g DW) and MeOH extracts(4.07 mg caffeic acid/g DW). The second one named group b houses only H_2O extracts (21.88 mg caffeic acid/g DW). Both groups differed from each other statistically significant (Figure 17).

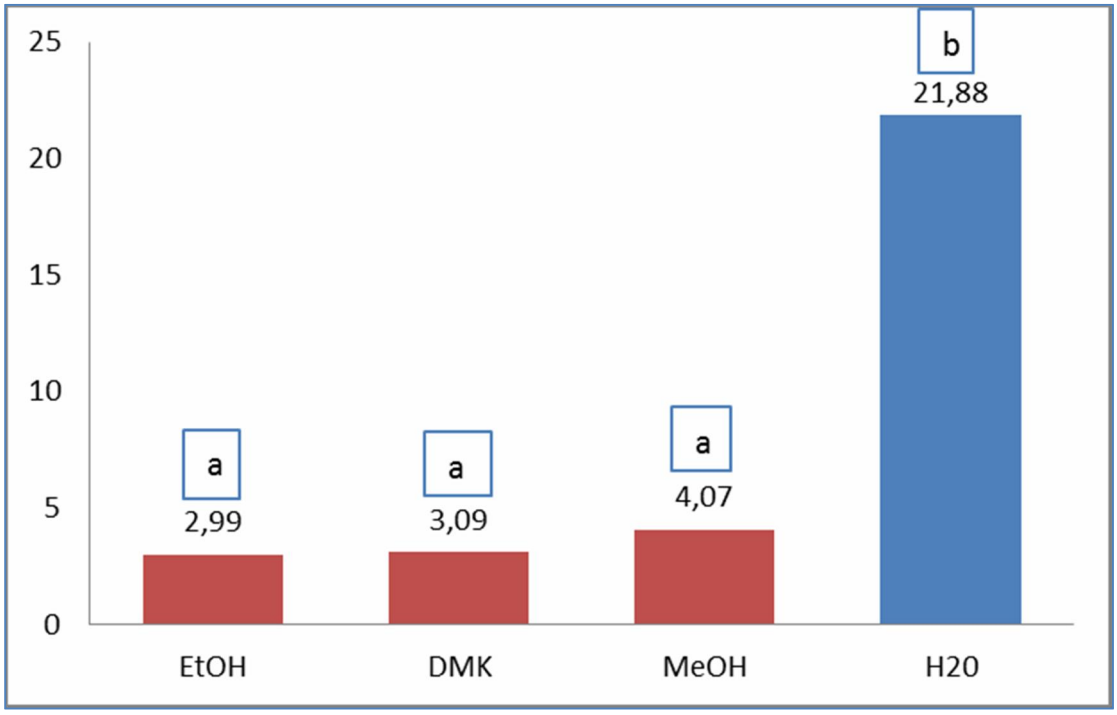

Figure 17: Ferric reduction subgrouping, two groups were established, namely group a and group b (values in mg caffeic acid/g DW)

3.3.2. Leaf samples

The antioxidative activity was again tested in terms of comparability amongst the extract solutions MeOH, EtOH and DMK. The results showed that all the various extract solutions differed from each other statistically significant.

3.3.2.1. Total phenolics

The results from the total phenolic contents showed that the various extract solutions all differed from each other statistically significant. Again subgroups were formed as observable in Figure 18. Group a inhabited the total phenolic content of the DMK extracts (1.24 mg caffeic acid/g DW), group b of the EtOH extracts (4.82 mg caffeic acid/g DW) and group c of the MeOH extracts (12.49 mg caffeic acid/g DW). All three groups differed from each other statistically significant (Figure 18).

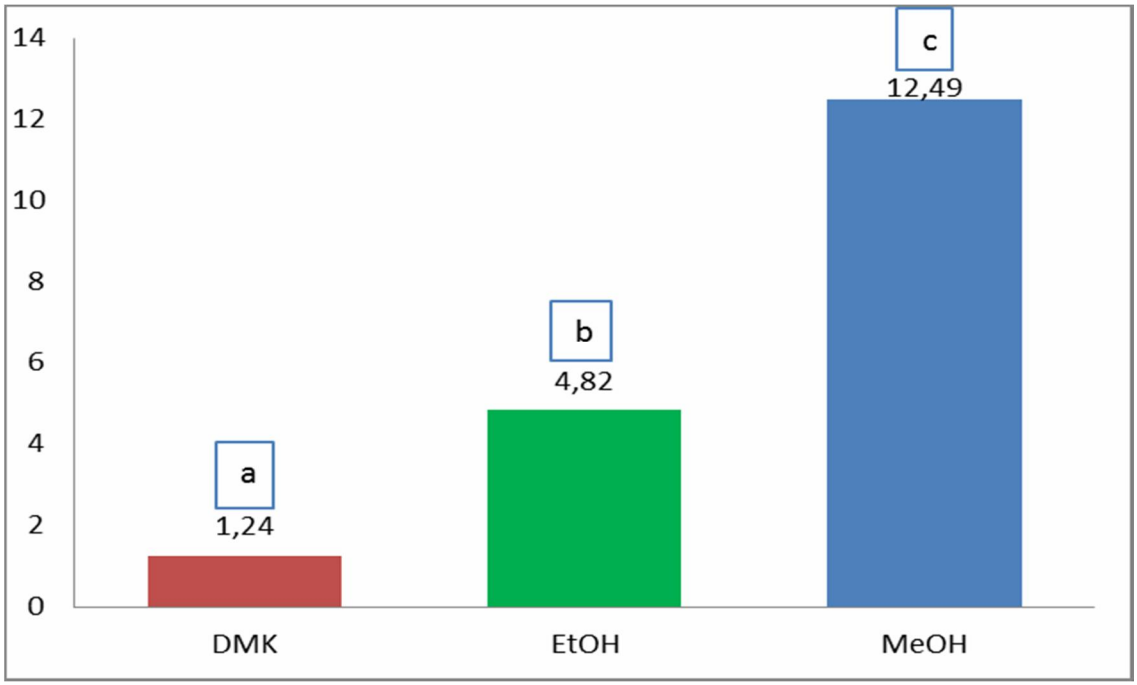

Figure 18: Total Phenolic content subgrouping, three groups were established, namely group a, group b and group c (values in mg caffeic acid/g DW)

3.3.2.2. DPPH

The DPPH results showed that the antioxidant activity in all the various extract solutions differed from each other statistically significant. Subgroups were again formed and the antioxidant activity in the DMK extracts (0.24 mg Trolox/g DW) named group a , in the MeOH extracts (5.98 mg Trolox/g DW) group b and in the EtOH extracts (7.69 mg Trolox/g DW) group c. Figure 19 visualizes this quite nicely.

