CHEMICAL CONSTITUENTS OF *CUSCUTA CAMPESTRIS* YUNCKER

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KUALA LUMPUR

2012

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DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE
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ABSTRACT

Cuscuta campestris Yuncker (Golden Dodder) is commonly known as Rumput Emas in Malaysia. *Cuscuta campestris* is a weed which has become a serious threat to agriculture.

A phytochemical study on the chemical constituents of sample (*C. campestris*) was performed. The sample was collected along the roadside at Pekan Nanas, Pontian, Johore (N 1° 20’ to N 2° 35’ - E 102° 28’ 59.9” to E 104° 33’ 52.9”). After separation from host plants, the sample was dried and extracted with ethanol. Chromatography method was used in separation and isolation of chemical constituents of sample. Six compounds were isolated *viz.*, sitosterol **99**, pinoresinol **18**, arbutin **22**, kaempferol **30**, quercetin **53**, and astragalin **50**. The structures of these compounds were elucidated through NMR spectra.

The allelopathic potential of ethanolic extract of sample was manifested through inhibition of seed germination and seedling growth. The ethanolic extract of *Cuscuta campestris* inhibited lettuce seed germination when exposed to higher concentration (500 ppm and above). The growth of shoot length and root of all three crops (lettuce, radish and weedy rice) showed significant reduction after treatment with the ethanolic extract. The same bioassay method was used to test the potential of three allelochemicals isolated, *viz.* kaempferol **30**, sitosterol **99** and pinoresinol **18**. All the samples showed stimulatory result on plant growth which could be due to hormesis effect. The response of all assayed species was dose-dependent.

SEBATIAN KIMIA DARIPADA *CUSCUTA CAMPESTRIS* YUNCKER

ABSTRAK

Cuscuta campestris Yuncker juga dikenali sebagai Rumput Emas di Malaysia. *Cuscuta campestris* adalah rumpai yang menjadi satu ancaman serius pada pertanian.

Satu kajian fitokimia ke atas sampel (*C. campestris*) telah dijalankan. Sampel telah dikumpulkan di tepi jalan Pekan Nanas, Pontian, Johor (N 1° 20' - N 2° 35' dan E 102° 28' 59.9" – E 104° 3' 52.9"). Setelah sampel diasingkan daripada perumahnya, proses pengeringan dan pengekstrakan dengan etanol dilakukan. Kaedah kromatografi telah digunakan untuk mendapatkan komponen kimia dalam sampel. Terdapat enam komponen telah dipencilkan, iaitu sitosterol **99**, pinoresinol **18**, arbutin **22**, kaempferol **30**, quercetin **53**, dan astragalin **50**. Struktur komponen-komponen ini telah dikenalpasti melalui spektrum NMR.

Potensi allelopati dalam ekstrak etanol sampel telah dimanifestasikan melalui cara perencatan percambahan dan pertumbuhan biji benih. Ekstrak ethanol *Cuscuta campestris* merencatkan percambahan biji salad apabila terdedah pada kepekatan yang tinggi (500 ppm dan ke atas). Pertumbuhan panjang pucuk dan pemanjangan akar bagi ketiga-tiga tanaman (salad, lobak dan padi angin) menunjukkan pengurangan yang ketara selepas didedahkan kepada ekstrak etanol. Kaedah bioassai yang sama digunakan untuk mengkaji potensi tersebut dalam ketiga-tiga komponen, kaempferol **30**, sitosterol **99** dan pinoresinol **18**. Semua sampel memberi keputusan yang memberangsangkan ke atas kadar pertumbuhan tumbuhan tersebut di mana ia juga boleh dijadikan sebagai penggalak hormon. Tindak balas semua spesis adalah bergantung kepada dos kepekatan.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my heartfelt thanks to both of my supervisors, Prof. Dr. Khalijah Awang and Prof. Dr. Baki bin Bakar for their supervision, guidance and patience in helping me to complete this research.

Besides, I am very grateful to all phytolab members for their help and guidance all the way in my study especially in analyzing NMR spectrum and also on the methods of chromatography. The consideration and encouragement from them enabled me complete this research smoothly.

Apart from that, I would like to thank all the staffs of Chemistry Department, University of Malaya in processing the documents and guiding me in using the instruments like NMR. The staffs like Pak Din, Rafly and Teoh also helping me when separating the plants and storing the plants. Thanks to them.

Thanks to Remy and Adam from Institute Science Biology for their sharing of knowledge about agriculture and software SPSS.

Finally, I would like to express my gratitude to my family members for their support and consideration during my study.

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ABBREVIATIONS

^{13}C	Carbon-13 NMR
^{1}H	Proton NMR
C.	*Cuscuta*
CD_3OD	Deuterated methanol
$CDCl_3$	Deuterated chloroform
COSY	H-H correlation spectroscopy
d	Doublet
dd	Doublet of doublet
DEPT	Distortionless enhancement by polarization transfer
FT	Fourier transforms
g	Gram
HMBC	Heteronuclear multiple bond coherence
HSQC	Heteronuclear single quantum coherence
HPLC	High Performance Liquid Chromatography
Hz	Hertz
IR	Infrared

I.	*Ipomoea*
J	Coupling constant
m/z	Mass to charge ratio
MeOH	Methanol
ml	Mililitre
MS	Mass spectrum
nm	Nanometre
NMR	Nuclear magnetic resonance
OH	Hydroxyl
ppm	Parts per million
s	Singlet
TLC	Thin layer chromatography
UV	Ultraviolet
α	Alpha
β	Beta
δ	Chemical shift
Δ	Delta
λ	Lambda (maximum wavelength)

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Malaysia has about 150,000 recorded species of vascular plants of which only a minority have been the subject of chemical with biological studies[1], and only part of them have been investigated for their medicinal potentials. This huge diversity of the Malaysia flora gives us an opportunity to identify the well-diverse chemical structures from their secondary metabolites, and chemical diversity become the plus factors that makes natural products as excellent candidates for any screening programme[2] to detect potential drug candidates.

The explorations of the plant kingdom for chemical compounds of medicinal value has been going on for thousands of years, from herbalism to folk medicine, from ancient to modern, and have been the source of much useful therapy. During the 19th century organic chemists took up the study of many plant principles, the physiological effect of which had been recognized. Although the structures of many well-known herbal drugs are now known, there is still plenty of scope for finding new and useful drugs[3] from natural resources.

Besides, natural products are also a source of compounds that might be used directly as pesticides or as a template starting point for new synthetic pesticides. Examples of recent successful commercial pesticides discovered from natural products include the spinosad and avermectin insecticides, the strobilurin fungicides, and the triketone herbicides[4]. The environmental half-life of many natural compounds is shorter, and they are generally less toxic to the environment than many synthetic herbicides[5].

Studies of natural substances not only increase and deepen our scientific knowledge, but also provide a basis for a highly developed industry providing people jobs, and help to raise living standards and to treat or prevent disease. In this way excellent services are rendered to humanity[6].

Cuscuta campestris is an annual obligate stem parasitic weed[7] that grows in abandoned area like shrubs and bushes on roadside in Malaysia. It is found to grow commonly on many different species of weeds like *Melastoma malabathricum*, *Mimosa pudica*, *Mikania micrantha*, and *Asystasia intrusa*. This special nature of *Cuscuta* suggests that it may possess herbicidal potential. Hence, this study concerns the chemical and biological assay evaluation of *C. campestris* that was collected from Pontian, Johore in order to understand its potential.

1.2 Family Convolvulaceae

The Convolvulaceae, commonly known as the bindweed or morning glory family, comprising about 57 genera and more than 1,600 species[8]. They are mostly twining and erect herbs, with a few woody vines, trees, and shrubs[8].

Some members of the family are well known as showy garden plants (e.g. morning glory) and as troublesome weeds (e.g. bindweed)[9].

1.2.1 Distribution and Habitat

The Convolvulaceae is a cosmopolitan family. The Convolvulaceae distributed nearly world-wide from North America to Oceania[10] and occupying a broad range of ecological habitats[11]. It is most diverse in tropical and subtropical regions, with representatives having ranges extending into north and south temperate regions; particularly abundant in tropical America and tropical Asia[12]. There are four major centres of diversity for this family, normally Mexico, South America, tropical Africa and Southeast Asia[13].

