PHYTOCHEMICALS, ANTIOXIDANTS AND ANTIMICROBIAL PROPERTIES OF CORN SILK HAIR

By

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CERTIFICATE

This is to certify that the dissertation entitled **"PHYTOCHEMICALS, ANTIOXIDANTS AND ANTIMICROBIAL PROPERTIES OF CORN SILK HAIR"** is the bonafide record of research work done by **WAN MAZNAH BT WAN ISHAK** during the period from July 2007 to March 2008 under our supervision.

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LIST OF ABBREVIATIONS

The following abbreviations were used in the text:

µl	microlitre
µm	micromolar
^0C	degree centrigrade
CFU	colony forming unit
cm	centimeter
g	gram
hrs	hours
mg	miligram
min	minute
ml	mililitre
mm	millimeter
Na_2CO_3	sodium carbonate
nm	nanometer
ppm	part per million
Pet.Ether	Petroleum ether
sec	second
UV	ultraviolet
v/v	volume per volume
w/v	weight per volume

PHYTOCHEMICALS, ANTIOXIDANTS AND ANTIMICROBIAL PROPERTIES OF CORN SILK HAIR

ABSTRACT

The corn silk hairs were extracted with four different organic solvent using the Soxhlet extraction method. Petroleum ether, chloroform, methanol and water solvent were used as solvents for extraction. Four types of corn silk extracts were obtained. They were petroleum ether extract, chloroform extract, methanol extract and aqueous extract. This plant extract were screened for phytochemical properties followed by antioxidant and antimicrobial test.

The extracted of corn silk hair were studied for phytochemical properties to detect the present of chemical constituent. Five phytochemical tests for the detection of different classes of secondary metabolites in plant extract were conducted. They were alkaloid, flavonoid, coumarin, saponin and tannin/phenolic tests. The preliminary pyhtochemical test revealed the presence of saponin, coumarin, flavonoids and tannin/phenolic in methanol and aqueous extract while absence in the petroleum ether and chloroform extract. Methanol extract showed an absence of alkaloid group. This result was contradicting with the previous study. So, further study using another method should be done to ensure the validation of result.

Total phenolics content and antioxidative activity based on Folin-Ciocalteu method and Ferric Reduction Antioxidant Power (FRAP) method respectively was conducted. The increasing order of concentration of extracts were petroleum ether extract > chloroform extract > aqueous extract > methanol extract. So, methanol extract recorded the highest phenolic content in the corn silk hairs compared to other extracts.

For FRAP assay, the concentration of plant extracts used were ranging from 200 ppm to 1000 ppm. The reducing power of the corn silk extracts were compared against the standard synthetic antioxidant, butylated hydroxytoluene (BHT).The result shows that the chloroform, methanol and aqueous extract were enhanced over than the BHT when the concentration was increased to 800 ppm. Petroleum ether extract showed the lesser antioxidant activity compared to the BHT even the concentration was increased up to 1000 ppm. There were positive correlation between the antioxidant activity and phenolic content together with phytochemical compound in corn silk extracts.

Antimicrobial effects from the extracted of corn silk were studied against three different bacteria. The Minimum Inhibitory Concentration (MIC) was tested towards *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) to determine the minimum concentration of the extract that can inhibiting the bacteria growth. The standard concentrations used were 0.05 mg/10ul, 0.25 mg/10ul, 0.50 mg/10ul, 0.75mg/10ul and 1.00 mg/10ul respectively by using the agar diffusion technique. However, all bacteria shows non susceptible against the corn silk extract.

SIFAT-SIFAT FITOKIMIA, ANTIOKSIDA DAN ANTIMIKROB PADA RERAMBUT JAGUNG

ABSTRAK

Rerambut jagung diekstrak dengan menggunakan empat pelarut organik yang berbeza menggunakan keadah pengestrakan Soxhlet. Petroleum eter, kloroform, metanol dan air digunakan sebagai pelarut dalam proses pengekstrakan .Empat jenis ekstrak rerambut jagung diperolehi iaitu petroleum ether ekstrak, kloroform ekstrak, metanol ekstrak dan akueus ekstrak. Ekstrak tumbuhan ini telah disaringkan untuk ujian sifat-sifat fitokimia diikuti oleh ujian antipengoksida dan ujian antimikrob.