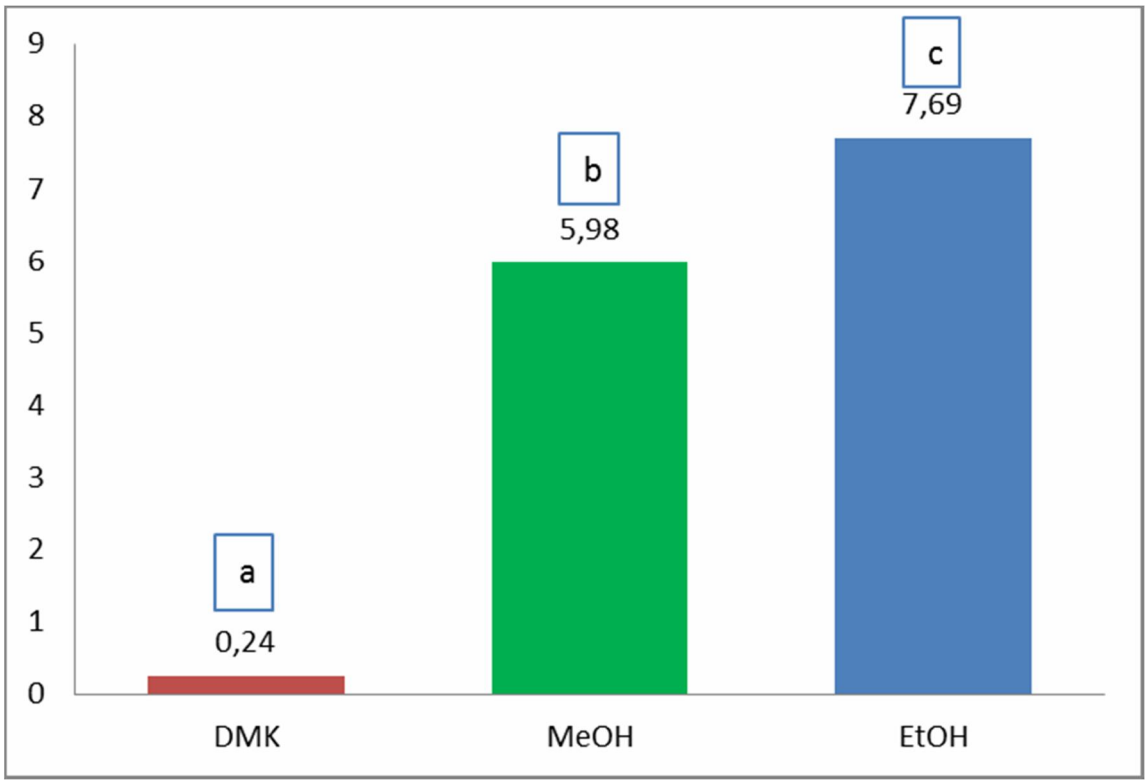

Figure 19: DPPH subgrouping, three groups were established, namely group a, group b and group c (values inmg Trolox/g DW)

3.3.2.3. Ferric reduction (FRAP)

The third method the ferric reduction presented only two groups, namely group a and b statistically differed from one another. The antioxidant activity of the DMK extracts (1.43 mg caffeic acid/g DW) and the EtOH extracts (1.73 mg caffeic acid/g DW) were placed into group a, whereas MeOH extracts (6.94 mg caffeic acid/g DW) were put into group b. This grouping is depicted in Figure 20.

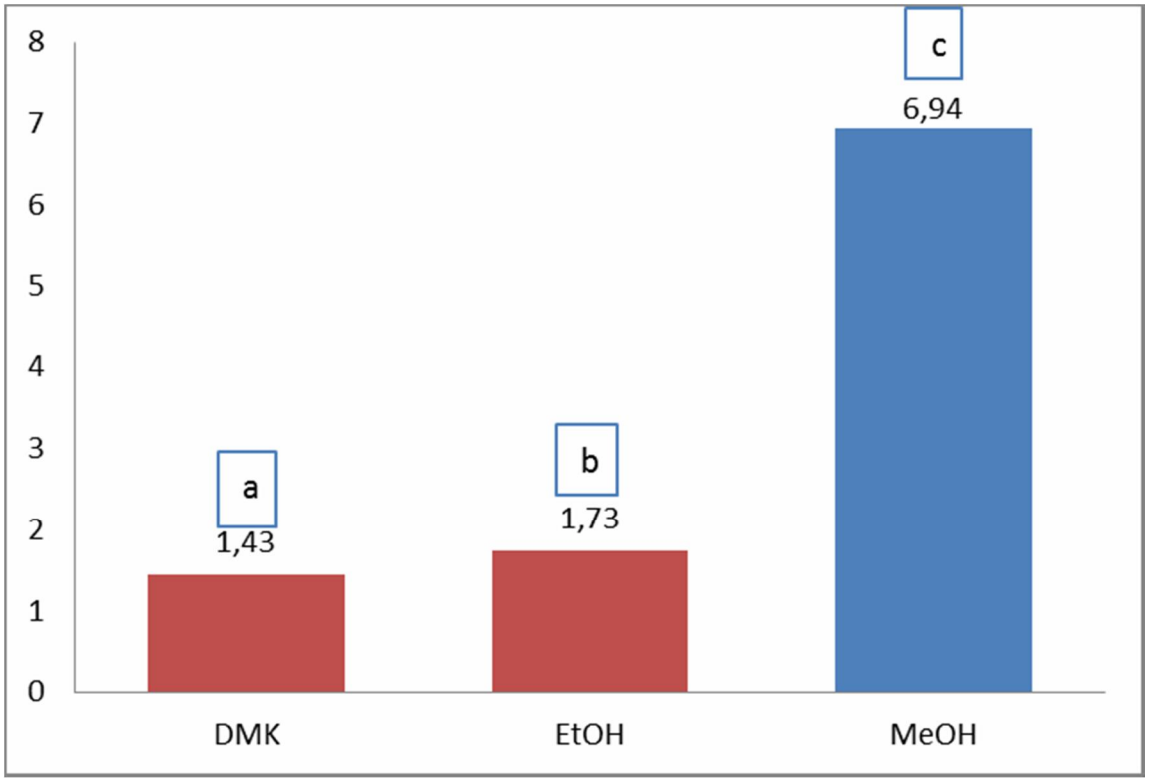

Figure 20: Ferric reduction subgrouping, two groups were established, namely group a and group b (values in mg caffeic acid/g DW)

3.4. Results in terms of comparability amongst the origin of the samples

The origin of the samples is wide spread. There are the market samples from Bobo-Dioulasso and the capital Ouagadougou and there are the reference samples from 4 different regions of the country. Because the few leaf samples were only ever acquired in the capital of Burkina Faso it was not considered in this issue.

3.4.1. HPLC Results

3.4.1.1. Fagaramide

The concentration of fagaramide among the samples was compared in regard to the origin of the sample. The results and the following subgrouping was the same for all five extract solutions. The applied test showed that there were two subgroups a and b. The group a was inhabited by the reference samples and by the market samples from Bobo-Dioulasso. On the other hand the market samples from Bobo-Dioulasso were also put in group b together with the samples from Ouagadougou. This is expressed in Figure 21.