Since morning glories are mostly vining heliophytes, they prosper best in open, tropical deciduous forests, where short-statured neighboring plants provide adequate support for vining growth and allow the entry of light near ground level. Shaded understories of tropical evergreen forest generally prohibit the establishment of most Convolvulaceae, excepting a few tall lianas (e. g. *Ipomoea phillomega*, *I. reticulata*, *I. santillanii*), and weedy species that exploit riparian and disturbed habitats of this vegetation type (e. g. *I. alba*, *I. batatas*, *Odonellia hirtiflora*)[13].

1.2.2 Morphology[12, 14-17]

Convolvulaceae are herbs or shrubs with annual or perennial vines, commonly with milky sap. The rootstocks are swollen and fleshy to fibrous. The stem of these plants is usually winding, hence its Latin name (Convolvere = to wind).

The leaves are minute and simple, entire to pinnately lobed, pectinate, or palmately compound, exstipulate.

Flowers are solitary and arranged in pedunculate bracteates cymes. Flowers are are often large and showy, ephemeral, usually with intrastaminal disc. There are five distinct and persistent sepals fused at the base occasionally accrescent in fruit. The corolla is funnel shape and showing induplicate valvate aestivation. The flowers consists of five stamens that alternating with corolla lobes, adnate to corolla. The filaments are filiform and the anthers are longitudinally dehiscing. The pollen is smooth or finely spiny. Ovary has two ovules in each locule. Styles are very short or even absent.

Fruits capsules are dehiscing by valves, circumscissile, or irregularly shattering, less often a berry or nutlike and the seeds usually smooth or pubescent.

1.2.3 Tribe[18]

Austin[17] classified Convolvulaceae into nine tribes. However, recent molecular studies that based on multiple data sets from plant genomes had reclassified this family into twelve tribes. The Table 1.1 showed the comparison of the tribes of Convolvulaceae according to traditional (Austin 1973) and newly proposed phylogenetic classification[19].

Table 1.1 Tribes of Convolvulaceae According to Traditional and Newly Phylogenetic Classification[19]

Austin Modified (1973) Tribes	**Phylogenetic Classification Tribes**
i) Argyreiae	i) Aniseieae
ii) Convolvuleae	ii) Cardiochlamyeae
iii) Cressea	iii) Convolvuleae
iv) Cuscuteae	iv) Cresseae
v) Dichondreae	v) Cuscuteae
vi) Ericybeae	vi) Dichondreae
vii) Ipomoeae	vii) Erycibeae
viii) Merremioids	viii) Humbertieae
ix) Poraneae	ix) Ipomoeeae
	x) Jacquemontieae
	xi) Maripeae
	xii) Merremieae

1.2.4 Genera[18]

Table 1.2 showed the genera of Convolvulaceae according to the newly proposed phylogenetic classification.

Table 1.2 Genera of Convolvulaceae

Tribe	**Genera**
Aniseieae	• *Aniseia* Choisy • *Iseia* O'Donell • *Odonellia* K.R.Robertson • *Tetralocularia* O'Donell
Cardiochlamyeae	• *Cardiochlamys* Oliv. • *Cordisepalum* Verdc. • *Dinetus* Buch.-Ham. ex Sweet • *Poranopsis* Roberty • *Tridynamia* Gagnep.
Convolvuleae	• *Calystegia* R.Br. - Bindweed, Morning glory • *Convolvulus* L. - Bindweed, Morning glory • *Polymeria* R.Br.
Cresseae	• *Bonamia* Thouars • *Cladostigma* Radlk. • *Cressa* L. • *Evolvulus* L. • *Hildebrandtia* Vatke • *Itzaea* Standl. & Steyerm. • *Neuropeltis* Wall. • *Neuropeltopsis* Ooststr. • *Sabaudiella* Chiov. • *Seddera* Hochst. • *Stylisma* Raf. • *Wilsonia* R.Br.

Cuscuteae	• *Cuscuta* L. - Dodder
Dichondreae	• *Calycobolus* Willd. ex Schult. • *Dichondra* J.R.Forst. & G.Forst. • *Dipteropeltis* Hallier f. • *Falkia* Thunb. • *Metaporana* N.E.Br. • *Nephrophyllum* A.Rich. • *Porana* Burm.f. • *Petrogenia* • *Rapona* Baill.
Erycibeae	• *Ericybe* Roxb
Humbertieae	• *Humbertia*
Ipomoeeae	• *Argyreia* Lour. - Hawaiian baby woodrose • *Astripomoea* A.Meeuse • *Blinkworthia* Choisy • *Ipomoea* L. - Morning glory, Sweet potato • *Lepistemon* Blume • *Lepistemonopsis* Dammer • *Paralepistemon* Lejoly & Lisowski • *Rivea* Choisy - Coaxihuitl • *Stictocardia* Hallier f. • *Turbina* Raf.
Maripeae	• *Dicranostyles* Benth. • *Lysiostyles* Benth. • *Maripa* Aubl.
Jacquemontieae	• *Jacquemontia* Choisy
Merremieae	• *Decalobanthus* Ooststr. • *Hewittia* Wight & Arn. • *Hyalocystis* Hallier f. • *Merremia* Dennst. ex Endl. - Hawaiian woodrose • *Operculina* Silva Manso • *Xenostegia* D.F.Austin & Staples
Not placed in tribe	• *Pentacrostigma* K.Afzel.

1.3 Genus *Cuscuta* (Dodder - Common Name)

Dodder is classified as a member of the Morning-glory family (Convolvulaceae) in older references, or as a member of the Dodder family (Cuscutaceae) in the more recent publications[20]. Dawson *et al*. (1994) suggested that the vernacular name, "dodder," may come from an old German word, "dotter," used to describe the yolk of an egg[21].

Dodder is phanerogamic stem parasite that able to grow on a plethora of different host plants[22] including those important agricultural plants, ornamental plants and weeds to absorb water, minerals and carbohydrates from the respective host plants.

Other names of this parasite include love vine, strangleweed, devil's-guts, gold-thread, pull-down, devil's ringlet, hellbine, hairweed, devil's-hair, and hailweed[23].

1.3.1 Distribution and Habitat

Cuscuta is a genus of cosmopolitan occurrence, mostly distributed in temperate to subtropical regions. The preference towards a temperate climate is probably related to the cycle of dormancy undergone by the seeds in the soil[24].

There are more than 150 species of dodders worldwide. In Sweden, it reaches the 64th parallel, and it has even found its way to Greenland (Schmucker 1959). The largest number of species is recorded from the southern Canada to Chile and Argentina is without the genus (Yuncker 1932). In southern Argentina, *C. pauciflorum* is known from as far south as 47° Latitude (Hunziker 1949-1950). Dodders are abundant in Europe and Africa also, but less so in Australia and the Indo-Malayan region. Intriguing, the genus has not been found in the Philippine Islands[25].

Cuscuta appears to be well adapted to cultivated situations and succeeds with many crops. However, growth is greatly reduced in shaded areas[26].

It is interesting to note that some species have a narrow host range (e.g., *C. epilinum*), while others (e.g. *C. campestris*) are capable of parasitizing numerous species from various families[24]. Gaertner (1950) reviewed the literature and studied host relationships experimentally for 609 species in 75 families for 10 dodder species. She found no single dodder species that was specific for a single host[25]. Dodders may also parasitize on other dodders.

1.4 *Cuscuta campestris* Yuncker

Cuscuta campestris, otherwise also known as dodder belongs to the family Convolvulaceae. It is an annual obligate angiosperm parasite with golden yellow color[7]. This parasite twines on other plants and attaches to the above-ground parts of a wide range of host plants[27]. A single plant of *C. campestris* may attack many host plants at a time. In this research, *C. campestris* is collected from Johore (N 1° 20’ - N 2° 35’- E 102° 28’ 59.9” - E 104° 33’ 52.9”), peninsular Malaysia. It can grow on more than 80 types of host plants, and we can commonly found it on *Asystasia gangetica* and *Mikania micrantha*. In Johore, this plant can be found in abandoned area like shrubs and bushes along the roadside. We collected *C. campestris* along the way from Pontian to Pekan Nanas. *Cuscuta campestris* that we collected were long branched, succulence, entangled with its own and the host plants[20].

Local people in Johore used *C. campestris* as a traditional medicine to treat impotence and as a tonic for seminal emission. According to previous research, *C. chinensis*, *C. australis*, and *C. japonica* that are similar used as herbs in the traditional Chinese medicine[28].

1.4.1 Distribution and Habitat[24, 25]

Cuscuta campestris is the most widely distributed species within the genus and also the most troublesome. *Cuscuta campestris* is a true indigene of North America and it spreads to Africa, Europe, South America, China, and Australia (Holm *et al.* 1997). This species is semi-cosmopolitan that exhibit from temperate to subtropical region.

