Faculty of Resource Science and Technology

Antimicrobial Properties of *Elephantopus Scaber* Against Crop Pathogenic Fungi

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Declaration

I declare that no portion of this research work had been submitted to support the application of other degree or qualification at any other universities or institutions of higher learning.

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Antimicrobial Properties of *Elephantopus scaber* Against Crop Pathogenic Fungi

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ABSTRACT

Harmful effect of synthetic fungicides towards environment and other organisms have become important issues nowadays. Research on the plant product to be developed as the alternative for synthetic fungicides has attracted interest of many scientists. *Elephantopus scaber* previously was reported to contain antimicrobial properties. The aim of this study is to determine the effect of antimicrobial properties of *Elephantopus scaber* against six crop pathogenic fungi viz., *Glomerella cingulata*, *Fusarium oxysporum, F. solani*, *Pestalotiopsis* sp, *Colletotrichum* sp and *Pyricularia oryzae*. 80% of methanols were used as solvent to extract the phytochemical content of *E. scaber*. Different concentrations of the methanol crude extracts were used in fungal growth inhibition test. Result showed methanol crude extract at 15% concentration retard the mycelia growth on four crop pathogenic fungi, *P. oryzae*, *F. solani, G. cingulata* and *Colletotrichum* sp. *F. oxysporum* and *Pestalotiopsis* sp. did not showed growth retardation at any concentration used.

Keywords: *Elephantopus scaber*, synthetic fungicides, crop pathogenic fungi, retardation

ABSTRAK

Hasil penggunaan fungisid sintetik telah memberikan kesan buruk terhadap persekitaran dan juga hidupan yang lain. Penyelidikan produk berasaskan tumbuhan untuk dijadikan sebagai alternatif penggunaan fungisid sintetik telah menarik perhatian ramai saintis. Tumbuhan Elephantopus scaber dilaporkan mempunyai ciri-ciri antimikrobia. Tujuan kajian ini dijalankan adalah untuk menentukan kesan ciri-ciri antimikrobia E. scaber terhadap enam kulat patogen tanaman dikenali sebagai Glomerella cingulata, Fusarium oxysporum, F. solani, Pestalotiopsis sp, Colletotrichum sp dan Pyricularia oryzae. 80% metanol digunakan sebagai pelarut untuk mengekstrak kandungan fitokimia E. scaber. Kepekatan ekstrak metanol yang berbeza digunakan didalam pertumbuhan ujian perencatan. Hasil menunjukkan kepekatan ekstrak metanol pada 15% dapat merencatkan pertumbuhan miselia terhadap empat kulat patogen tanaman iaitu P. oryzae, F. solani, G. cingulata dan Colletotrichum sp. Manakala, F. oxysporum dan Pestalotiopsis sp. tidak menunjukkan sebarang perencatan tumbesaran bagi setiap kepekatan yang digunakan.

Kata kunci: Elephantopus scaber, fungisid sintetik, kulat patogen tanaman, perencatan

1.0 Introduction

Losses in crop production field due to plant diseases have become one of the most important issues nowadays. Plant disease can be defined as a harmful alteration of the normal physiological and biological development of a plant. Plants diseases predominantly caused by infection of plant pathogens such as fungi, bacteria, viruses, nematodes and parasitic plants. Any parts of the pathogen that can cause diseases is known as inoculum and the example of it includes fungal spores, bacterial cells, virus particles, or nematode eggs (Karasevicz, 1995). Common infectious agents in plants are pathogenic fungi that causing alterations during developmental stages including post-harvest since they are the most widespread and destructive parasites of plants (Alrajhi, 2013).

The uses of antifungal control agents such as synthetic fungicides are required to reduce the damage to crops (El-Khateeb *et al*., 2013). Synthetic fungicides have been used for over 200 years to protect plants against diseases attack by pathogenic fungi (Brent & Hollomon, 2007). The effective usage of synthetic fungicides leads to a series of problems. Fungi have developed resistance or tolerance to the synthetic fungicides in the area where these synthetic fungicides are applied heavily. Higher concentrations of synthetic fungicides had been used to overcome the fungi which have gained resistance or tolerance against the chemicals. Increased usage of the chemical subsequently increases the risks of high-level of toxic residue in the products that are harmful to human and environment (Shrestha & Tiwari, 2009). The mentioned problems with the usage of synthetic fungicides have led to the search of the new natural compounds that are safe for human and environmentally friendly (El-Khateeb *et al*., 2013).