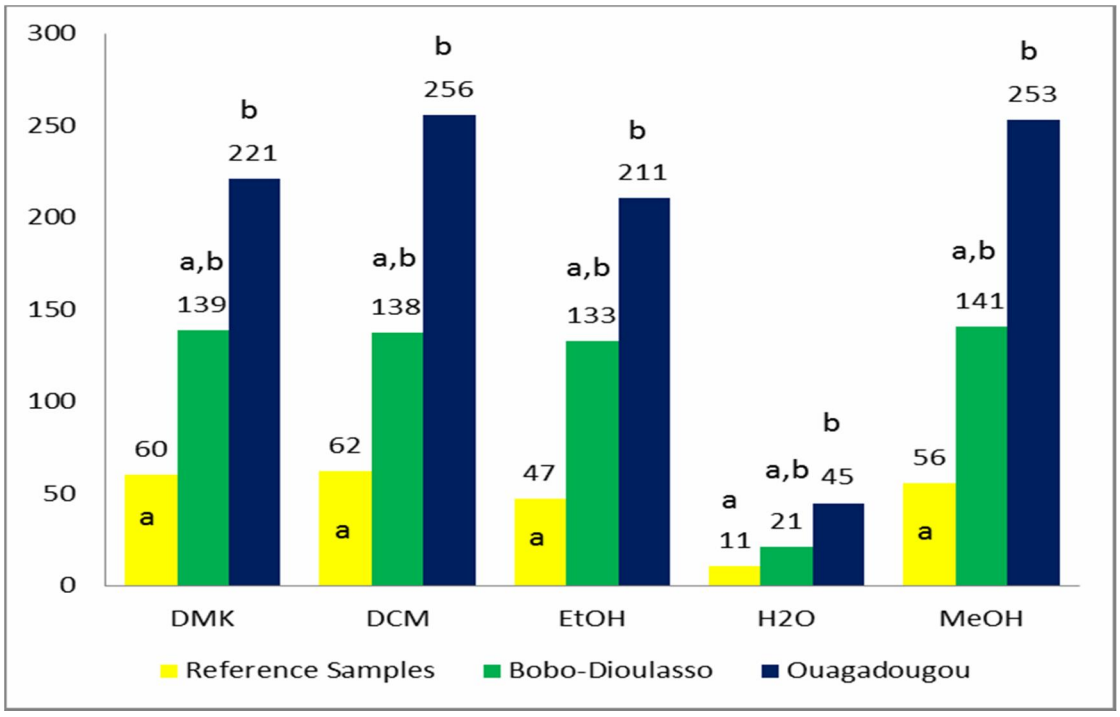

Figure 21: Concentrations of fagaramide regarding the origin of the samples in group a, b or both (µg/ml)

3.4.1.2. Sesamin

The concentration of Sesamin among the samples was compared in regard to the origin of the sample. In case of the DMK extracts the applied statistical test showed that there were two subgroups group a and group b. Group a was only inhabited by the reference samples. The second group was called group b. In this group the market samples from Bobo-Dioulasso and from Ouagadougou were placed. The same phenomenon was observable with the extracts from DCM, eventually giving the same grouping as previously obtained with the DKM samples. The EtOH-samples presented three different subgroups a, b and c.

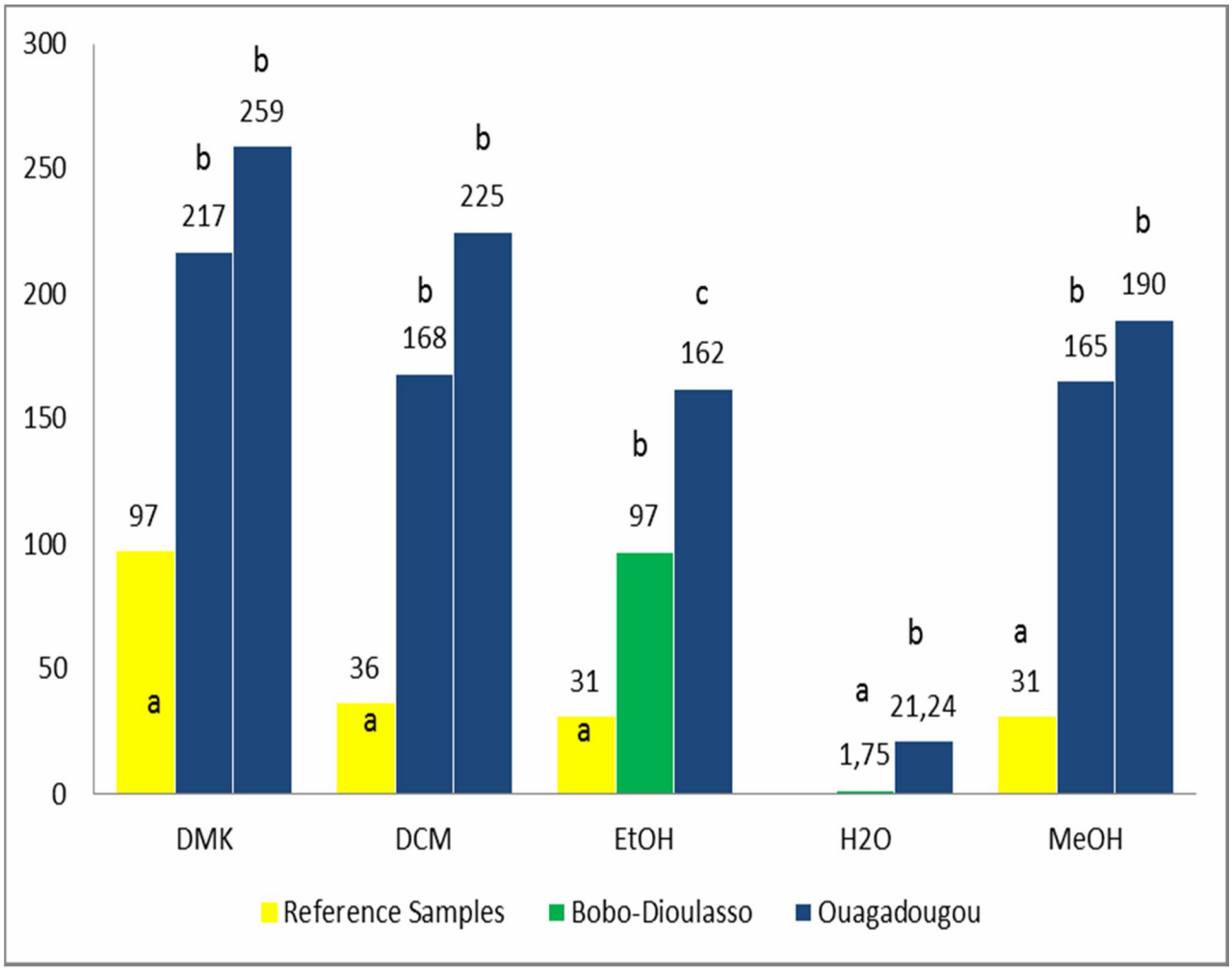

Figure 22: Concentrations of sesamine regarding the origin of the samples in group a, b or c (µg/ml)

The reference samples were put into group a, the samples from Bobo-Dioulasso were placed into group b and the samples from Ouagadougou were set in group c. In the reference samples sesamine could not be detected in the H_2O extracts. Sesamine was found only in the market samplesand the samples were placed into two different subgroups group a and b, due to a Tukey-B-Test. The MeOH extracts presented two different groups. Group a was inhabited only by the reference samples and group b by both of the market samples.