1.4.2 Morphology[29-31]

Stems of *C. campestris* are slender and medium (0.40 – 0.60 mm thick) with yellow to orange color, smooth or tuberculate (Fig. 1.1).

Flowers are (4 – 5)-merous, 2.1 – 4.6 (–5) mm long, white color, membranous, with pellucid, gland-like laticiferous cells evident in the calyx. Corolla is with campanulate tube, ca. 1.5 - 1.9 mm long with triangular-lanceolate lobes. Stamens are exerted; anthers are broadly elliptic and about 0.3 – 0.5 mm long; filaments are 0.4 – 0.7 mm long. Stamens are shorter than corolla lobes, filaments longer than or equaling anthers. Scales are large, adnate only at base, much-fringed apically and almost or quite reaching the anthers. Ovary is globose; styles slender and becoming thicker and conspicuous in fruit. Styles are filiform, about 0.8 – 1.6 mm. Capsules are indehiscent or irregularly dehiscent, depressed-globose to depressed, with size of 1.3 - 3 × 1.9 - 3.5 mm, with a large and conspicuous interstylar aperture, with the withered corolla surrounding the lower part.

Fruit is like a subglobose capsule up to 4.2 mm in diameter. Seed subglobous or ovoid shape, about 1mm, usually flattened on one side.

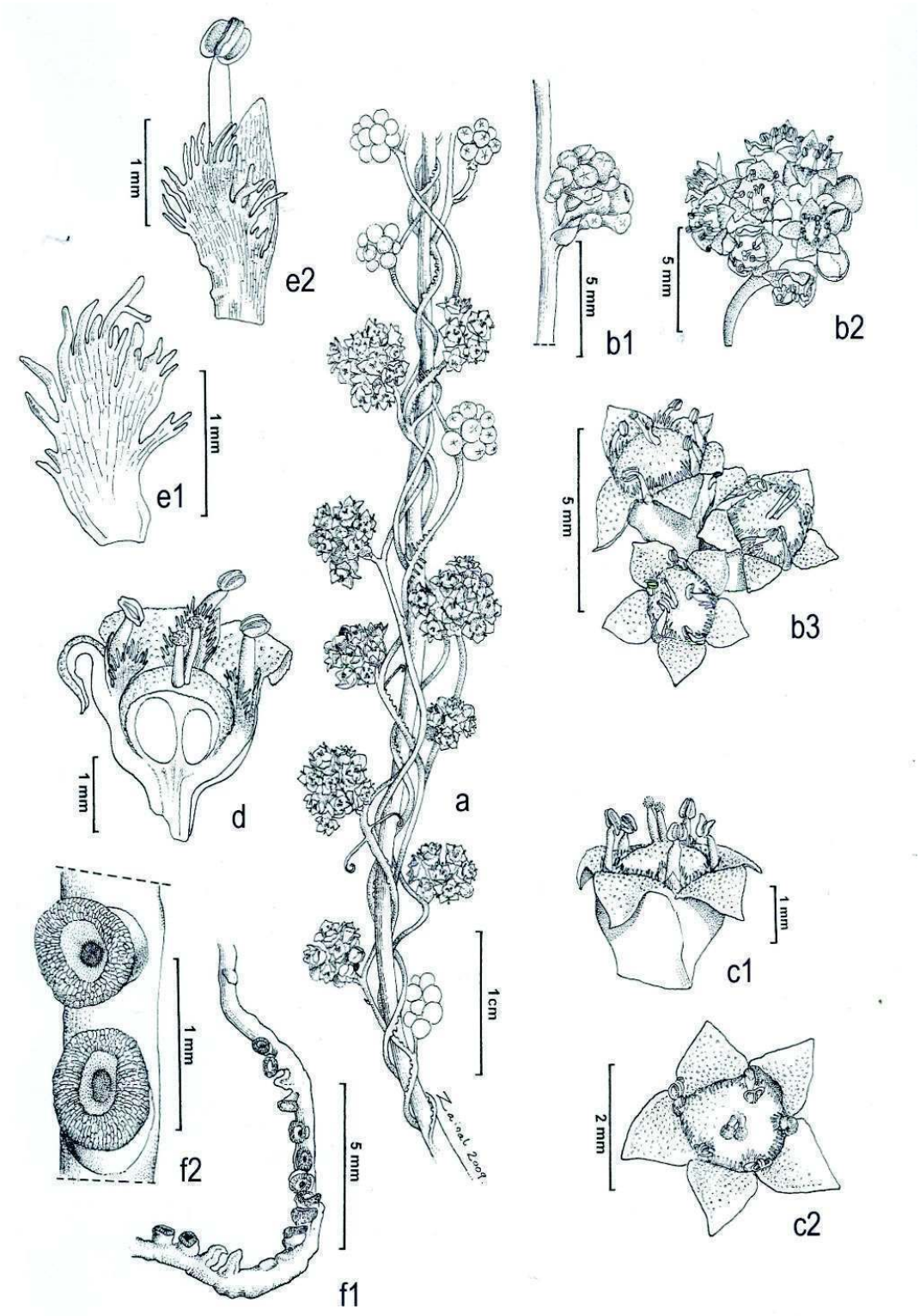


Figure 1.1 ***Cuscuta campestris*** **Yuncker. (a) flowering branch, (b1) detail of inflorescence, (b2) floral cluster, (b3) top-view of a cluster of three flowers. (c1) side-view of a flower, (c2) top-view of a flower showing exposed stigmas and stamens, (d) flower, longitudinal section, (e1, e2), appendage enveloping pollen tube, (f1) branch with haustoria, and (f2) closer-view of haustoria**

1.5 Biological and Pharmacological Importance of *Cuscuta*

Cuscuta is a famous traditional herb in Asia. For example, *Cuscuta chinensis* is used in China to treat deficient of kidney and liver causing vision disorders, as well as chronic diarrhea that due to deficiency of spleen Yang[28]. The following paragraphs will briefly discuss on the biological activities of some *Cuscuta* spp. and compounds isolated from them.

1.5.1 Antibacterial Activity[32]

The methanol extract of *C. reflexa* at the dose of 125 μg/ mL showed significant antibacterial activity against *Staphylococcus aureus*, *Shigella boydii*, *Pseudomonas aeruginosa*, *Shigella dysenteriae* and *Escherichia coli* with a zone of inhibition ranging from 16 to 24 mm.

1.5.2 Antiviral Activity[33]

Mahmood *et al.* (1997) tested the aqueous extract of *C. reflexa* on its anti-HIV activity and obtained a positive result. They isolated 9 constituents consisting flavanones and caffeoyl quinic acid. However, the activity of flavanones was found to be more toxic and less active. They suggested that the anti-HIV activity of crude extract may be the result of combined effects with compounds of different modes of action.

Awasthi (1981) reported that a protein with molecular weight around 14,000 to 18,000 Daltons had been isolated from aqueous extract of *C. reflexa* plants. The antiviral activity of the protein was increased several folds. This protein prevented the infection of several isometric and anisometric viruses in their hypersensitive and systemic hosts.

1.5.3 Antioxidant Activity[28]

Yen *et al.* (2007) observed the ethanol extract of *C. chinensis* by assessing the DPPH (1,1-diphenyl-2-picryl hydrazine) free radical scavenging, superoxide anion scavenging, anti-superoxide anion formation, and anti-lipid peroxidation abilities. The results showed that the ethanol extract of *C. chinensis* was an effective antioxidant for preventing free radical damage to cell membranes through scavenging of free radicals, and by inhibiting the lipid peroxidation process.

1.5.4 Enzyme Inhibition Studies[34]

Anis (2002) examined the enzyme inhibitory activity against α-glucosidase type VI of some constituents that was extracted from *C. reflexa*. The result showed that 7'-(3',4'-dihydroxyphenyl)-*N*-[(4-methoxyphenyl) ethyl] propenamide and 7'-(4'-hydroxy, 3'-methoxyphenyl)-*N*-[(4-butylphenyl) ethyl] propenamide showed strong inhibitory activity. Besides, 6,7-dimethoxy-2*H*-1-benzopyran-2-one and 2-(3-hydroxy-4-methoxyphenyl)-3,5-dihydroxy-7-*O*-β-D-glucopyranoside-4*H*-1-benzopyrane-4-one showed moderate inhibitory activity.

