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UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION OF ALKALOIDS OF PEPPER (*Piper nigrum)* AND KADOK (*Piper sarmentosum)* AND THE BIOTRANSFORMATION OF PIPERINE USING *Aspergillus niger*

LIM CHYI MEEI

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By

LIM CHYI MEEI

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, In Fulfilment of the Requirement for the Degree of Master of Science

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Abstract of the thesis presented to the Senate of Unversiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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Chairman : Associate Professor Gwendoline Ee Cheng Lian, PhD

Faculty : Science

Studies have been carried on the roots of *Piper nigrum* and the aerial parts of *Piper sarmentosum.* Their alkaloidal components and biological properties are reported in this thesis. The major compound of the *Piper nigrum*, piperine **(85)** was biotransformed by *Aspergillus niger* FTCC 5003 yielded one piperine derivative, a new natural product 5-[3,4-(methylenedioxy)phenyl]-pent-2-ene piperidine **(88).** These alkaloidal components were isolated using common chromatographic techniques and were identified using spectroscopic experiments such as NMR, MS, IR and UV.

The roots of *Piper nigrum* furnished eight alkaloids, Cepharadione A **(81),** Piperolactam **(82),** 2,4-tetradecadionoic acid isobutyl amide **(83),** (E)-1-[3',4'-(Methylenedioxy)cinnamoyl]piperidine **(84)**, Piperine **(85)**, Pellitorine **(1)**, Sylvamide **(86)** and Paprazine **(87)**. The compounds 2,4-tetradecadionoic acid isobutyl amide **(83)** and (E)-1-[3',4'-(Methylenedioxy)cinnamoyl]piperidine **(84)** were first obtained as natural products and new to this species of plants. Beside that, Paprazine **(87)** never had

been isolated from this species of plant. Meanwhile from the biotransformation of *Aspergillus niger* FTCC 5003 yielded one new compound, **(88).** Up to now, biotransformation research has only been carried out on the terpenoid compounds and a few on alkaloids. Meanwhile, investigations on the aerial parts of *Piper sarmentosum* gave one alkaloidal compound, 1-nitrosoimino-2,4,5-trimethoxybenzene **(89).** This compound was first isolated from this plant and also a new found natural product

The petroleum ether and chloroform extracts of *Piper nigrum* roots were active against HL-60 cell line with the IC_{50} values of 9.8 and 11.2 μg/ml, respectively. However the ethyl acetate extract of *Piper nigrum* was inactive to HL-60 cell line. Meanwhile, the hexane and ethyl acetate extracts of *Piper sarmentosum* showed good cytotoxic activity with the IC_{50} values of 14.4 and 9.8 μg/ml respectively on MCF-7 cell line. Meanwhile the methanol extract of the aerial parts of *Piper saramentosum* was inactive on MCF-7 cell line. Cytotoxic test on HeLa cell line also been carried out on the various extracts of *Piper sarmentosum.* However, only hexane extract gave active result with the IC_{50} value of 11.6 μg/ml. Two pure compounds from *Piper nigrum* (E)-1-[3',4'-(Methylenedioxy)cinnamoyl]piperidine **(84)** and Pellitorine **(1)** were tested on HeLa and MCF-7 cell lines. Pelltorine **(1)** showed good activities against MCF-7 and HeLa cell line with the IC_{50} value of 1.8 and 13 μg/ml, respectively. The extracts of *Piper sarmentosum* and pure compounds of *Piper nigrum* were never tested on HeLa and MCF-7 cell lines. This is the first report on them.

The antimicrobial assay was carried out towards four pathogenic bacteria, Methicillin Resistant *Staphylococcus aures, Pseudomonas aeruginose, Staphylococcus typhimurium*

and *Bacillus subtilis*. The antifungal activity testing of the plant extracts were carried out against the fungi *Candida albican, Apergillus ochraceaus, Sacchoromycs cerevisiae* and *Candida lypolytica*. No activity was observed for all the extracts on these two assays.