3.4.2. Photometric Analysis

3.4.2.1.Total Phenolic

The results from the Tukey-B test in accordance with the DMK samples show that all three sample origins were placed into the same group (reference samples: 1.33 mg caffeic acid/g DW, Bobo-Dioulasso: 1.34 mg caffeic acid/g DW and Ouagadougou 1.48 mg caffeic acid/g DW). On the other hand the EtOH samples all presented the diverse data which resulted in forming three different subgroups. Group a is composed of the reference samples (1.52 mg caffeic acid/g DW) and of the market samples from Bobo-Dioulasso (2.32 mg caffeic acid/g DW). Although those market samples were also put into group b with the samples from Ouagadougou (3.01 mg caffeic acid/g DW).

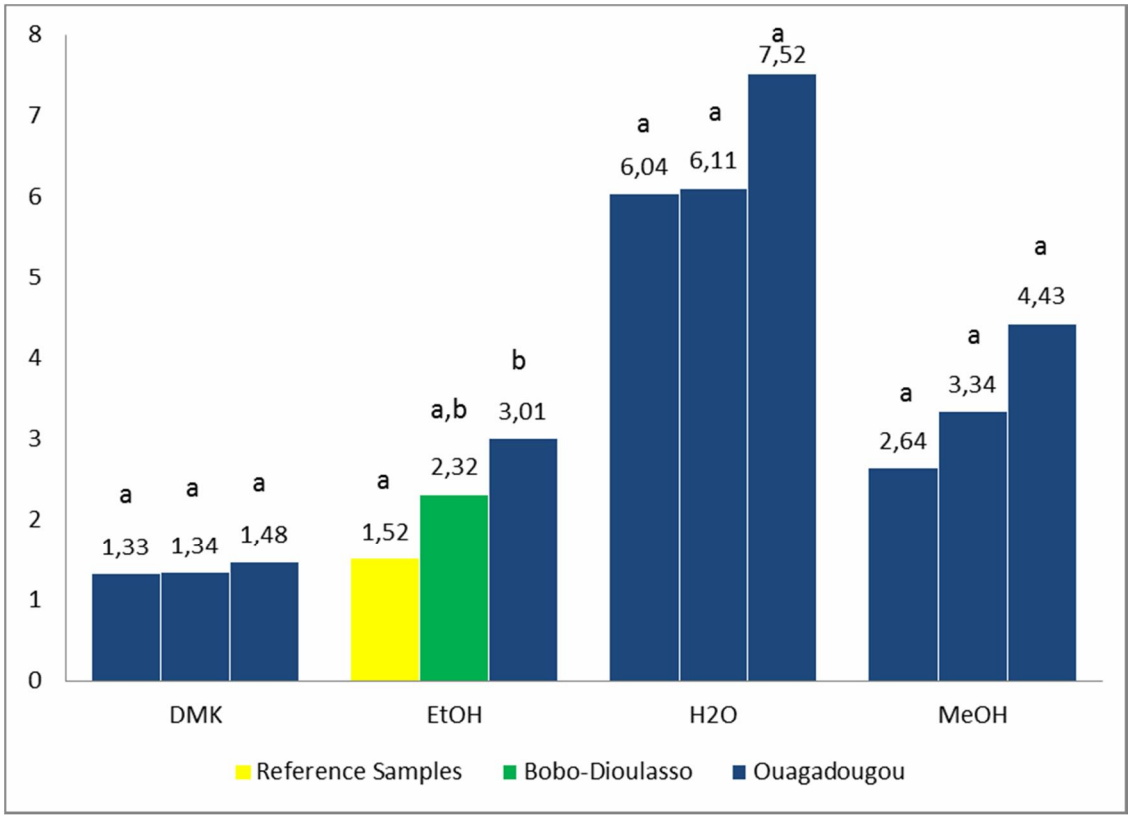

Figure 23: Total phenolics content subgrouping of all four extract solutions regarding the origin of the samples; only within the EtOH samples there were three groups established, namely group a and group b, additionally the market samples from Bobo-Dioulasso were assigned to both subgroups (values in mg caffeic acid/g DW)

The H_2O samples showed that all three origins were put into the same subgroup in accordance to their total phenolics concentration (reference samples: 6.04 mg caffeic acid/g DW, Bobo-Dioulasso: 6.11 mg caffeic acid/g DW and Ouagadougou 7.52 mg caffeic acid/g DW). The MeOH samples presented the same result for as the H_2O samples.

Therefore there is also only one subgroup (reference samples: 2.64 mg caffeic acid/g DW, Bobo-Dioulasso: 3.34 mg caffeic acid/g DW and Ouagadougou 4.43 mg caffeic acid/g DW).

3.4.2.2. DPPH

The results from the Tukey-B test in case of the antioxidant activity measured with DPPH in DMK extracts show that all the market samples from Bobo-Dioulasso and from Ouagadougou were placed into the same group (Bobo-Dioulasso: 1.28 mg Trolox/g DW and Ouagadougou: 1.87 mg Trolox/g DW). This group was named group a. The reference samples (4.76 mg Trolox/g DW) were put in to the singular group b