1.5.5 Neurite Growth Activity[35]

The extract of *C. chinen*sis showed neuro activity. *C. chinensis* glycoside significantly promoted the neurite growth and increased AChE activity in PC12 cells in a dose-dependent manner.

1.5.6 Immunity[36]

Pan *et al.* (2005) reported that ethanol extract of Semen *Cuscuta* significantly enhanced the mitogen and OVA-stimulated splenocyte proliferation in OVA-immunized mice. The ethanol extract of Semen *Cuscuta* is effective on Th1 and Th2 cell at suitable dose. The result showed that ethanol extract of Semen *Cuscuta* could significantly enhance a specific antibody and cellular response against OVA in mice.

1.5.7 Effect on Alopecia[37]

Pandit *et al.* (2008) reported that the petroleum ether extract of *C. reflexa* and its isolate can be used to treat androgen-induced alopecia by inhibiting the enzyme 5-α-reductase. The extract exhibited hair growth-promoting activity as reflected from follicular density, anagen/telogen ratio, and skin sectors. 5-α-reductase activity was inhibited and the result suggested that the extract reversed androgen-induced alopecia by inhibiting conversion of testosterone to dihydrotestosterone.

1.5.8 Anti-inflammatory and Antipyretic Activities[38]

Backhouse *et al.* (1996) examined the anti-inflammatory and antipyretic properties of infusion and methanol extract of *C. chilensis*. The experiment they did was based on the reduction of bacterial pyrogen-induced fever in rabbits and carrageenan-induced paw edema in guinea pigs. They found that the infusion of *C. chilensis* reduced bacterial pyrogen-induced fever, which showed that it exerted strong antipyretic effect, with an area reduction of 43%, but the methanol extract gave negative result. Besides, both extracts exhibited strong anti-inflammatory activity.

1.5.9 Insect Growth Regulatory Effect[39]

Maragenin was isolated from petroleum ether extract of *C. reflexa* by Srivastava (1990). This triterpenoid was found to possess growth regulatory effects on *Dysdercus cingulatus* nymphs.

1.6 General Aspects on Allelopathy[40, 41]

Allelopathy (rootwords: allelon and pathos) is derived from the Greek allelon, 'of each other', and pathos, 'to suffer'; hence it means: the injurious effect of one upon another. The term denotes that body of scientific knowledge which concern on the production of biomolecules by one plant, mostly secondary metabolites, that can induce suffering in, or give benefit to, another plant. The phenomenon could also be considered as a biochemical interaction among plants. The concept suggests that biomolecules (specifically termed allelochemicals) produced by a plant escape into the environment and subsequently influence the growth and development of other neighbouring plants.

Most of the allelopathic compounds released are hydrophilic, such as phenolic acids, alkaloids, flavonoid glycosides, etc. Many compounds were identified by various workers in different habitats. (Bode, 1940; Del Moral and Muller, 1969; Rice, 1984; Chou, 1999; Cutler and Cutler, 1999; Kohli *et al.,* 2001; Macias, *et al*., 1997).

There is a myriad of plant, microbe, and animal natural products with an enormous range of structural diversity (Henkel *et al.*1999) that arose from co-evolution between competing organisms. Thus, most secondary metabolites are biologically active. Historically, natural products have been used as pesticides, either directly as crude preparations, as pure compounds, or as structural leads for the discovery and development of natural product-based pesticides. The impact of natural products have

historically been greater on the development of fungicides and insecticides than on herbicides (Dayan, 2002), but the potential benefits of natural product-based herbicides remain underestimated.

The mode of action of allelochemicals can broadly be divided into indirect and direct actions. The indirect action may include effects through alteration of soil property, its nutritional status and an altered population and/or activity of harmful/beneficial organisms like microorganisms, insects, nematodes, etc., and this is relatively a less studied aspect. On the other hand, the direct mode of action, which includes effects of allelochemicals on various aspects of plant growth and metabolism, has received fairly wide attention.

As noted by Winter (1961), the visible effects of allelochemicals on plant processes are only secondary signs of primary changes. Therefore, studies on the effects of allelochemicals on germination and/or growth are only the manifestation of primary effects occurring at the molecular level. Although a strong tendency is being developed to look into the actual mechanism of action, the experimental work is in its infancy.

Bioassay is the simplest forms used in studies of allelopathy by quantify germination and/ or emergence of seedlings and to measure the length of the radical and shoots or their equivalents.

Many annual and perennial weeds have allelopathic activities and can affect crop survival and productivity[42]. *Cuscuta* spp. are found to contain a lot of chemical constituents, in which some of them are synthesized by the parasite itself and some are diverted from the host plants. Phenolic constituents were found to be synthesized by the parasite itself and according to previous research, polar constituents contains allelopathic effects[43]. Hence, one of the purposes of this research is to determine the allelopathic effect of *Cuscuta* through bioassay.

1.7 Host Plant Effects on *Cuscuta* Spp.

The relationship between parasitic species and their hosts can be described as a kind of compatible/antagonistic one since both involve chemicals that stimulate germination and attachment and others incompatible phytotoxic or prevent germination, attachment, growth or development of the parasite.

Cuscuta is a type of parasite that is capable in attacking a broad range of host species. Thigmotropic responses and chemical recognition cause *Cuscuta* spp. to develop haustoria when attached to suitable host plants. This parasite will connect the xylem and phloem of the host plants to divert resources (water and nutrients) from the hosts to itself through the haustoria[44].

Khan (1968) found that the four host plants (*Zizyphus jujube, Clerodendron inerme, Citrus medica* and *Accacia arabica*) of *C. reflexa* will not influence the chemical built up of *Cuscuta*[45].

Soluble phenolic compounds from *C. reflexa* and *C. platyloba* showed no qualitative or quantitative influence from the hosts on the phenylpropanoid patterns. Hence, the soluble phenolic compounds are assumed to be synthesized exclusively by the parasite itself[46].

However, *Cuscuta campestris* harvested from different host plants were found to contain different chemical constituents, which indicated that host plants may influence the metabolism of the parasite plant[47]. For example, D-mannitol was isolated from *C. reflexa* that grows on *Santalum album* whereas this compound was not found on *C. reflexa* that grows on other hosts[48]. In addition, Wink (1993) also discovered that quinolizidine alkaloids were present in *C. reflexa* that grows on *Lupinus angusufolius* whereas the latter is known to be a rich source of these alkaloids[49].

1.8 Thesis Structure

The work in this thesis was reported in six chapters. Chapter 1 of this thesis embodied the general introduction. This was followed by Chapter 2 which discussed the general chemical aspects, while Chapter 3 reported the results and discussions of the chemical constituents. The ensuing chapter discussed the allelopathic potential of *C. campestris*. The conclusions formed the body of Chapter 5, while the final chapter discussed the experimental parts of this research project.

1.9 Objectives

The objectives of this study are:

(i) To isolate the chemical constituents from the ethanol extract of *C. campestris*.

(ii) To elucidate the structures of chemical constituents of *C. campestris* by spectral ^{1}H, ^{13}C, COSY, HMBC, HSQC NMR, IR, MS and UV.

(iii) To evaluate the allelopathic potential of the ethanol extract and selected compounds isolated from *C. campestris* on seed germination and seedling growth of tested plants.

CHAPTER 2

GENERAL CHEMICAL ASPECTS

2.1 General Definition

Cuscuta spp. is known to contain numerous amounts of chemical substances depending on different host effects[20]. Scientists from different fields like chemists, biologists, and botanists had done a lot of researches on *Cuscuta* spp. in order to obtain bioactive constituents from them. In this chapter, the general chemical characteristics and chemical constituents of *Cuscuta* spp. will be discussed.

2.2 Aromatic Compounds[50, 51]

Phenolic compounds are a large group of molecules that play different functions in plant growth, plant development, and plant defense system. Phenolic compounds including those signaling molecules, pigments and flavors can attract or repel, as well as those that can protect the plant against insects, fungi, bacteria, and viruses.

Based on the number of carbons in molecule, Harborne and Simmonds (1964) had classified the compounds into group (Table 2.1).