Plants produce bioactive molecules or phytochemicals which will evolve as chemical for defence against predation or infection (Javale & Sabnis, 2010). These bioactive molecules also can be known as secondary metabolites which possess insecticidal, antifungal, antibacterial and cytotoxic activities (Rhouma *et al*., 2009). An example of bioactive molecules are phenols, flavonoids, quinons, tannins, alkaloids, saponins, and sterols which are actively against fungal and bacteria pathogen. These molecules are biodegradable to non-toxic product and safer (Soylu, Soylu & Kurt, 2006), also potential as alternative to replace synthetic fungicides. They have minimal environmental impact and harmless to the consumer (Satish, Mohana, Raghavendra & Raveesha, 2007).

Elephantopus scaber is a medicinal plant which is known for its medicinal properties and had been reported to possess phytochemical compounds with antimicrobial activity (Kamalakannan *et al*., 2012). *E. scaber* is a species of flowering plant in the Asteraceae family (Kabiru, 2013). It is distributed in Malaysia, Southeast Asia, mainland China, Japan and Indo-China. *E. scaber* is also known as tutup bumi (Malaysia), elephant's foot (English), and rickly-leaves elephant's foot (Chinese) (Ali, Samah, Mustapha & Hussein, 2010).

This study aims to evaluate the crude extracts from *E. scaber* in inhibiting the growth of a selection of crop pathogenic fungi.

1.1 Problem Statement

i. Synthetic fungicide increases the resistance pathogenic fungi, harmful to the environment, consumer and is costly.
ii. Limited studies have been done on the antimicrobial properties of *Elephantopus scaber* against crop pathogenic fungi.

1.2 Objectives

i. To obtain the methanol crude extracts of *Elephantopus scaber*.
ii. To test the effect of methanol crude extracts at different concentration on the growth of crop pathogenic fungi.

2.0 Literature review

2.1 Biofungicides

Hazardous effect due to the usage of synthetic fungicides leads to the finding of alternative based on natural product from plant to control diseases. There are specific chemical compounds in plant that involved in defence against pathogen attack. Examples of the antimicrobial compounds are alkaloids, steroids, tannins, and phenolic compounds (Ciocan & Bara, 2007). When these antimicrobial compounds had been isolated from the plant extract, it is potentially to be developed into biological fungicides or biofungicides (Dubey, 2011). Biofungicides are composed of beneficial microorganisms and naturally occurring substances that control plant pathogens and the diseases they cause (Thomas, 2009; Francis & Keinath, 2010).

The advantages of using biofungicides are biodegradable in nature, no effect on non-target species, much cheaper than synthetic fungicides, and less toxic (Dhiraj, Shiv, Aanand & Dayaram, 2014). One of commercial biofungicides is known as Timorex Gold^{TM}. This biofungicides is form based on natural product of *Melaleuca alternifolia* and it is used to control fungal diseases in edible crops, such as powdery mildews, downy mildews, early blight, late blight and black sigatoka (Reuveni, 2010).

2.2 *Elephantopus scaber*

2.2.1 Chemical constituents

Various kinds of solvents had been used before to isolate the chemical constituents in the *Elephantopus scaber* such as aqueous, methanol, ethanol, and acetone. Main chemical constituents in *E. scaber* include sesquiterpene lactones, phenolic acids, flavonoids, triterpenoids, steroids, essential oil, salt and minerals (Table 1).

Table 1: Chemical constituents of *Elephantopus scaber*

Chemical constituents	Isolated compounds	Reference
Sesquiterpene lactones	Isodeoxy-elephantopin isoscabertopin Scabertopin Deoxyelephantopin 17, 19-dihydrodeoxyelephantopin Iso-17, 19-dihydrodeoxyelephantopin	Taizong et al., 2014; Ahmad, Alkarkhi, Hena, & Lim, 2009
Phenolic acids and Flavonoids	3,4-dihydroxy benzaldehyde p-coumaric acid vanillic acid p-hydroxybenzoic acid Luteolin Luteolin-4'-O-β-D-glucoside Trans-p-coumaric acid Methyl trans-caffeate	Farha & Remani, 2014
Triterpenoids Steroids Salt	Epifriedelanol Lupeol Stigmasterol Potassium chloride	Ali, Samah, Mustapha & Hussein, 2010
Minerals	Calcium Magnesium Iron Zinc	Farha & Prathapan, 2014
Essential oil	Hexadecanoic acid Isopropyl dimethyl tetrahydronaphthalenol β-sesquiphellandrene, octadecadienoic Phytol	Li, Shuguang, Peng & Yang, 2004