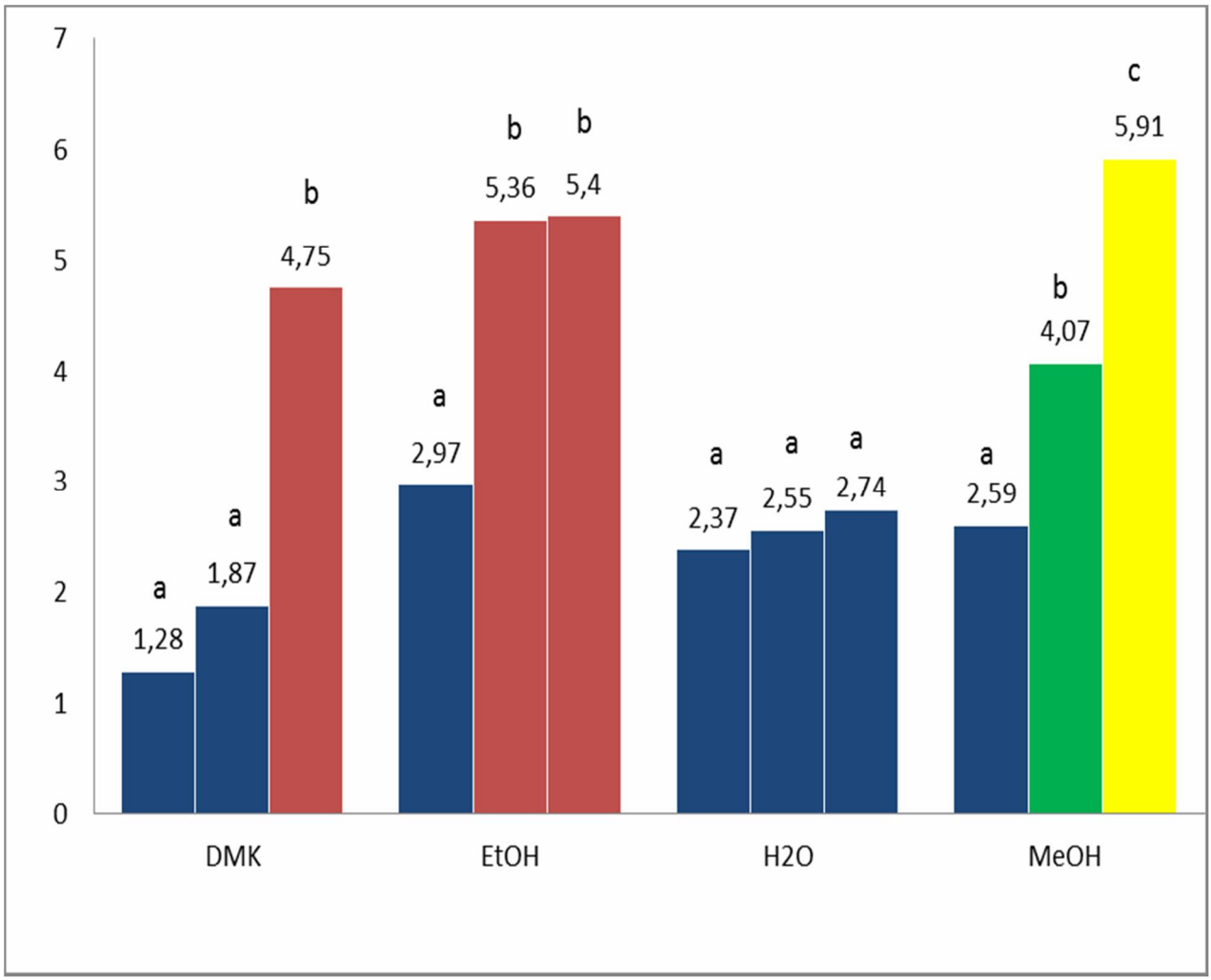

Figure 24: DPPH subgrouping regarding the origin of the samples, the data from DMK and EtOH samples produced two soupbgroups each, whereas the MeOH samples provided three subgroups, namely group a, group b and group c (all values in mg Trolox/g DW)

On the other hand the EtOH samples presented data which resulted in forming two different subgroups. The market samples from Bobo-Dioulasso were put into group a (2.97 Trolox/g DW). Additionally group b is inhabited by the reference samples (5.36 mg Trolox/g DW) and by the market samples from Ouagadougou (5.40 Trolox/g DW). The H_2O samples showed that all three origins were put into the same subgroup in accordance to their total phenolic concentration (reference samples: 2.56 mg Trolox/g DW, Bobo-Dioulasso: 2.74 mg Trolox/g DW and Ouagadougou 2.37 mg Trolox/g DW). In the case of MeOH samples the result presented three different subgroups: group a with the Bobo-Dioulasso samples (2.59 mg Trolox/g DW), group b with the Ouagadougou samples (4.07 mg Trolox/g DW) and group c with the reference samples (5.91 mg Trolox/g DW). This all is observable in Figure 23.

3.4.2.3. FRAP

Regarding the DMK samples in the ferric reducing antioxidative power (FRAP) tests the results from the Tukey-B test present that all three sample origins were placed into the same group (reference samples: 3.16 mg caffeic acid/g DW, Bobo-Dioulasso: 2.65 mg caffeic acid/g DW and Ouagadougou 3.26 mg caffeic acid/g DW). Additionally the same results were observed in case of the H2O samples group (reference samples: 27.45 mg caffeic acid/g DW, Bobo-Dioulasso: 16.10 mg caffeic acid/g DW and Ouagadougou 20.32 mg caffeic acid/g DW).

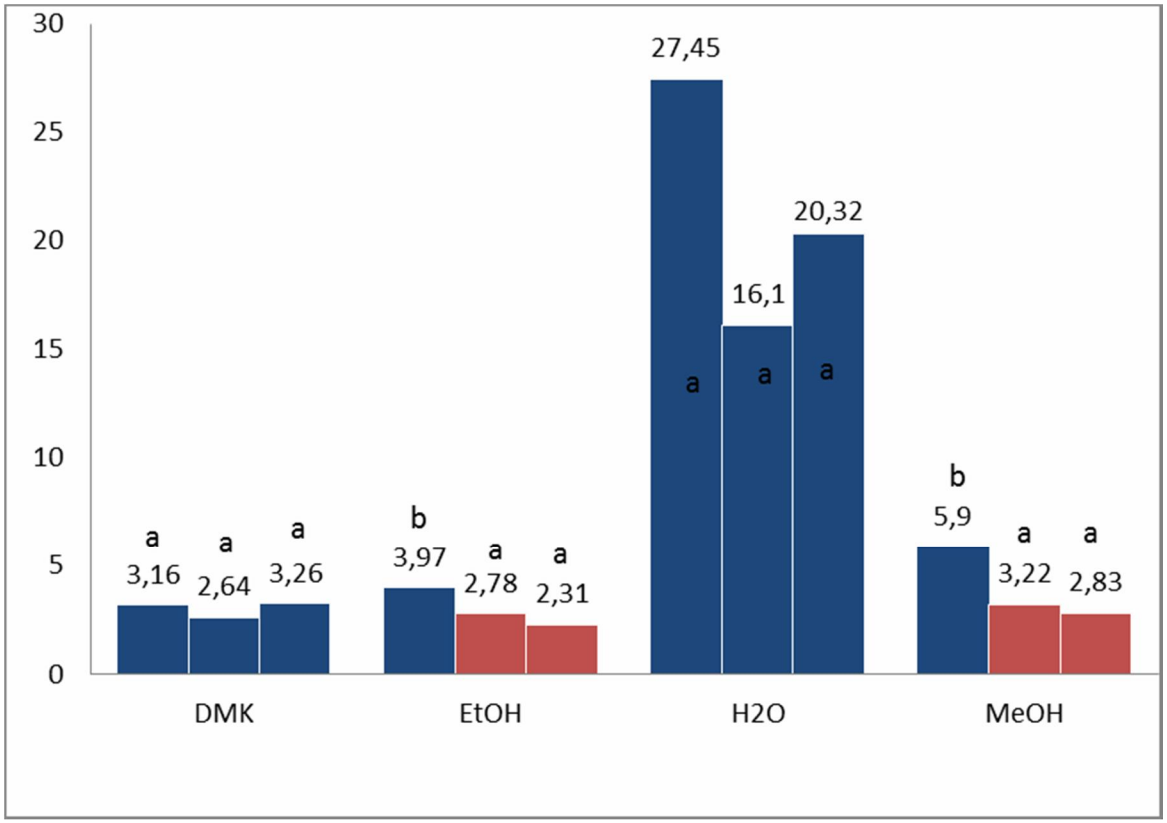

Figure 25: FRAP subgrouping regarding the origin of the samples in group a or b (mg caffeic acid/g DW)

On the other hand the EtOH samples all presented the diverse data which resulted in forming two different subgroups. Group a is inhabited by the market samples from Ouagadougou (2.30 mg caffeic acid/g DW) and by the market samples from Bobo-Dioulasso (2.78 mg caffeic acid/g DW). On the other hand reference samples were put into group b (3.97 mg caffeic acid/g DW).