The larvicidal tests performed against the larvae of *Aedes aegypti*. The acid base treated ethanol extract of *Piper nigrum* showed a strong activity against the larvae with the LC_{50} value of 1.88 µg/ml. The pure compound, pellitorine **(1)** also gave good activity as the LC_{50} value is 6.22 µg/ml. The hexane and ethyl acetate extracts of *Piper sarmentosum* gave strong activities with the LC_{50} values of 8.08 and 6.94 µg/ml, respectively. Meanwhile the methanol extract of *Piper sarmantosum* was not active towards the larvae of *Aedes aegypti*. The acid base treated ethanol extract of *Piper nigrum* was first performed against the larvae of *Aedes aegypti* in this study.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains.

ALKALOID-ALKALOID DARI *PIPER NIGRUM* DAN *PIPER SARMENTOSUM* DAN BIOTRANFORMASI PIPERIN

Oleh

LIM CHYI MEEI

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Kajian telah dijalankan ke atas akar *Piper nigrum* dan bahagian atas *Piper sarmentosum*. Semua aktiviti biologi and komponen alkaloids telah dilaporkan dalam tesis ini. Sebatian utama *Piper nigrum*, piperine **(85)** telah ditranformasi oleh *Apsergillus niger* FTCC 5003 dan memberikan satu sebatian semulajadi baru iaitu product 5-[3,4-(metilenadioksi)fenil]-pent-2-ena piperidin **(88).** Struktur sebatian-sebatian ini ditentukan dengan menggunakan eksperimen spekstroskopi seperti NMR, IR, UV dan MS.

Dari akar *Piper nigrum* telah memberikan lapan alkaloid iaitu, Cefaradion A **(81),** Piperolaktam D **(82),** 2,4-tetradekadionik asid isobutil amida **(83),** (E)-1-[3',4'-(Metilenadioksi)cinnamoyl]piperidin **(84)**, Piperin **(85)**, Pelitorin **(1)**, Silvamida **(86)** and Paprazin **(87)**. Manakala dari proses biotransformasi telah memberikan satu sebatian baru **(88).** Sehingga kini, kajian biotransformasi hanya dijalankan terhadap sebatian terpen and sesetengah alkaloid. Manakala, kajian terperinci terhadap bahagian atas *Piper*

sarmentosum telah memberikan satu sebatian alkaloid 1-nitrosoimina-2,4,5-trimetoksibenzena **(89).** Dua sebatian ini, 2,4-tetradekadionik asid isobutil amida **(83),** (E)-1-[3',4'-(Metilenadiosi)cinnamoyl]piperidin **(84)** ialah kali pertama diekstrak daripada tumbuhan. Manakala sebatian ini, Paprazin **(87)** ialah kali pertama didapati daripada tumbuhan spesies ini.

Ekstrak petroleum eter and kloroform *Piper nigrum* adalah aktif terhadap sel HL-60 dengan memberikan nilai IC_{50} masing-masing 9.8 and 11.2 μg/ml. Ektrak hexana dan etil asetate *Piper sarmentosum* menunjukkan aktiviti sitotosik yang baik dengan nilai IC_{50} masing-masing ialah 14.4 dan 9.8 μg/ml terhadap sel MCF-7. Manakala ekstak metanol *Piper sarmentosum* adalah tidak aktif terhadap sel MCF-7. Kajian sitotosik terhadap sel HeLa telah juga dijalankan ke atas semua ekstrak *Piper sarmentosum*. Akan tetapi, hanya ekstrak hexana menunjukkan keputusan aktif dengan nilai IC_{50} 11.6 μg/ml. Dua sebatian tulen diperoleh dari *Piper nigrum*, (E)-1-[3',4'-(Metilenedioksi)sinnamol]piperidin **(84)** dan Pellitorin **(1)** diuji dengan sel MCF-7 dan HeLa. Pelltorin **(1)** memberikan aktiviti yang baik terhdap sel MCF-7 dan HeLa dengan nilai IC_{50} ialah 1.8 dan 13 μg/ml masing-masing. Sebelum ini, tidak ada kajian dijalankan ke atas kanser sel HeLa dan MCF-7 menggunakan sebatian-sebatian tulen dari *Piper nigrum* dan ekstrak *Piper sarmentosum*. Kajian ini adalah kali pertama dilaporkan.

Ujian anti-mikrobial dijalankan dengan menggunakan bakteria jenis Methicillin Resistant *Staphylococcus aures, Pseudomonas aeruginose, Staphylococcus typhimurium* dan *Bacillus subtilis*. Aktiviti anti-fungal ekstrak tumbuhan telah dijalankan ke atas

Candida albican, Apergillus ochraceaus, Sacchoromycs cerevisiae dan *Candida lypolytica*. Tiada aktiviti diperhatikan ke atas semua ektrak untuk kedua-dua jenis ujian ini.