Table 2.1 Classification of the Aromatic Constituents in Plants[52]

Structure	Class
C_6	simple phenolics
C_6-C_1	phenolic acids and related compounds
C_6-C_2	acetophenones and phenylacetic acids
C_6-C_3	cinnamic acids, cinnamoyl aldehydes, cinnamoyl alcohols
C_6-C_3	coumarins, isocoumarins, and chromones
C_{15}	chalcones, aurones, dihydrochalcones

C_{15}	flavans, flavones, flavanones, flavanonols
C_{15}	anthocyanidins, anthocyanins
C_{30}	biflavonyls
C_6-C_1-C_6, C_6-C_2-C_6	benzophenones, xanthones, stilbenes
C_6, C_{10}, C_{14}	quinones
C_{18}	betacyanins
Lignans, neolignans	dimers or oligomers
Lignin	polymers
Tannins	oligomers or polymers
Phlobaphenes	polymers

2.2.1 Biosynthesis of Aromatic Compounds

There are three major biosynthesis pathways of aromatic compounds. They are:

(i) From glucose via Shikimic acid[51]

This pathway is a key intermediate for the biosynthesis of C_6-C_3 units (phenyl propane derivative) from carbohydrates. Shikimic acid serves as precursor for the biosynthesis of amino acids; while being also an intermediate in production of tannins, flavones, coumarins, and vanillin. This pathway is thus responsible for all life.

(ii) From acetic acid/ polyketide[50, 51]

This pathway starts from acetate and malonate residues that are aligned in a regular head-to tail fashion, -CO-CH_2-CO-CH_2-; acetate occupies a central position in relation to the general metabolism of plants. Acetate condensation occurs in many possible routes, which give rise to a variety of aromatic compounds.

(iii) From mevalonic acid[51]

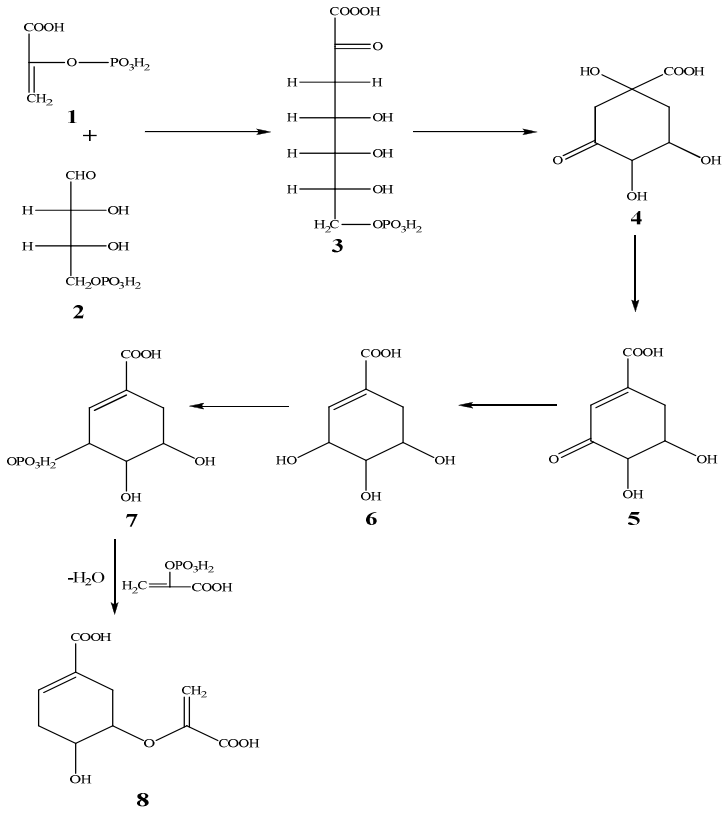
This pathway starts with mevalonic acid and produces a variety of aromatic terpenoids, carotenoids, and steroids.

Majority of the aromatic chemical constituents that are found in *Cuscuta* spp. are formed through Shikimic pathway. Hence, Shikimic pathway will be discussed in this chapter.

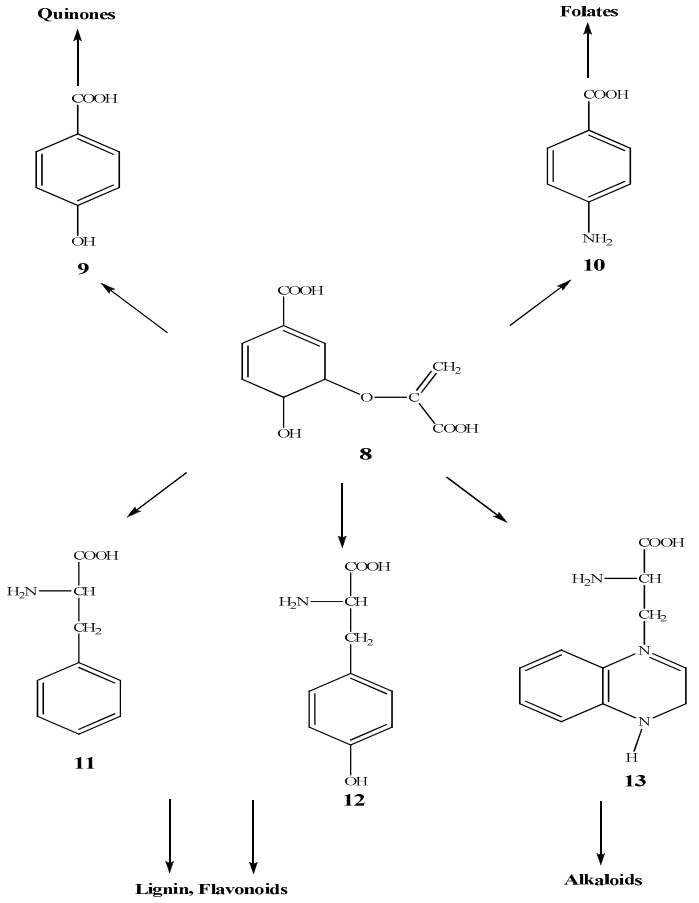
2.2.2 The Shikimic Pathway[50, 53, 54]

Shikimate is synthesized from the substrates phosphoenolpyruvate **1** and erythrose 4-phosphate **2**. The aldol-type condensation between these two precursors by enzyme DAHP synthase gives 3-deoxy-D-arabinoheptulosonic 7-phosphate (DAHP) **3**. Ring closure in DAHP results in the formation of dehydroquinic acid (DHQ) **4** by the enzyme 3-dehydroquinate synthase. DHQ **4** undergoes reversible dehydration to give dehydroshikimic acid **5** in the presence of the enzyme 3-hydroquinate dehydratase. Finally, shikimic acid **6** is synthesized by the enzyme shikimate dehydrogenase (Scheme 2.1).

Chorismic acid **8** is the branch point for the biosynthesis of aromatic amino acids. Reduction of chorismic acid **8** and its incorporation with ammonia (from the amino acid glutamine) via anthranilic acid leads to the formation of tryptophan **13**. The formation of phenylalanine **11** and tyrosine **12** proceeds via prephenic acid that is formed from chorismic acid **8** involves Claisen rearrangement. The phenylalanine **11** and tyrosine **12** are the precursors of the vital class of phenolic compounds-phenylpropanoids, and also other classes of phenolic compounds (Scheme 2.2).



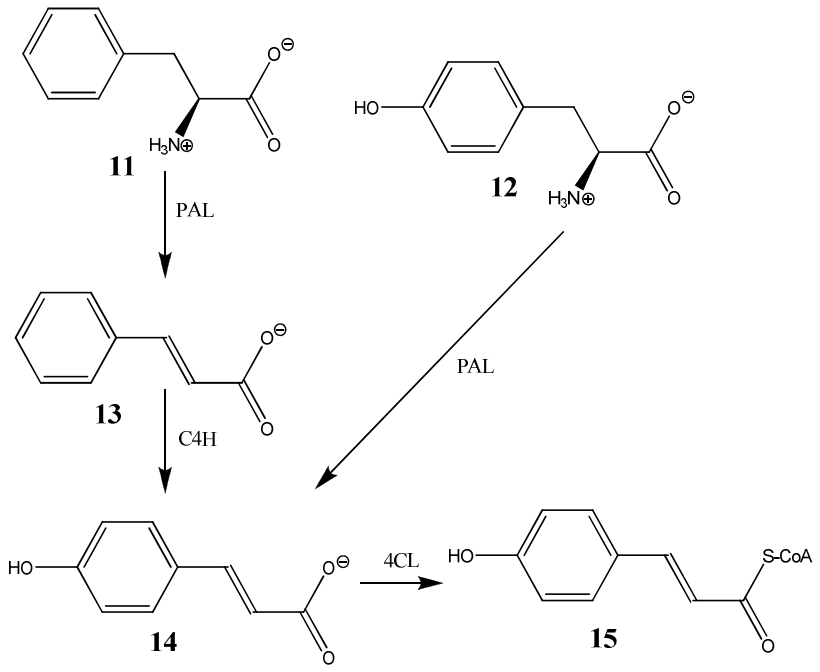
Scheme 2.1 Formation of Chorismic Acid[54]