The MeOH samples presented the data that led to the formation of two subgroups, group a and group b. Here the market samples from Ouagadougou (3.22 mg caffeic acid/g DW) and the market samples from Bobo-Dioulasso (2.83 mg caffeic acid/g DW) were placed into group a. The reference samples were put into group b (5.90 mg caffeic acid/g DW).

4. Discussion

4.1. A first glance

When first looking at results in terms of comparability it might seem quite intricate to evaluate the data coming from the water samples, as their results are so frequently off the charts and produce a staggering overall standard deviation. This might derive from a commonly known difficulty to maintain comparable results from water extractions (Lamien-Meda, personal communication). Nevertheless, in some cases it has proven itself valuable to include a water extraction in the laboratory work.
Another problem that manifested, was the fact that due to the high costs in obtaining the reference compounds – such as lupeol and sanguinarin – the window of opportunities in this matter was quite narrow, because their chemical structure in pure substance is quite delicate to handle. Nevertheless all the necessary "lab-work" was completed in all conscience within 3 months.

4.2. Mobile phase for the TLC ("the running solution dilemma")

Whilst running the first couple of TLCs it became clear, that the used mobile phase was not quite suitable for these plants compounds. The separation in the beginning was not going very well and it soon became clear, that a different solvent mixture would be needed.

With a lot of effort, research and the help provided by Dr. Roland Meda a new mobile phase was found, which separated the various compounds to our satisfaction. The best mobile phase used in this thesis was achieved as follows:

- 30,8 ml Ethyl Acetate
- 5,2 ml MeOH
- 4,0 ml H_20

But due to the many failed attempts in establishing a correct chemical compostion of the

running solution much of the expensive reference compounds had to be sacrificed. Additionally the amount sanguinarine was more limited from the beginning.

4.3. Comparison of Root and leaf samples

The comparison between leaf and root samples among the various extracts presented following results:

- **Methanol extracts:** Total phenolic concentrations did actually present data that indicated a statistical difference between root and leaf samples. The same results were established with the results from the ferric reduction.
- **Ethanol extracts:** The total phenolic results showed a statistical difference between the 2 different type of samples. On the contrary in the case of ferric reduction it seems that there are no statistical differences.
- **Acetone extracts:** In comparison between leaf and root samples there seems to be no statistical difference in the case of total phenolic on the one hand, but there appears to be one regarding the ferric reduction on the other.
- **Extracts from H_20:** Hereby there was again a statistical difference detectable with the total phenolic deliverables as well as the ones from the ferric reduction. .

In case of the DPPH samples the results were quite simple. All of the different extract solutions showed a statistical difference amongst the root and leaf samples.

Overall it seems that methanol might be the most useful solvent for extracting the various compounds, in particular phenolic substances, in a satisfying way and enriching them. It is not amazing, that those substances once enriched in polar solvents like MeOH furthermore achieve better results in tests for antioxidant activity such as DPPH and FRAP than extracts of more apolar solvents. This was shown quite nicely in ferric reduction antioxidant power test: even though the samples are in statistically significant different groups they appear to maintain a steady, but still quite high level of mean values in recognition to their TROLOX

concentration. Against this no other extract solution was able to perform as well as this particular solution.

4.4.Compounds in terms of origin

4.4.1. Photometric Analysis

As shown above the cohorting proved itself to be quite successful and show an overall distinction between the samples obtained in the west of Burkina Faso around the city of Bobo-Dioulasso and the ones that were procured in the capital of the country in Ougadougou. Furthermore there was also a difference in the quantity of the compounds either bought or collected. The quality of the product from *Zanthoxylum* appears to be higher and more beneficial when bought on the market and even more when acquired on one in the capital of the country.

This phenomenon is quite interesting as most of the plants are home and harvested in the western regions of Burkina Faso around the city of Bobo-Dioulasso. This is also the origin of all the field samples used in this project. One can definitely assume that the long hours of transportation to the capital markets and the probably poorly manner of conserving the product would result in some sort of impairment on the product. Nevertheless this is not the case. On possibility is that the markets import a certain amount of plants from other countries in West Africa. That could give an explanation to these circumstances. Whether or not this hypothesis is accurate or false cannot be ascertained.

In the case of the total phenolics concentration it was established that the highest amount can be found in market samples. Beneficial to the quantity is the usage of an alcoholic solution, such as ethanol or methanol, though methanol seems to exceed the prior in this matter with an overall average caffeic acid of 10.4 mg/g in an Ouagadougou market sample. This surpasses all the other mean values up to over three times. Furthermore the methanol samples are also more successful in maintaining a high concentration in light of antioxidant activity. This is documented in their performance in the methods ferric reduction and DPPH. In both cases the methanol extract obtained the highest levels of TROLOX concentration. Interestingly enough the field samples were also able to perform very well within these methods. This concludes a contradictory opinion that market samples might not always have the edge over the field samples in terms of compound

quantity. Evidently in the case of DPPH methanol and ethanol competed admirably for the best quantity. Recapitulatory surprisingly ethanol was in the ascendancy in the end.

4.4.2. Chromatographic Analysis

As established the occurring problems with the mobile phase maintained a problem throughout the chromatographic analysis. Especially sanguinarine presented itself difficult to detect. Even though it is mentioned and well established that sanguinarine is a known compound of Zanthoxylum it could not be found in the samples. There was evidently a certain pattern in the samples that could maybe be this sought out compound because it presented a similar color. Nevertheless the pattern presented from the reference compound deviated too extensively. On the one hand sanguinarine could be overlapped by sesamine in that specific TLC system, interacted with some property of the experiment or on the other hand did not anymore have its original chemical integrity and construction.
For caffeic acid hexane/ethyl acetate (seven+ three) was not the appropriate mobile phase which can be seen at the starting point of the TLC plate where a great amount of the substance remained.

Vanillic acid could not be detected in the Zanthoxylum z. samples by means of TLC. Maybe this compound was under the detection limit or overlayed by other compounds. However, three substances that are known compounds of this plant were actually detected by means of TLC. Lupeol, fagaramide and sesamine were almost always identifiable on the sprayed plates. According to the brightness of color and the thickness of the bar patterns numbers were assigned to the samples. Water solutions proofed themselves to be inadequate to be appropriated for this method, especially for the lupeol. Amongst the three fagaramide had the highest concentration and achieved the highest score the most often.

The quantification of fagaramide and sesamine proofed to be very effective in the HPLC. Fagaramide values were highest in the market samples from the capital of Burkina Faso. To emphasize the quality of market products sesamine concentrations were also highest in the market samples from this region. Unfortunately lupeol, though definitely very prominent in the TLC was not detectable in the HPLC under the described conditions, even

though a thorough search in the results from the HPLC was performed, to maybe after all unkennel lupeol.