Ujian larva telah dijalankan dengan menggunakan larva jenis *Aedes aegypti*. Ekstrak etanol *Piper nigrum* menunjukkan aktiviti yang kuat terhadap larva dengan nilai LC_{50} 1.88 µg/ml. Sebatian tulen Pelltorin **(1)** juga memberikan aktiviti yang baik dengan LC_{50} ialah 6.22 µg/ml. Ekstrak hexana dan etil asetate *Piper sarmentosum* memberikan aktiviti kuat dengan nilai LC_{50} masing-masing ialah 8.08 dan 6.94 µg/ml. Manakala, metanol ekstrak tidak menunjukkan sebarang aktiviti terhadap larva. Ekstrak etanol adalah kali pertama dijalankan ke atas larva *Aedes eagypti* dalam kajian ini.

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I certify that an Examination Committee has met on 18th September 2008 to conduct the final examination of Lim Chyi Meei on her degree thesis entitled "Alkaloids of *Piper nigrum* and *Piper sarmentosum* and Biotransformation of Piperine" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the relevant degree.

Members of the Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date:19 DECEMBER 2008

DECLARATION

I hereby declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universtiti Putra Malaysia or at any other institutions.

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LIM CHYI MEEI

Date: 11 OCTOBER 2008

CHAPTER 1

INTRODUCTION

1.1 Natural Products

For centuries, the plant kingdom has been an important source of natural products. In ancient times, people depend greatly on flora for their survival. Various parts of plants such as berries, leaves and roots were experimented to determine what effects these parts have on human beings. As a result, many plants were discovered by local healers to have medicinal uses. These have led to the development of Western medicine as well as Chinese Traditional medicine and the Ayuverda.

In recent years, a renewed interest in obtaining biologically active compounds from natural sources has been revived, as natural products are low or absent in toxicity and possess complete biodegradability. Their availability from renewable sources, and, in most cases, their low-cost when compared with those of compounds obtained by total chemical synthesis are added advantages. In developing countries, this is sustained by search for biologically active compounds obtainable from locally available plants, particularly with a view to reducing public health costs which have significantly been raised due to acquisition of synthetic drugs from industrialized countries.

Many medical practitioners with training in pharmacology and /or pharmacology are well aware of the number of modern therapeutic agents that have been derived from tropical forests. In fact, over one-hundred and twenty pharmaceutical products currently

in use are plant-derived, and some 75 per cent of these were discovered by examining the use of these plants in traditional medicine. Thus biologically active plant derived chemicals can be expected to play an increasingly significant role in the commercial development in pharmaceuticals.

The other reason for seeking potential lead compounds from natural products is the increasing number of cancer patients world wide. Cancer treatment is hindered by failure of chemotherapy. Some cancers are intrinsically resistant to chemotherapy whereas others, although are initially sensitive, develop resistance during treatment. Many cancers may also develop resistance to the synthetic medicines during prolonged chemotherapy. Therefore, with natural products as an excellent source of complex chemicals with a wide variety of biological activities, this overcoming the problem of resistance to synthetic drugs can be overcome.

As the world's population is increasing, effective methods of pest control are needed to solve urgent problems of producing enough food to feed the population. Controls involving the use of insecticidal compounds are a major contribution for the foreseeable future. However the current array of insecticides is based on a narrow range of chemical classes. As use of each class of compounds increases, the proportion of insects becoming resistant to them also increases. Furthermore, there have been changes in the target pest species as a consequence of changes in cropping systems and climatic changes. Since then, natural products have played an important role in crop protection. This is because natural products have provided the agrochemical industry with important lead compounds and offer a wider source of chemical diversity than synthetic compounds.

These compounds have activity against a range of target species e.g. mites and flies are low against non-target species which is beneficial to insects and mammals and most importantly are able to breakdown in the environment to non-toxic products. Thus natural products are in accordance to the recently well practiced 'green wave', (Choudhary *et al*., 1997) and (Khambay *et al*., 1997).