2.2.2 Medicinal value of *Elephantopus scaber*

Elephantopus scaber is a medicinal plant which is used a lot in traditional medicine. In Malaysia, the root decoction of *E. scaber* is used to accelerate contraction of abdominal area and prevent inflammation after childbirth. The whole parts of this plant also get boiled with red bean to remove flatulence. Other countries such as in Taiwan use this species to treat hepatitis and in Mauritius use to treat diarrhea, urinary problems and pimples (Wan *et al*., 2009).

Three products in Chinese traditional medicine were formulated using natural product from *E. scaber* as the important active ingredient. The products namely Teng-Khia-U, Yi-Gan-Yin and Dang Gui Lu Hui (Wan *et al*., 2009). The paste is useful for skin diseases, and children suffering fever. Crushed root can be given to patients with heart and liver trouble, the same remedy also can be applied to cattle wounds (Jenny *et al*., 2012). In the Middle America, this plant is one of the most popular cough remedies. This medicinal plant is also used for diuresis, antipyresis and to eliminate bladder stones. *E. scaber* contain hydroxylated germacranolides molephantin and molephantinin that possess cytotoxic and antitumor properties (Mohan, Chenthurpandy & Kalidass, 2010).

2.2.3 Antimicrobial properties report previously

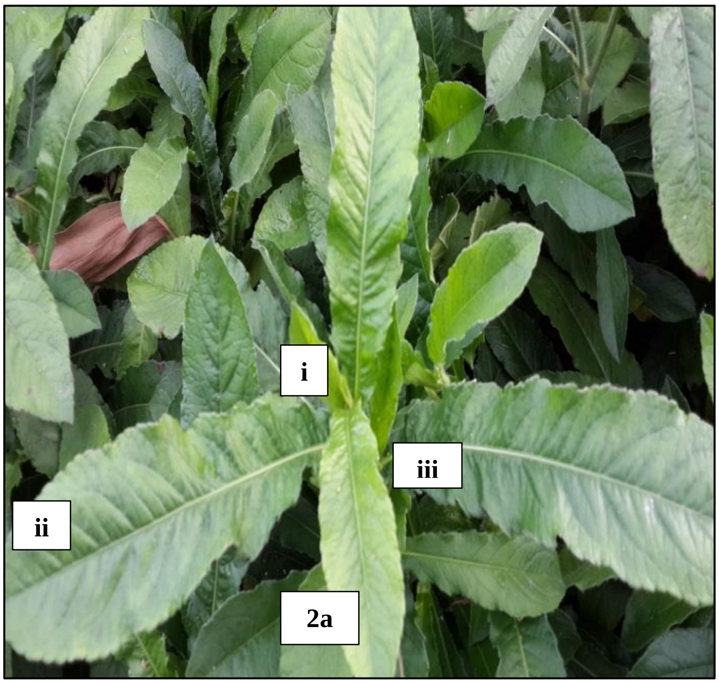
A few experiments had been done before to show antimicrobial properties of *Elephantopus scaber*. Different extract of the plant give a different inhibition zone against target microorganisms. Methanol extract of *E. scaber* showed inhibition zone on several pathogens, *Staphyloccocus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Proteus vulgaris*. Aqueous extract showed low antimicrobial activity against *E. coli, S. aureus, Streptococcus pyogenes, P. aeruginosa, Leuconostoc lactis* and *Salmonella* *typhi* (Farha & Prathapan, 2014). Test for the inhibition zone of aqueous and methanol extract against *Aspergillus falvus, Mucor indicus, Aspergillus niger* and *Rhizopus indicus* showed that there is no antimicrobial activity observed on aqueous extract rather than methanol extract (Kamalakannan *et al.*, 2012).