Ekstrak rerambut jagung dikaji untuk sifat-sifat fitokimia untuk mengetahui kehadiran sebatian kimia tertentu. Lima ujian fitokimia untuk mengesan kelas-kelas dalam ekstrak rerambut jagung digunakan. Ujian-ujian tersebut adalah ujian alkaloid, ujian flavonoid, ujian koumarin, ujian saponin dan ujian tannin/fenolik. Ujian penyaringan fitokimia menunjukkan kehadiran saponin, koumarin, flavonoid dan tanin/fenolik dalam ekstrak metanol dan akueus manakala ketidakhadiran dalam ekstrak petroleum eter dan kloroform. Ekstrak metanol menunjukkan ketidakhadiran kumpulan alkaloid. Keputusan yang diperoleh bercanggah dengan kajian terdahulu. Jadi, kajian lebih lanjut menggunakan kaedah yang lain harus dijalankan untuk memastikan kesahihan keputusan tersebut.

Untuk ujian antipengoksida, jumlah sebatian fenolik dan aktiviti antipengoksida masing-masing berdasarkan Kaedah Folin-Ciocalteu dan ujian Kuasa Antipengoksida Penurunan

Ferum (FRAP) dilakukan. Kepekatan ekstrak yang diperoleh dalam susunan menaik adalah seperti berikut: ekstrak petroleum eter > ekstrak kloroform >ekstrak akueus > ekstrak metanol. Hal ini menunjukkan ekstrak metanol memberikan jumlah sebatian fenolik yang tertinggi dalam rerambut jagung jika dibandingkan dengan ekstrak yang lain.

Untuk ujian FRAP, kepekatan ekstrak rerambut jagung yang digunakan adalah antara 200 ppm sehingga 1000 ppm. Kuasa penurunan ekstrak rerambut jagung ini dibandingkan dengan standard sintetik antipengoksida, butylated hydroxytoluene (BHT). Keputusan menunjukkan ekstrak kloroform, metanol dan akueus meningkat melebihi BHT apabila kepekatan ditambah kepada 800 ppm. Hanya ekstrak petroleum ether sahaja yang menunjukkan pengurangan aktiviti antipengoksida apabila dibandingkan dengan BHT walaupun kepekatan ditingkatkan sehingga 1000 ppm. Didapati, terdapat positif kolerasi/hubung kait antara aktiviti antipengoksida dengan jumlah fenolik dan sebatian fitokimia dalam ekstrak rerambut jagung.

Kesan-kesan antimikrob dari ekstrak rerambut jagung dikaji terhadap tiga jenis bakteria yang berbeza. Ujian Kepekatan Perencatan Minimal (MIC) dijalankan ke atas bakteria *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) dan *Pseudomonas aeruginosa* (ATCC 27853) untuk menentukan kepekatan yang paling kecil yang dapat merencatkan pertumbuhan bakteria. Kepekatan yang digunakan untuk semua bahagian ekstrak rerambut jagung dipiawaikan masing-masing kepada 0.05 mg/10ul, 0.25 mg/10ul, 0.50 mg/10ul, 0.75mg/10ul dan 1.00 mg/10ul menggunakan teknik penyerapan agar. Walau bagaimanapun, semua bakteria tidak dipengaruhi oleh ekstrak rerambut jagung

1.0 INTRODUCTION

Medicinal plants are rich source form which antioxidant and antimicrobial agents may be obtained. Plants are used medicinally in different countries and are source of many potent and powerful drugs to combat serious disease (Srivastava *et al.*, 1996). Approximately 60-80% of the world's population still relies on traditional medicines for the treatment of common illnesses (Ramzi *et al.*, 2008). The herbal drug *Maydis stigma* (dried cut stigmata of maize female flowers, *Zea mays* L.ssp. *mays*, Poaceae) is recognized and used both in traditional and official medicine as mild diuretic, urinary demulcent, to pass stones and gravel from kidneys and urinary bladder against benign prostatic hyperplasia, cystitis, gout, chronic nephritis and similar ailments (Tucakov, 1990., British herbal Pharmacopoeia, 1996., Czygan, 1997).