1.2 Piperaceae

The genus *Piper* studied in this work belongs to the Piperaceae family and has over 700 species distributed in both hemispheres. *Piper nigrum*, and *Piper sarmentosum* belong to the Piperaceae family. There are five genera in the family of Piperaceae, which consists of *Piper*, *Lepianthes*, *Piperomia*, *Macropiper*, and *Triannaeopiper*. Piperaceae is either shrubs or herbs, often with swollen nodes and are usually aromatic. Leaves alternate are opposite or whorled and connate to the petiole. Flowers are minute unisexual with terminal catkin-like spikes subtended by a peltate bract. Fruits are small and indehiscent in the 1-celled species or have follicles in the many-carpelled species. Seeds are globes, ovoid or oblong. (Ng, 2005)

Piperaceae species are mostly pioneer shrubs with economic and medicinal importance and are widely spread out in the tropical regions.(Benevides *et al*., 1999). Economically, Piperaceae is important in the worldwide spice market.

1.3 *Piper nigrum*

1.3.1 Botany

In Malaysia, *Piper nigrum* is predominantly planted in Sarawak, which account for 95% of the production, with some plantings in Sabah and Johor. *Piper nigrum* is known locally as black pepper. It is a branching vine with a smooth, woody, articulate stem swollen at the joins. A woody climber, it may reach heights of 10m by means of its aerial roots. The leaves are from 4 to 6 inches long, broad and are shiny green or dark green in color. The petiolate leaves are alternately arranged, glossy above, paler beneath, 5 to 7 nerved and the nerves are connected by lesser transverse ones or veins and are prominent beneath. The flowers are whitish, small, not stalked, and are borne in spikes opposite the leaves, chiefly near the upper ends of branches. The flowers have five petals about 2.5 cm wide, white with blue veins appearing between June and September. They yield a seed capsule with five compartments each topped by a spike. The compartments open when dried to disperse the seeds.

Black pepper grows in almost all types of soil. However, it thrives well in loose, well-drained soil. It is best suited to humid climate with a rainfall of 100 to 250cm per year and in an elevation above sea level.

The berry-like fruits, or peppercorns, are small ovoid single-seeded berries. Fruits are about 6mm in diameter. The fruits are initially green, then yellowish-orange, and turn red when ripe. Black pepper is the dry unripe berry. The corns are wrinkled and spherical, about 5mm in diameter. White peppers are allowed to ripe more fully on the

vine. The outer shell is then removed by soaking the berries in water until the shell falls off, or are held under flowing water, yielding a whiter, cleaner pepper. White pepper is hotter, less subtle and mildly fermented. Green pepper is harvested before they mature. The pungency of pepper is due to chavicine (an acid amide yielding piperine and chavicinic acid), (Duke, 2002)

Figure 1.1 The leaves and fruits of *Piper nigrum*

Figure 1.2 The tree of *Piper nigrum*

1.3.2 Medicinal uses of *Piper nigrum*

Black pepper helps to improve digestion and promote intestinal health. Black pepper stimulates the taste buds in which an alert is sent to the stomach to increase hydrochloric acid secretion, thereby improving digestion. Thus black pepper has been recognized as a carminative (a substance that helps prevent the formation of intestinal gas).

Pepper is said to possess febrifuge properties. It acts as a stimulant and is especially evident on the mucous membrane of the rectum, it is good for constipation as well as for the urinary organ problem. Externally it is valued for its ruberfacient properties and is useful in relaxing prolapsed rectum.

The fruit has a sharp, pungent, slightly bitter taste and is useful in relieving toothache, inflammation as well as pain in the liver and the muscles. Black pepper is much employed as an aromatic stimulant in cholera, weakness following fevers and as an alternative in paraplegia and arthritic diseases. It has also been used in the treatment of vertigo and paralysis. It is also used for diarrhea, cholera, scarlatina, and in solution as a wash for *Tinea capititis*.

In recent studies, it has been proven that black pepper has also demonstrated impressive antioxidant and antibacterial effects. Besides that black pepper can helps in imrpoving the digestive tract. The outer layer of the peppercorn stimulates the breakdown of fat cells thus helping in the slimming process and provides energy as well, (Mat-Salleh *et al*., 2002).