Another test performed on *S. aureus*, *E. coli*, *B. subtilis, P. aeruginosa, P. vulgaris* showed inhibition zone occur due to the presence of sesquiterpene dialactone isodeoxy elephantopin in the plant (Kumar, Perumal & Suresh, 2004). The compounds, 17, 19 dihydrodeoxyelephantopin and iso-17, 19 dihydrodeoxyelephantopin from *E. scaber* had showed antimicrobial activity against *S. aureus* (Farha & Prathapan, 2014). Against *E. coli*, *S. aureus* and *β-streptococcus*, sesquiterpene lactones, scabertopin, isoscabertopin, deoxyelephantopin and isodeoxyelephantopin from *E. scaber* are involved in the antimicrobial activity (Jack, 2011; Farha & Prathapan, 2014).

2.2.4 Herbarium specimen collection of *Elephantopus scaber*

According to Royal Botanic Garden (KEW) (n.d.), herbarium (Figure 1) is the collection of the preserved plants that stored, catalogued and arranged systematically to be used as a reference in the future (Royal Botanic Garden (KEW), (n.d.). *Elephantopus scaber* is an erect, stiff, perennial herb that can grow up to 60 cm in height. Leaves (Figure 2a) are radical in basal rosette on the ground, while certain are cauline, finely dentate, hairy, obovate-oblong, short petioles (Das & Mukherjee, 2014; Wan *et al*., 2009). Flowers (Figure 2b) small, actinomorphic, epigynous, purple to dull pink, each clusters supported by a rigid ovate leaf like bract; inner bracts leafy, distinct, pale green. cuneate below; pappus bristly, hairy (Das & Mukherjee, 2014).

Figure 1: Herbarium specimen collection of *Elephantopus scaber*. i: Leaves, ii: Flower, iii: Stem, iv: Roots



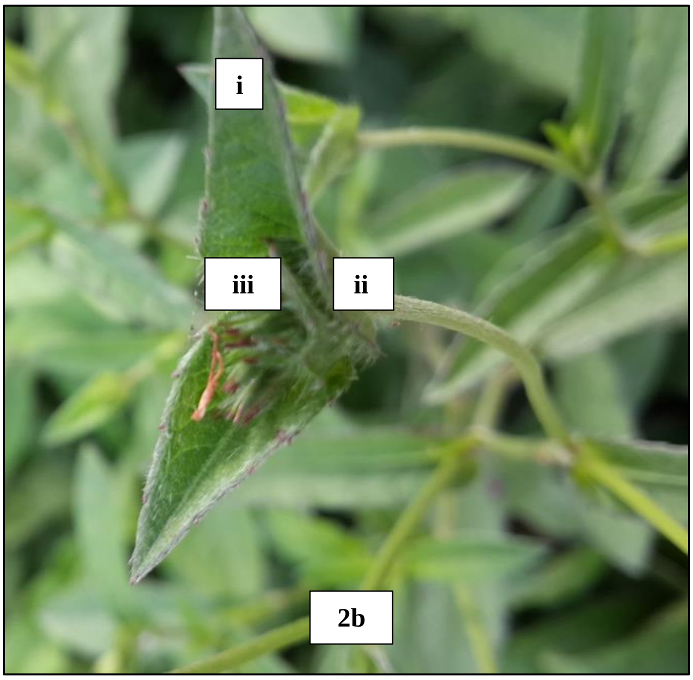


Figure 2: *Elephantopus scaber* plants. Leaves (2a), i: basal rosette on ground, ii: finely dentate, iii: obovate-oblong. Flower (2b), i: cuneate below, ii: rigid ovate leaf like bract, iii: inner bracts leafy.

2.3 Crop pathogenic fungi

2.3.1 *Glomerella cingulata*

Genus *Glomerella* is in the phylum of Ascomycota and it is a group consists of mostly pathogenic plant-associated fungi. *G. cingulata* (anamorph: *Colletotrichum gloeosporoides*) has been associated with diseases on a wide range of host plants. It is a pathogen and entophytes in tropical crops and trees, and it also infecting tree seedlings in temperate forests. Basically, it can infect fruits, flowers, leaves and other non-lignified tissue (The Regents of the University of California, 2014). Many tropical fruits such as mango and papaya are infected with *G. cingulata* that cause anthracnose diseases. Diseases caused by *G. cingulata* can cause spoilage and rotting in plants (Bussaman, Namsena, Rattanasena, & Chandrapatya, 2012).