Scheme 2.2 Chorismic Acid as a Precursor[53]

2.2.3 The Phenylpropanoid Pathway[50, 52]

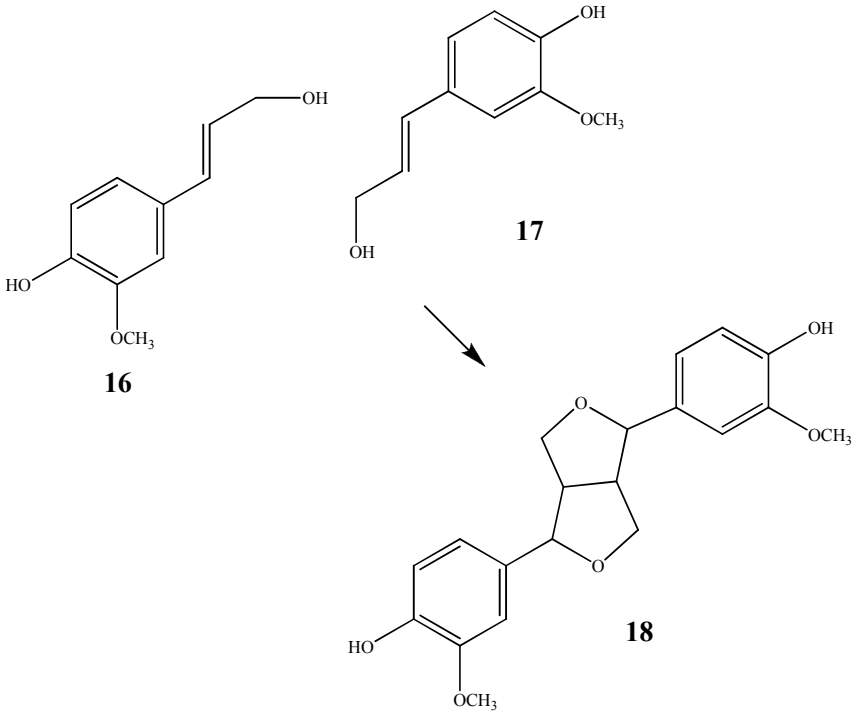
This pathway generates a number of phenylpropanoid compounds including flavonoids, monolignols, hydroxycinnamic acids, sinapoyl esters, coumarins, and stilbenes. Elimination of ammonia from phenylalanine **11** (generated via shikimate pathway) is catalyzed by the enzyme phenylalanine ammonia lyase (PAL) leading to the formation of cinnamic acid **13**. Cinnamic acid **13** is then hydroxylated by cinnamic acid 4 –hydroxylase (C4H) and resulting in *p*-coumaric acid **14**. However, *p*-coumaric acid **13** may also be the product derived by loss of ammonia from tyrosine **12**. *P*-coumaric acid **14** is converted to *p*-coumaryl coenzyme A **15** by the enzyme 4-coumaric acid: CoA ligase (4CL). This compound may be synthesized to specific classes in further reactions (Scheme 2.3).



Scheme 2.3 The Phenylpropanoid Pathway[50]

2.2.4 Biosynthesis of Lignan[55]

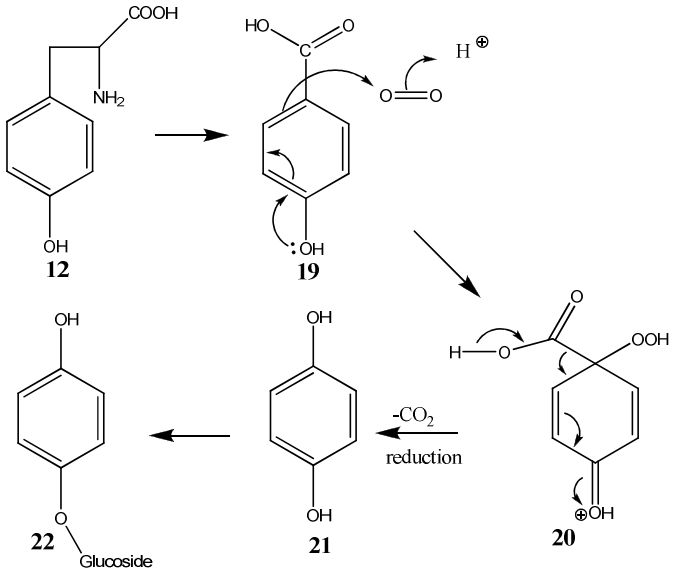
Lignan are dimmers formed from the same monomeric species and invariably optically active. They are probably formed by stereospecific, reductive coupling between the two central carbon atoms of cinnamyl side-chains. Lignan are synthesized from the oxidative coupling of monolignol radicals that are generated from the reaction of laccases or peroxidases (Scheme 2.4).



Scheme 2.4 Formation of Pinoresinol[55]

2.2.5 Biosynthesis of Arbutin[55]

Arbutin is the β-D-glucoside of hydroquinone. *P*-hydroxybenzoic acid is first formed from tyrosine. Then, oxidative decarboxylation subsequently leads to hydroquinone and hence arbutin (Scheme 2.5).



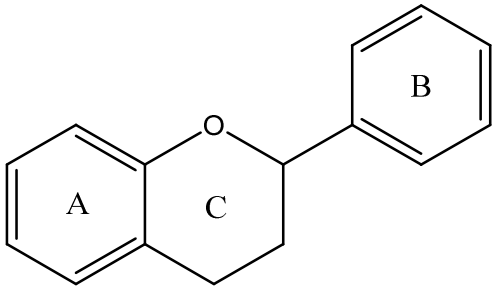
Scheme 2.5 Formation of Arbutin

2.3 Flavonoids[56, 57]

Flavonoids and their conjugates form a very large group of natural products. They are found in many plant tissues, where they are present inside the cells or on the surfaces of different plant organs[58]. These compounds are found in virtually all plants and their role in pollination is well known. In other instances, flavonoids are important in limitation or selectivity of herbivory. Flavonoids may play a role by screening harmful ultraviolet light from sensitive areas within plants. Many flavonoids appear to be involved in nitrogen fixation[41].

They are formed in plants from the aromatic amino acids phenylalanine and tyrosine, and malonate[59]. The word flavonoid coins from Greek word “flavus” meaning yellow, is originally applied to the yellow plant pigments but with the revelation of thousands of structures[60]. The term “flavonoid” is generally used to describe a broad collection of natural products that include a $C_6C_3C_6$ carbon framework, or more specifically a phenylbenzopyran functionality[58]. The 15 carbon atoms are arranged in 3 rings, labeled as rings A, B and C. The various classes of flavonoids differ in the level

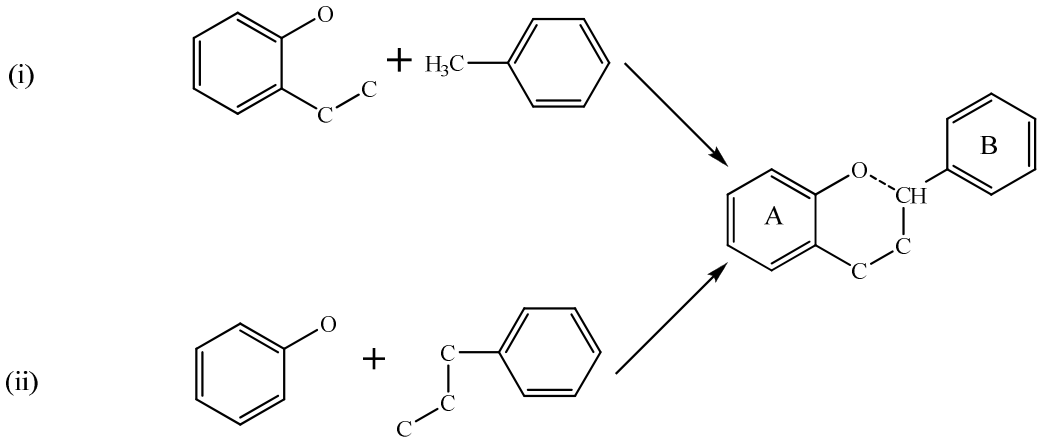
of oxidation and pattern of substitution of the C-ring, while individual compounds within a class differ in the pattern of substitution of the A and B rings[59].