Some constituents in black pepper have already been proven to be phenolic compounds. Present in green pepper (*Piper nigrum*) are 3,4-dihydroxyphenyl ethanol glucoside and 3,4-dihidroxy-6-(N-ethylamino) benzamide which were found to inhibit the growth of the foodborne pathogens, *Salmomneli typimurium*, *Straphyloccus aureus*, *Bacillius cereus* and *Eschericha coli*, (Pradhan *et al*.,1999).

Black pepper is also found to have extensive usage in the Ayurvedic system of medicine. (Piperine possesses a variety of biological properties such as CNS stimulant, analgesic, antipyretic and antifeedent activities, (Miyakado *et al*., 1979).

Black pepper has been used to treat seizure disorders in traditional Chinese medicine. A derivative of piperine, antiepilepsirine has also been used in China to treat this disorder as well.

1.3.3 Essential oils of *Piper nigrum*

An essential oil is a concentrated, hydrophobic liquid containing volatile aromatic compounds extracted from plants. These are hydrocarbons (monoterpenes and sequiterpenes), alcohol, ester, aldehydes, ketones, oxides and lactones. It may be produced by distillation, expression, or solvent extraction. Essentials oils are used in perfumery, aromatherapy, cosmetics, incense, medicine, household cleaning products, and as flavor for food items. They are valuable commodities in the fragrance and food industries Essential oil is also known as volatile oil and ethereal oil. It may also be

referred to as “oil of the raw plant material” from which it was extracted, such as oil of clove.

Essential oils form black pepper has a sharp and spicy aroma. Common uses include stimulating the circulation and relief for muscular aches and pain. Skin application is useful for bruises, since it stimulate the circulation.

The most important constituents in the essential oils of *Piper nigrum* are limone (18.8%), β-caryophyllene (15.4%), sabine (16.5%) and β-pinene (10.7%). Monoterpenes hydrocarbons are the main group of the constituents in the essential oil of *Piper nigrum* (Martins *et al*., 1998)

1.4 *Piper sarmentosum*

1.4.1 Botany

Piper sarmentosum is commonly known as kaduk. It is a creeping terrestrial herb about 20cm tall, closely allied in morphological characters to the betel leaf plant, thus is often mistaken for its cousin *Piper betle* leaf plant. Its soft stem has a diameter of 0.3-0.5 cm and is swollen at the joins where roots are formed. The leaves are thin, dark green, ovate, deeply equally cordate, lobes, round with nerves, 18 cm long and 3.6-7.6 cm wide, are alternate and the stipules are joined to the edge of the leaf-stalk. It has single flower spikes with very small flowers that grow opposite a leaf. It has very small fruits. Kaduk is commonly found in all the moist and warm parts of the world and grows easily in tropical lowlands. It grows wild in the forests and is cultivated in villages. It is found

from China to Borneo, Java and throughout the Peninsular Malaysia. It is commonly found as undergrowth in shady and moist areas. (Arifin, 2005)

1.4.2 Medicinal values of *Piper sarmentosum*

Traditionally, the leaves of *Piper sarmentosum* are used to treat malaria, cough and cold, backache and joints pain, toothache and worm. The leaves are boiled and the decoction is rubbed to cure weakness and pain in bones. The decoction of the boiled leaves is also used in treatment of cough, flu, rheumatism, pleurisy and lumbago. The leaf poultice is used externally for headache and as an embrocation for discoloration of the skin. It has been reported that the leaves have very high antioxidant activity. Thus Kadok leaves are an excellent source of natural antioxidant. The leaves have high antioxidant potential and good reducing power that can neutralize free radicals and keep the oxidative stress state in balance. Consumption of the leaves can prevent oxidative related chronic conditions and maintain good health and fitness. Heated leaves are used for poulticing the chest and the throat for cough.

The roots are chewed with areca nuts to cure longstanding cough and asthma, with ginger for toothache and with nutmeg and ginger for pleurisy. The roots can also be used to treat toothache and are an effective remedy for, fungoid dermatitis on the feet. The whole plant of Kadok can be used to treat fever and aids digestion. Meanwhile the fruits can be used as an expectorant.