Over the past decade, there has been an increased interest in phytochemicals for the purpose of human health and for benefits in the food industry (James, 2004). The active principles of many drugs found in plants are secondary metabolites (Ghani, 1990., Dobelis, 1993). Although secondary metabolites products may have variety of functions in plants, it is likely that their ecological function may have some bearing on potential medicinal effects in humans (James, 2004). Therefore, basic phytochemical investigation of plant extract for their major phytoconstituents is also vital.

In the last years, interest in the antioxidant activity of plant extracts has become larger and very important due to the fact that free radicals e.g reactive oxygen species (ROS) can be responsible for various disease, e.g heart disease, stroke, arteriosclerosis and cancer as well

as for aging process (Ramzi *et al.*, 2008). Although there are some synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), these compounds are associated with some side effects (Ito *et al.*, 1983). Corn silk has been reported to have polyphenol substances which should be consider as an important feature of herbal drug *Maydis stigma* (Maksimovic and Kovacevic, 2003). The phenolic compounds have some of interest as antioxidants in humans from food (James, 2004).

Scientists have focused on increasing human infections caused by microorganism. The microorganisms have unfavourable effects on the quality and safety of life. Synthetics chemicals are widely used against these microorganisms; unfortunately they develop resistance to many antibiotics (Service, 1995., Mukherjee *et al.*, 2002). Therefore, the use of plant extract is less damaging to the human health and environment (Isman, 2000., Misra and Pavlovstathis, 1997).

Due to the lack of information about antioxidant activity of corn silk coupled with inadequate data on its phytochemistry, hence, in the present study, organic extracts of corn silk were phytochemically analysed and tested for antioxidant activity using Folin-Ciocalteu method and Ferric Reduction Antioxidant Power (FRAP). The antimicrobial activity of the corn silk extracts using agar diffusion method also was examined.

1.1 BOTANICAL CHARACTERISTIC OF CORN

Zea mays Linnaues is an herbal plant which belongs to the family of Gramineae or Poaceae. The details of the systemic position of this plant are shown in Table 1.1

Table 1.1: The hierarchical level of *Zea mays*

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Poales
Family	Gramineae/poaceae
Genus	Zea
Species	*Zea mays*

(http://www.gramene.org/species/zea/maize_taxonomy.html)

Zea mays known as maize throughout most of the world and as corn in the United States. It has so many common names such as Corn, Indian corn, Maize, Mais, Thurah Safrah, Sweet corn and so on (Derrida, 1997). It is the premier cash crop in the United States and it also cultivated in other country like in the West Indian Islands, Australia, Africa, India and now in France. It cultivation, genetics, processing, financing, and distribution on a national and international scale is pervasive and complex (http://www.aphis.usda.gov).

The production of this plant in the world in 1987/1988 was 439 million metric tons, of which the United States produced 179, China 76, Brazil 23, and France 12. Corn is grown commercially in almost all States of the United States (Jewell, 1989). United States

production in 1987 was 7064 million bushels, of which the top State producers were Iowa (1306), Illinois (1201), Nebraska (812), Minnesota (635), and Indiana (632). Corn has the highest value of production of any United States crop; 1987 value was 12.1 billion dollars, compared to soybeans at 10.4, hay at 9.1, wheat at 5.4, and cotton at 5.0 (http://www.aphis.usda.gov).