G. cingulata also caused Glomerella leaf spot. Brazil was first reported the diseases in 1988 followed by United States (1998). In 2011, China spotted the diseases at Fengxian, Jiangsu Province. The leaf has necrotic lesions and the diseased leaves becoming dark and shed (Wang, Zhang & Li, 2012). Moisture and temperature will influence the infection and development. As the temperature increases, the severity of the diseases will also increases (Yoder, 2012).

2.3.2 *Colletotrichum* sp.

Plant diseases known as anthracnose comes from the causal agents of the fungi of genus *Colletotrichum* and their teleomorph *Glomerella* spp. Pathogens of this genus can severely infect cereals, legumes, ornamentals, vegetables and fruit trees. Under prolonged warm and wet weather condition, diseases outbreak can occur rapidly and yield loss can be severe. This genus is also considered as important postharvest pathogen (Grahovac *et al*., 2012; Wharton & Uribedon, 2004).

Most of the *Colletotrichum* sp. can infect more than one host and also different kinds of *Colletotrichum* sp can infect in a single host. As mention by Roberts, Pernezny and Kucharek (2012), in Florida, there are two kinds of *Colletotrichum* sp which are known as *C. gloeosporioides* and *C. acutatum* which cause anthracnose on a single host, pepper. *C. gloeosporioides* infected on matured fruit while *C. acutatum* on immatured fruit (Roberts, Pernezny & Kucharek, 2012).

2.3.3 *Fusarium oxysporum*

Fusarium oxysporum can be classified under class Deuteromycetes and this is a ubiquitous soil borne fungus that includes pathogenic and non-pathogenic (Groenewald, 2005). Pathogenic strain of *F. oxysporum* causes wilt, root rot and crown rot diseases on variety of crops. The non-pathogenic strain can be used as biological control agents. Most strains of this species are saprotrophic (Bogale, Wingfield, Wingfield & Steenkamp, 2006). Normally, individual isolates within these fungi have a narrow host range. *F. oxysporum* have been divided into forma specialis based on the pathogenicity on a host plant (Llic, Cosic, Jurkovic, & Vrandecic, 2013). Forma specialis is an informal taxonomic grouping allowed by the international Code of Nomenclature for algae, fungi, and plants (English Encyclopedia, 2014). Some of the forma specialis that had been classified are known as *F. oxysporum* f. sp. *pisi*, *F. oxysporum* f. sp. *ciceri*, *F. oxysporum* f. sp. *psidii*, *F. oxysporum* f. sp. *dianthi*, and *F. oxysporum* f. sp. *cucumerinum* (Correll, 1991; Khilare, & Ahmed, 2012; Gupta, Misra, & Gaur, 2010; Llic, Cosic, Jurkovic, & Vrandecic, 2013).

F. oxysporum is an anamorph that can produce three types of spores known as microconidia, macroconidia, and chlamydospores. Microconidia usually can be found on the infected plants, while macroconidia usually can be found on surface of plant that had been. Chlamydospores are one type of spores that can remain dormant in the soil and infected the host for as long as 30 years (The New York Botanical Garden, 2013).

Several studies had been done to determine favourable conditions that affect the growth of *Fusarium oxysporum* such as pH, temperature, and media. According to Khilare and Ahmed (2012), *F. oxysporum* performs growth at optimum pH within the range of 6.5-7.0. Their growth inclined gradually as the pH decreased or increased from the optimum pH. The authors also found that *F. oxysporum* have maximum growth on Richard's agar and potato dextrose agar. Maximum growth and sporulation were showed at temperature ranges from 27 °C – 30 °C (Gupta, Misra, & Gaur, 2010).

One of the important fungal diseases cause by *F. oxysporum* is Fusarium wilt (Panama disease) (Daly, 2006). Banana i**s** among the crops that are susceptible to *F. oxysporum*. The infect banana is called *F. oxysporum* f.sp. *cubense.* This forma specialis is responsible for huge losses of Gros Michel export bananas in Central America (Groenewald, 2005). Symptoms showed on the infected plants are vascular discoloration, yellowing and wilting. The symptoms yellowing and wilting are due to the ability of the fungus to establish itself systemically in the xylem vessel (Kidane & Laing, 2010).