A few pharmacological studies have been carried out on Kaduk. It has been to have many applications in modern medicine. Kaduk shows anti-microbial activity against

Escherichia coli and *Bacillus subtilis* by inhibiting the growth of the bacteria. A compound namely β-sitosterol isolated from *Piper sarmentosum* demonstrated significant anti-tumor activity. (Vimala *et al*., 2003)

Figure 1.3: The leaves of *Piper sarmentosum*

Figure 1.4: The stalk of *Piper Sarmentosum*

1.5 Alkaloids

From ancient time man has utilized alkaloids as medicines, poisons and magical potions. It has only been four decades ago, since precise knowledge about the chemical structures of many of these interesting alkaloidal compounds have been obtained. They are usually basic, nitrogen-containing compounds of plant origin. However, in the last two decades, amines produced by animals and fungi have also been called alkaloids. Alkaloids can be defined as any nitrogen-containing base has complex molecular structures and they manifest significant pharmacological activity. Such compounds occur only in certain genera and families and are rarely universally distributed in large groups of plants.

Alkaloids are usually derivatives of amino acids, and many have a bitter taste. They are found as secondary metabolites in plants, animals and fungi. In plants they naturally occur in seed-bearing plants and are found in berries, bark, fruits, roots, and leaves.

Alkaloids are usually classified by their common molecular feature, based on the metabolic pathway used to construct the molecule. When not much was known about the biosynthesis of alkaloids, they were grouped under the names of known compounds, even some non-nitrogenous ones (since the structures of these molecules appear in the finished product; the opium alkaloids are sometimes called "phenanthrenes", for example), or by the plants or animals they were isolated from. When more is learned about a certain alkaloid, the grouping is changed to reflect the new knowledge, usually taking the name of a biologically-important amine that stands out in the synthesis process. So far there are nine groups of alkaloids defined. These groups are the pyridine

group, pyrrolidine group, tropane group, quinoline group, isoquinoline group, phenethylamine group, indole group, purine group and terpenoid group.

These groups of alkaloids can be purified from crude extracts by acid-base extraction. Although numerous alkaloids exist, they have similar properties when separated. In general, they are colorless, crystalline solids that are basic, have a ring structure, and have definite melting points. The molecular weight for alkaloids is in the range of 100-900. Alkaloids without oxygen atoms in the structures are usually liquid at ordinary temperature. Alkaloids with oxygen atoms are crystalline. Alkaloids have a bitter taste. Most alkaloids are chiral molecules, and they are optically active.

Alkaloids are by products of normal plant metabolism. It is also thought that alkaloids may provide a means of defense against insects and animals. Alkaloids may be also be a reservoir for molecules that plants often use.

The physiological effects of alkaloids have made them important compounds in the field of medicine. They have been used as painkillers, stimulants, muscle relaxants, tranquilizers, and anaesthetic. The four types of alkaloids that have the most important economic impact include opium, cocaine, caffeine and nicotine.

Alkaloids give characteristic colors when tested with certain reagents. For example, the orange color on the TLC plates shows the presence of alkaloids when the TLC plate is sprayed with the Dragenoff reagent. Meanwhile, the Mayer and Wager reagents give white precipitate for a positive test of alkaloids in the sample, (Kuvian *et al*., 2007) and (Daniel., 2006).

1.6 Microbial Transformations

Throughout the history of mankind, microorganisms have been of tremendous social and economic importance. Men have been using microorganisms in the production of food and beverages before 6000 B.C. The Egyptians used yeast for baking bread. During the course of time, mankind discovered that the microorganism could modify certain compounds by simple, chemically well-defined reactions which further catalyzed by enzymes. These reactions are called microbial transformations. A microbial transformation is a chemical modification (or modifications) carried out by an organism. Microbial transformations do not change the chemical structures of the substrate and the products resemble one another in the process. Bio-catalysis is a relatively green technology. Enzyme reactions can be carried out in water at ambient temperature and neutral pH, without the need for high pressure and extreme conditions, thereby saving energy. Biocatalysts which have proven to be a useful supplementary technology for the chemical industry, allows reactions for which are not easily conducted by classical organic chemistry. In the years to come, it appears that enzyme-based processes will gradually replace conventional chemical-based-methods. Biocatalysts enable functional or chiral groups to be introduced into molecules by utilizing H_2, O_2 or CO_2. In comparison to fermentation processes, enzymatic microbial transformations has fewer side-product formed and expensive fermenters are not required, aeration, agitation and sterility need not necessarily be maintained.

Microbial transformation processes are environmentally friendly as chemical synthesis often results in environmentally unfriendly production processes and lacks substrate