There are two ways in the formation of the C_6-C_3-C_6 flavonoid skeleton, *viz.*

(i) Condensation of a C_6C_2 unit (2-hydroxyacetophenone) with a C_6C_1 unit (aromatic aldehyde); (Scheme 2.6)

(ii) Acylation of phenols (C_6 unit) with a cinnamic acid derivative or its equivalent (C_6C_3 unit) (Scheme 2.6)



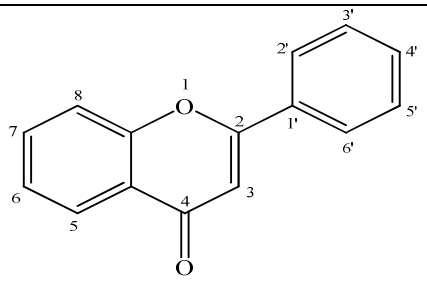
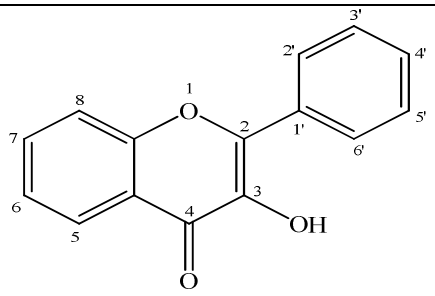
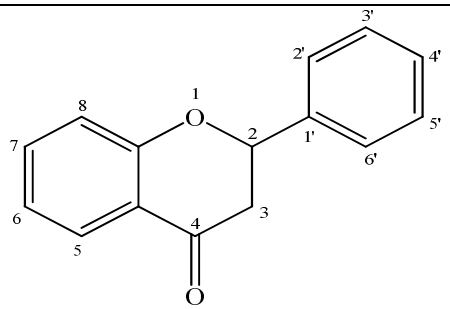
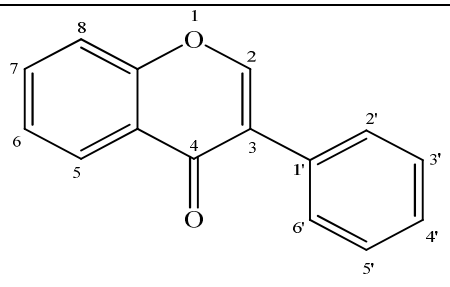
Scheme 2.6 Formation of the C_6-C_3-C_6 Flavonoid Skeleton[56]

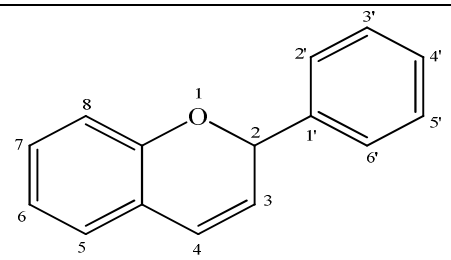
The family includes flavonols, flavonones, anthocyanidins, flavones, and isoflavones (Table 2.2). Along with the phenyl propanoids or hydroxycinnamic acid derivatives (C_6C_3), flavonols, and to lesser extent flavones, are found in almost every plant. While flavonones and flavones are often found together (e. g., in citrus fruits) and are connected by specific enzymes, there is a certain mutual exclusion between flavones

and flavonols in many plant families and anthocyanins are almost absent in flavanone-rich plants[61].

The class, general structure and substitution pattern of flavonoids are displayed in Table 2.2.

Table 2.2 Classification of Flavonoids[56]

Class	General Structure	Flavonoid	Substitution Pattern
Flavone		Chrysin	5,7-OH
		Apigenin	5,7,4'-OH
		Rutin	5,7,3',4'-OH, 3-rutinose
		Luteolin	5,7,3',4'-OH
Flavonol		Kaempferol	3,5,7,4'-OH
		Quercetin	3,5,7,3',4'-OH
		Myricetin	3,5,7,3',4',5'-OH
		Isorhamnetin	3'-Me
Flavanone (Dihydro-flavone)		Naringin	5,4'-OH,7-rhamnoglucose
		Naringenin	5,7,4'-OH
		Taxifolin	3,5,7,3',4'-OH
		Eriodictyol	5,7,3',4'-OH
Isoflavone		Genistein	5,7,4'-OH
		Daidzein	7,4'-OH
		Orobol	5,7,3',4'-OH
		Tectorigenin	5,7,4'-OH,6-OMe

Anthocyanidin		Apigenidin	5,7,4'-OH
		Cyanidin	3,5,7,3',4'-OH
		Delphinidin	3,5,7,3',4',5'-OH
		Pelargonidin	3,5,7,4'-OH

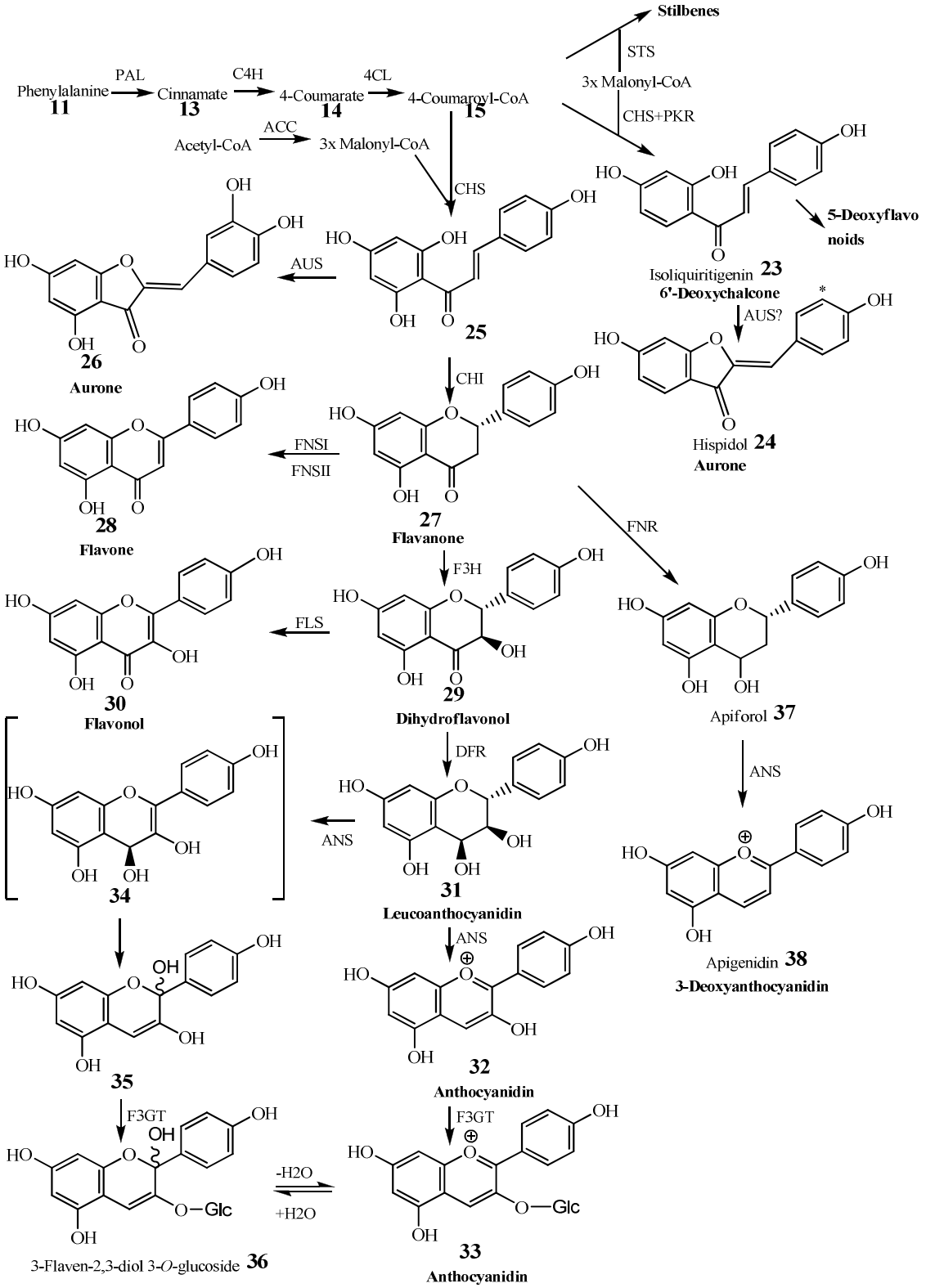
2.3.1 Biosynthesis of Flavonoids[55, 62]

Flavonoid biosynthetic pathways form a lot of branches, and the best characterized being those leading to the colored anthocyanidins and proanthocyanidins (Pas) and also the generally flavones, flavonols, and isoflavonoids.