Many valuable phytochemical data about Zanthoxylum z. were collected in this thesis. Over all according to the data presented in this project it can surely be maintained that the market samples in combination with the location Ouagadougou and an alcoholic extract solution (preferably MeOH) is the most beneficial to achieve highest concentration of the phytochemical compounds used in this study.

5. Abstracts

5.1. In English

To this day Malaria is still a very common threat, especially in West Africa and there in Burkina Faso. Fighting this disease has been the center of attention in many traditional concoctions made to treat this disease. Especially among native tribes the usage of the surrounding flora, such as the plant Zanthoxylum zanthoxyloides, has been well established and was successful. The research focused mainly on the developing and investigating the various methods to safely and easily determine this particular plant. Two different plant organs namely leaf and root were acquired from herbal markets in the capital Ouaguadougou and the city of Bobo-Dioulasso. The references samples were harvested and collected in four different regions in the west of Burkina Faso. Overall there were 25 different samples. Solutions were made with MeOH, EtOH, DMK, DCM and H2O as extract solvents. Fagaramide and sesamin were perfectly detectable in the chromatographic methods (TLC and HPLC), whereas lupeol was only detectable in the TLC. The quantification of fagaramide and sesamin in the HPLC did present some data that varied depending on the origin of the product and the extract solution used.

Additionally methods to determine the antioxidant activity were also performed such as total phenolics content, ferric reduction and DPPH. The results were statistically analyzed, which led to a homogeneous cohorting between the various extraction solvents to determine comparability Thereinafter the samples were also analyzed and compared regarding their origin. The thoroughly promising results showed that MeOH functions as a very considerable extract solvent for quantifying fagaramide and sesamine in an extract solution. On the other hand H_2O did not present itself reliable when it comes to procuring essential compounds for treating malaria.
The significance of creating a reliable method is incredibly important to ensure product quality. In case of Zanthoxylum zanthoxyloides was merely a first step.

5.2. In German

Bis zum heutigen Tag ist Malaria eine ständige Gefahr in vielen Ländern in West Afrika, wie zum Beispiel in Burkina Faso. Der Kampf gegen diese Krankheit stand bei vielen traditionellen Naturheilern eingeborener Stämme schon lange im Mittelpunkt des Interesses. Zur Anwendung kamen hierbei auch viele Pflanzen der lokalen Flora. Eine dieser Pflanzen ist Zanthoxylum zanthoxyloides, die auch im Fokus dieser Arbeit stand. Primär wurde Wert darauf gelegt Systeme und Methoden zu finden, die die Möglichkeit einer Identitäts-, Reinheits- und Qualitätsprüfung bieten, um sicher zu gehen, dass man tatsächlich diese Pflanze vor sich hat und es zu keiner Verfälschung kommen kann. Auf Märkten in der Hauptstadt von Burkina Faso, Ouaguadougou, und in der Stadt Bobo-Dioulasso wurden sowohlWurzel- als auch Blattproben dieser Pflanze gekauft. Desweiteren wurden Referenzproben in vier verschiedenen Regionen im Westen des Landes gesammelt. Aus den Proben wurden MeOH-, EtOH-, DMK-, DCM- und H2O-Extrakte hergestellt. Die in der Dünnschichtchromatographie (DC) gefundenen Substanzen Fagaramid und Sesamin wurden anschließend in der HPLC quantifiziert und deren Werte nach Herkunft der Droge und Extraktionsmittel verglichen, um Unterschiede zu eruieren. Das in der DC gefundene Lupeol konnte in der HPLC unter den für Fagaramid und Sesamin geeigneten Bedingungen nicht sicher detektiert werden.

Zusätzlich zu den chromatographischen Methoden wurden photometrische Methoden durchgeführt, um das antioxidative Potential zu untersuchen. Hierbei wurden die MethodenGesamtphenolgehalt, Eisen III reduktion und DPPH verwendet. Die Ergebnisse der Proben wurden statistisch ausgewertet und nach Fähigkeit der Extraktionsmittel und Herkunft der Proben gruppiert. Die durchaus erfreulichen Ergebnisse zeigten, dass Methanol als hervorragendes Extraktionsmittel fungiert, vor allem wenn es darum geht die Substanzen Fagaramid and Sesamin aus der Zanthoxylum Wurzel zu qunatifizieren. Eine reine H_2O Lösung wird jedoch nicht sehr erfolgreich sein können, wenn es sich um eine Malariatherapie handelt. Das Extraktionsvermögen ist unzureichend und die Löslichkeit der Wurzel und ihrer Komponenten in H_2O ist einfach zu gering.

Es ist von größter Wichtigkeit und Dringlichkeit, dass man sichere und möglichst simple Methoden zur Verifizierung von Pflanzenmaterial dieser Art etabliert. Diese Arbeit leistet hierbei einen kleinen Beitrag..

6. Appendix

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Figure 3 – 6: Pictures made by the project team (APPEAR 2014)

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Figure 10: OUATTARAA B, ANGENOTA L, GUISSOUB P, FONDUC P, DUBOISD J, FRÉDÉRICHA M, JANSENA O, HEUGENE J, WAUTERSA J, TITSA M. 2004. LC/MS/NMR Analysis of isomeric divanilloylquinic acids from the root bark of Fagara zanthoxyloides Lam. Photochemistry Volume 65 Issue 8: pages 1145–1151

Figure 11 – 24: Created by the author

All tables were created by the author.

6.3. Selected results of the photometric analysis

The results from the photometric analysis show a wide range of results, especially in the various solutions.

All tables were created by the author.

Appendix Table 1: Total Phenolics complete data

Sample		TP(mg caffeic acid/g DW)			
		MEOH	ETOH	Aceton	H2O
1	EM_Oua_114	4,64	3,16	1,63	8,32
2	EM_Bbo_196	2,68	2,50	1,28	5,49
3	EM_Oua_118	2,17	1,48	1,20	5,08
4	EM_Oua_120	5,24	3,25	1,42	6,70
5	EM_Oua_122	64,29	5,08	2,00	11,28
6	EM_Oua_124	3,28	2,68	1,15	5,69
7	EM_Oua_126	3,08	1,88	1,59	6,50
8	EM_Oua_128	12,02	4,71	1,73	12,02
9	EM_Oua_130	3,98	3,29	1,87	4,88
10	EM_Oua_132	2,77	2,08	1,01	9,26
11	EM_Bbo_183	3,77	1,29	1,13	5,15
12	EM_Bbo_185	2,06	1,73	1,67	5,89
13	EM_Bbo_186	2,52	1,73	1,13	7,04
14	EM_Bbo_189	13,17	5,01	1,32	No Result
15	EM_Bbo_190	11,82	4,64	1,18	No Result
16	EM_Bbo_193	2,13	1,30	1,28	6,36
17	EM_Bbo_194	2,72	1,56	1,46	5,76
A	RH_ZXR_1	3,16	1,65	1,00	5,82
B	RH_ZXR_2	4,66	2,89	1,66	7,71
C	RH_ZXR_3	3,95	2,01	1,36	7,64
D	RH_ZXR_4	3,96	2,14	1,00	7,17
E	RS_ZXR_1	3,71	2,04	1,33	7,37
F	RC_ZXR_1	1,75	2,83	0,94	6,50
G	RC_ZXR_2	3,23	2,37	1,67	1,85
H	RC_ZXR_3	2,29	2,67	1,77	4,81