2.3.4 *Fusarium solani*

Fusarium solani (teleomorph: *Nectria haematococca*) is a soil fungus that is the most frequent causal agents of root rot on many host plants. This species has both saprophytic and parasitic forms (Ondrej, Dostalova & Trojan, 2008).

Fusarium solani also produces the same spores as *Fusarium oxysporum* which are known as macroconidia, microconidia and chlamydospores. However, both fungi can be distinguished by the production of microconidia. *F. solani* produces microconidia on long monophialides (Kochman, 2007).

Root rots can cause damage ranges from 10% to 80% losses on different vegetables (Karima, Haggag, Nadia & El-Gamal, 2012). Other disease can caused by this pathogen is Fusarium wilt, a potential disease of potato in Pakistan and guava in India (Shafique & Shafique, 2012; Gupta *et al*., 2009).

2.3.5 *Pestalotiopsis* sp.

Pestalotiopsis Steyaert is the anamorph of *Pestalosphaeria* Barr belonging to the family *Amphisphaeriaceae*. *Pestalotiopsis* species have conidia that usually are fusiform. *Pestalotiopsis* species mostly are entophytes and could be found especially in tropical regions (Ai, Tong, & Liang, 2007). It is an important species that causes leaf blights, fruit rot and other post-harvest diseases. *Pestalotiopsis* species is also known to cause grey blight disease of tea (*Camellia sinensis*) in southern India and resulted in 17% production loss, while 20 % in Japan (Joshi *et al*., 2009) as cited by Hyde, 2011.

2.3.6 *Pyricularia oryzae*

Genus *Pyricularia* gets its name due to the pyriform shape of conidia. *Pyricularia oryzae* is commonly found on tropical grasses (Bussaban, Lumyong, Hyde & Mckenzie, 2003). *P. oryzae* causes rice blast disease. It attacks the leaves, culms, branches of the panicle and the floral structures (Gouramanis, 1997). Mostly, this pathogen will infect rice that especially grown in temperate regions or as an uplands crop in the tropics such as in West Africa, Malaysia, Iran, and the savannah of South America (Mackill & Bonman, 1986; Vanaraj *et al*., 2013).

Rice blast is also an important disease in China since many losses frequently occurred in rice-producing areas of China (Chen *et al*., 2001). Infected plant showed the symptom on leaves which begins as grey-green and water soaked lesions, and once the spots are completely expanded, it will form dark-green borders (Vanaraj, *et al*., 2013). This fungal pathogen can be controlled by fungicides such as Fongoren and also biological control by fungal antagonists such as *Trichoderma harzianum* and *Chaetomium globosum* (Gouramanis, 1997).

3.0 Methodology

3.1 Plant materials

Plants were collected from Tarat, and transplanted to the UNIMAS greenhouse. Soil beds (Figure 3) were prepared for 3 - 4 m^2. The soil is a mixture of 3: 2: 1 of top soils, organic soils and sands respectively. Nitrogen – Phosphorus - Kalium (NPK) fertilizer were applied once per month.

Figure 3: Sample preparation

3.2 Sample collection and preparation

Leaves were harvested from mature plants. 50 g of leaves were washed thoroughly with tap water and rinsed with distilled water to remove the contaminants. The leaves were then being air dried for five days. The air dried leaves (Figure 4) were grounded and homogenised into powder form using blender.

Figure 4: Air dried sample

3.3 Methanol crude extracts preparation

Fifty gram of grounded sample were immersed in 500 mL of 80% of methanol overnight in a beaker sealed with aluminium foil (Figure 5) at room temperature. The extracts obtained were then filtered by using filter paper. The filtered extracts were poured into a watch glass and let evaporated for a week to obtain crude extract of methanol. The pure crude extract will be weighed to obtain dried weight. Percentage of pure crude extract was calculated using the equation given in section 3.3.

Figure 5: Grounded samples were immersed with 80% methanol

Percentage of pure crude extract was calculated using the equation below:

$$\text{Crude extract (\%)} = \frac{\text{Sample 1}}{\text{Sample 2}} \text{ x } 100$$

Sample 1 = Weight of extract obtained after evaporated using watch glass

Sample 2 = Weight of grounded sample before extraction