Corn has been cultivated since the earliest historic times from Peru to central North America. The region of origin is now presumed to be Mexico (Gould, 1968). Dispersal to the Old World is generally deemed to have occurred in the sixteenth and seventeenth centuries (Cobley and Steele, 1976); however, recent evidence indicates that dispersal to India may have occurred prior to the twelfth and thirteenth centuries by unknown means (Johannessen and Parker, 1989).

1.2 CORN SILK AND THEIR USE

Zea mays or *Maydis stigma* require a warm climate and well-drained as well as moist soil. It prefers growing in light (sandy), medium (loamy), and heavy (clay) soils with acid and neutral soils (pH range from 5.5 to 6.8). It can growing annually up to 2m at a fast rate, hardy to zone 9 and is frost tender. It usually flowers from July to October and seeds ripen from September to October (Derrida, 1997).

Although corn always be seen in yellow color, but actually this plant comes in host in varieties range of color. One of these varieties is red, pink, black and blue. Other than have different varieties of color, corn also having many types. It often can be classified as dent corn, flint corn, sweet corn, flour corn, waxy corn and popcorn (Derrida, 1997).

1.2.1 DENT CORN

Dent corn is known scientifically as '*Zea mays* indentata' frequently referred to as 'field' corn. It is usually white or yellow in color. The kernels contain both hard and soft starch and become intended at maturity. It is often used as livestock feed, food and industrial products (Derrida, 1997).

1.2.2 FLINT CORN

Flint corn also called as 'Indian corn' mostly grown in Central and South America. It has different range of colors from white to red. Flint corn is known by scientific name as '*Zea mays* indurata' having hard, horny, rounded or short and flat kernels with the soft and starchy endosperm completely enclosed by a hard outer layer. This crop is used as similar purposes as dent corn (Derrida, 1997).

1.2.3 SWEET CORN

Sweet corn is considered as a distinct species (scientifically known as *Zea saccharata* or *Zea rugosa*), a subspecies (*Zea mays* rogusa) or a specific mutation of dent corn. It is called sweet corn because it contains more natural sugars compare to the other types of corn (Field corn contain 4% sugar while sweet corn contain 10% sugar). It is better to eat while it still fresh because almost 50% of this sugar will be converted to starch 24 hours after it is picked. The kernels of this corn contain high percentage of sugars in the milk stage. Sweet corn is a warm-season vegetable, grown easily in any garden but requires sufficient light, fertility, growing season and space. The successive planting are better to be done in early summer until frost if the weather cooperates. According to genetic background, sweet corn is divided

into three distinct types: normal sugar (SU), sugary enhancer (SE) and supersweet (Sh2). Standard sweet corn varieties contain a "sugary (SU) gene" that is responsible for the sweetness and creamy texture of the kernels are suitable for being picked, husked and eaten within a very short time. This sweet corn can be eaten fresh, canned or frozen for future consumption but occasionally used for feed or flour (Derrida, 1997).

1.2.4 FLOUR CORN

Flour corn also called 'soft' or 'squaw' corn or in scientific name known as '*Zea mays* amylacea'. The kernel has shape like flint corn and composed entirely of soft starch. This type of corn having sugary enhancer hybrid which is containing sugary enhancer (SE) gene that significantly raises the sugar content standard SUs gene while retaining the tenderness and creamy textures of standard varieties. This sugary enhancer is the gourmet corn of choices because it contains best quality both of SU and Sh2 types. Fresh from the garden, virtually all current SE releases have eating quality that is superior to all other types. No isolation from standard SUs is necessary. Supersweet hybrids have shrunken -2 genes (Sh2) and the sugar content is high compare to the SU varieties. The kernels of these extra-sweet varieties have a crispy, tough-skinned texture and low amount of the water-soluble polysaccharides that impart the creamy texture and 'corny' flavor to other sweet corn varieties. Lacking of the creamy texture will affects the quality of frozen and canned corn as does the toughness of the seed coat. This corn is wind-pollinated .It should be isolated at the distance of 500 feet or more especially downwind and separated from any other type of corn tasseling at the same time to ensure sweetness and tenderness. Their pollen is weak and