Appendix Table 2: Antioxidant Activity (DPPH) complete data

Sample		DPPH (mg trolox/g DW)			
		MEOH	ETOH	Aceton	H2O
1	EM_Oua_114	4,77	5,91	1,62	1,93
2	EM_Bbo_196	2,83	4,06	1,19	2,11
3	EM_Oua_118	2,56	3,23	1,44	0,16
4	EM_Oua_120	4,69	5,25	1,95	4,10
5	EM_Oua_122	6,49	8,15	2,82	5,32
6	EM_Oua_124	3,16	5,75	1,59	2,36
7	EM_Oua_126	3,21	4,92	1,99	3,08
8	EM_Oua_128	5,91	7,16	2,35	1,58
9	EM_Oua_130	4,36	6,02	2,45	0,66
10	EM_Oua_132	2,72	3,57	1,33	2,45
11	EM_Bbo_183	3,63	3,07	1,81	3,89
12	EM_Bbo_185	1,97	2,76	1,36	2,08
13	EM_Bbo_186	2,04	2,85	1,43	1,84
14	EM_Bbo_189	5,91	7,57	0,01	No Result
15	EM_Bbo_190	6,06	7,82	0,47	No Result
16	EM_Bbo_193	2,77	3,02	0,78	3,17
17	EM_Bbo_194	2,53	3,17	1,01	2,73
A	RH_ZXR_1	5,29	1,22	5,12	2,90
B	RH_ZXR_2	6,93	6,97	1,81	2,69
C	RH_ZXR_3	6,83	6,94	7,19	2,23
D	RH_ZXR_4	6,87	7,32	6,15	0,56
E	RS_ZXR_1	6,23	6,00	5,52	3,02
F	RC_ZXR_1	4,36	2,43	4,92	3,65
G	RC_ZXR_2	5,43	5,84	5,94	2,01
H	RC_ZXR_3	5,37	6,15	1,42	3,40

Appendix Table 3: Fe3+ Reduction (Trolox) complete Data

Sample		FRAP (mg Trolox/g DW)			
		MEOH	ETOH	Aceton	H2O
1	EM_Oua_114	2,96	0,89	3,48	14,41
2	EM_Bbo_196	4,57	4,45	2,97	4,18
3	EM_Oua_118	3,50	2,49	2,64	1,74
4	EM_Oua_120	3,17	1,16	3,31	23,37
5	EM_Oua_122	4,43	1,74	4,42	35,78
6	EM_Oua_124	1,93	3,40	3,30	21,27
7	EM_Oua_126	1,85	2,83	3,62	21,47
8	EM_Oua_128	3,27	1,86	3,87	37,29
9	EM_Oua_130	2,45	1,27	2,47	21,52
10	EM_Oua_132	4,12	2,97	2,47	22,18
11	EM_Bbo_183	2,51	2,47	3,38	18,90
12	EM_Bbo_185	3,58	3,09	3,00	16,14
13	EM_Bbo_186	2,98	2,63	2,41	13,56
14	EM_Bbo_189	7,02	2,35	1,31	No Result
15	EM_Bbo_190	6,85	1,12	1,56	No Result
16	EM_Bbo_193	3,54	2,56	2,25	18,04
17	EM_Bbo_194	1,56	3,16	2,19	13,87
A	RH_ZXR_1	5,30	3,12	2,36	25,36
B	RH_ZXR_2	8,84	4,79	4,18	38,77
C	RH_ZXR_3	7,16	4,13	3,49	31,22
D	RH_ZXR_4	7,75	4,68	3,56	32,01
E	RS_ZXR_1	6,42	4,11	3,17	28,41
F	RC_ZXR_1	2,80	4,13	2,39	28,15
G	RC_ZXR_2	4,54	3,46	3,26	17,02
H	RC_ZXR_3	4,38	3,36	2,92	18,67

6.4. HPLC data:

Appendix Table 4: Fagaramide concentrations from market samples (1-17) and reference samples (A-H) (values in µg/ml)

	MeOH	EtOH	Aceton	DCM	H20
1	82.46	68.29	79.43	89.16	6.99
2	125.88	68.29	126.45	189.05	23.28
3	53.32	33.41	43.48	48.04	4.96
4	47.07	47.93	45.15	58.52	12.76
5	271.25	62.93	227.61	270.71	50.24
6	382.80	347.93	312.35	288.49	96.40
7	345.46	293.08	362.50	439.32	72.72
8	388.29	379.76	307.89	377.54	69.33
9	427.58	426.89	334.08	415.61	70.11
10	415.70	390.55	377.02	384.59	51.31
11	93.74	54.44	97.36	84.63	10.83
12	173.89	205.27	199.50	210.38	26.23
13	168.82	149.70	133.34	128.53	26.86
14	No Results	No Results	No Results	No Results	No Results
15	No Results	No Results	No Results	No Results	No Results
16	226.07	210.62	222.81	223.32	32.02
17	45.76	45.50	43.55	43.17	9.57
A	27.59	22.38	17.31	23.18	2.51
B	98.54	84.60	123.03	114.67	13.70
C	46.93	31.03	59.27	63.36	11.62
D	44.67	35.29	35.66	39.58	10.22
E	112.84	76.31	100.01	111.41	18.59
F	16.03	55.97	23.41	60.80	16.19
G	78.06	46.79	64.50	58.63	9.94
H	28.51	24.94	62.53	27.04	5.51

Appendix Table 4: Sesamin concentrations from market samples (1-17) and reference samples (A-H) (values in µg/ml)

	Me OH	EtOH	Aceton	DCM	H20
1	218.44	203.83	341.66	278.98	34.57
2	113.71	103.11	145.75	165.55	4.25
3	98.73	70.49	146.54	No Results	No Results
4	136.09	155.18	160.95	160.11	155.18
5	192.16	157.60	219.95	202.12	6.72
6	175.10	168.79	197.64	157.68	No Results
7	211.36	205.01	371.76	305.71	6.11
8	389.11	243.07	212.83	240.22	5.66
9	144.28	110.40	148.19	No Results	No Results
10	230.52	209.45	224.91	169.75	No Results
11	132.51	74.58	170.63	123.17	No Results
12	276.13	No Results	364.66	354.82	4.53
13	No Results	No Results	288.96	249.95	4.23
14	No Results	No Results	No Results	No Results	No Results
15	No Results	No Results	No Results	No Results	No Results
16	172.92	172.37	173.69	178.15	No Results
17	245.62	238.52	299.46	223.30	No Results
A	21.82	26.06	85.30	33.17	No Results
B	44.47	48.34	167.00	53.89	No Results
C	33.56	25.96	122.67	47.99	No Results
D	42.80	82.08	94.51	37.92	No Results
E	38.65	15.06	90.63	31.21	No Results
F	7.11	23.76	51.90	39.21	No Results
G	19.93	0.00	21.53	6.81	No Results
H	45.14	32.36	46.83	41.33	No Results

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