Flavonoid biosynthesis is initiated from the condensation of 4-coumaryl-CoA **15** with three molecules of malonyl-CoA. Chalcone synthase (CHS) is the enzyme that synthesizes this reaction, and generated naringenin chalcone **25**. An aurone- aureusidin **26** is formed by aureusidin synthase from naringenin chalcone **25**. This product is the yellow pigments that commonly found in the flower petals.

Naringenin chalcone **25** catalyzed by chalcone isomerase (CHI) and converted into a six member ring flavanone naringenin **27**. Naringenin **27** is subsequently synthesized by flavanone 3-hydroxylase (F3H) and gives the dihydroflavonol-dihydrokaempferol **29**. This compound can be converted by flavones synthase (FLS) to yield flavonol kaempferol **30** (Scheme 2.7).

Alternatively, the dihydroflavonol can also yield anthocyanidins. This involves a few steps whereby they are first reduced to leucoanthocyanidins - leucopelargonidin **31** by the enzyme dihydroflavonol 4-reductase (DFR), followed by dehydration by the enzyme of anthocyanidin synthase (ANS) and also glycosylation. ANS is a 2-oxoglutarate-dependent oxygenase that is thought to abstract a hydrogen radical from C2 of leucoanthocyanidin to yield the radical. Following a second hydrogen abstraction



Scheme 2.7 The Biosynthesis Pathway of Flavonoids

at C3, the 2-flaven-3,4-diol **34** is formed. This reaction may also occur in reverse order, whereby abstraction of the hydrogen at C3 followed by the one on C2. The 2-flavene-3, 4-diol **34** is hydrated to 3-flavene-2,3-diol **35**, and yield anthocyanidin-pelargonidin 3-*O*-glucoside **33** under acidic condition. The glycosylation of anthocyanidins-pelargonidin **32** results in the formation of anthocyanins-pelargonidin 3-*O*-glucoside **33** by the enzyme flavonoid 3-*O*-glucosyltransferase (F3GT) (Scheme 2.7).

2.3.2 Flavones[63, 64]

Flavones and flavonols are the most abundant group among all the naturally occurring flavonoids. Flavones are a class of flavonoids based on the backbone of 2-phenyl-1-benzopyran-4-one.

A lot of higher plants have white, ivory or cream flowers; in addition, cyanic-flowered plants in cultivation often produce acyanic varieties. The vast majority of such flowers are pigmented by the common flavonols or flavones. The function of flavones is to add “body” to petals which would otherwise be translucent. These colorless flavones absorb strongly in ultra-violet, can be “seen” by bees and possibly other insects and presumably provide the flowers with a satisfactory means of attracting pollinating insects.

2.3.3 Flavonols[64]

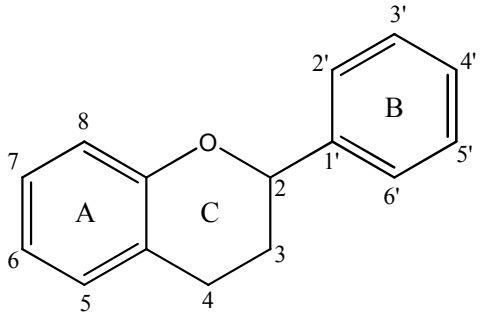
Flavonols are very similar to flavones; the only different is the substitution of a hydroxyl group on the 3-position of flavonols. Besides, the two groups can be distinguished also in their spectral and color properties. Flavones and flavonols are distinguishable by chromatographic method. Flavonols are known to be responsible for yellow flower color in a number of plants.

2.3.4 Flavone and Flavonol Glycosides[65]

Flavone and flavonol *O*-glycosides make up one of the largest classes of flavonoid constituents with over 2000 known structures. The most usual place of sugar attachment in flavonols is at the 3-hydroxyl and at the 7-hydroxyl in flavones but sugar have been found at all the other possible positions.

2.4 Identification of Flavonoids

Many flavonoids have been elucidated structurally by spectroscopic methods such as UV absorption and NMR. The following paragraphs shall discuss briefly on the general spectrum features of flavonoids.



2.4.1 Ultraviolet and Visible Absorption Spectroscopy[57, 64, 65]

UV spectroscopy has become a major technique for the structural analysis of flavonoids for two main reasons. One of the reasons is only a minute amount of pure material is required, and the second is that the amount of structural information gained from a UV spectrum is considerably enhanced by the use of specific reagents which react with one or more functional groups on the flavonoid nucleus.

The UV spectra of most flavonoids consists of two major absorption maxima, one of which occurs in the range 240 - 285 nm (band II) and the other in the range of

300 - 400 nm (band I). In general terms the band II absorption may be considered as having originated from the A-ring benzoyl system, and band I from the ring-B cinnamoyl system.

2.4.2 Proton NMR Spectroscopy

2.4.2.1 A-Ring Protons[57, 64, 67]

For 5,7-Dihydroxyflavonoids, the protons at C-6 and C-8 appear separately as doublets (d, *J*= 2.5 Hz) in the region δ5.7 - 6.9. The H-6 doublet always occurs at upfield compared with the H-8, and glycosylation at 7-hydroxyl group causes both signals to shift to lower field. The H-6 and H-8 signals can be distinguished from each other by their widely different paramagnetic-induced shifts.

The additional C-5 proton in 7-hydroxyflavonoids is strongly deshielded by the 4-keto group. Due to ortho-coupling with H-6, it appears as a doublet (d, *J*-ca, 9Hz) near δ8.0. Signals for H-6 (a quartet, q, *J*= 9 and 2.5 Hz) and H-8 (a doublet, d, *J*= 2.5 Hz) occur at lower field compare to that in the 5,7-dihydroxyflavonoids, and may even reverse their positions relative to one another.

2.4.2.2 B-Ring Protons[57, 64, 67]

In 4'-oxygenated flavonoids, the protons at position of C-2', 3', 5' and 6' exist as two pairs of ortho-coupled doublets (d, *J*= ca, 8.5 Hz) in the region δ6.5 - 7.9 due to free rotation in B-ring. The H-3', 5' doublet always occurs more upfield compared with the H-2', 6' doublet due to the shielding effect of the oxygen substituent and also the deshielding influence from C-ring on H-2' and 6'. The chemical shift of the H-2', 6' doublet is dependent upon the level of oxidation of the C-ring.

In 3',4'-dioxygenated flavonoids, the C-5' proton in 3',4'-dioxygenated flavones and flavonols appears as a doublet (d, *J*= 8.5 Hz) in the range δ6.7 - 7.1. However, in 3',4',5'-trihydroxylated flavonoids, H-2' and H-6' are equivalent, and appear as a two proton singlets in the range δ6.5 - 7.5.

2.4.2.3 C-Ring Proton

Flavones[57, 64, 67]

The C-3 proton in flavones usually appears as a sharp singlet near δ6.3. However, we may confuse it with C-6 or C-8 proton signals in 5,6,7-, 5,7,8- or 5,6,7,8-oxygenatedd flavones. Farid (1968) has suggested that a distinction may be made between the H-6 and H-3 signals by means of signal intensity.

Hydroxyl Protons[57, 64, 67]

Fully trimethylsilylated flavonoids, by definition, do not contain phenolic hydroxyl protons, and thus cannot be used for their detection. Anhydrous DMSO-d_6 exists as the ideal solvent for the detection of phenolic hydroxyl protons due to the presence of phenolic hydroxyl protons in it.

2.4.2.4 Sugar Protons

Flavonoid Monoglycosides[57, 64, 67]

The signal of C-1 proton (H-1'') normally can be found more downfield from the bulk of sugar signals. The site of glycosylation and the nature of sugar can be known from the exact chemical shift. For instance, the H-1'' signal in spectra of flavonol 3-*O*-glycosides at δ5.7 - 6.0 is well distinguished from those in flavonoid 7-, 5-, 4'-*O*-glycosides which occur in the region δ4.8 - 5.2.

The diaxial coupling constant of H-1'' with H-2'' in the β-linked glucosides is about 7 Hz, and this is particularly well defined when the sugar moiety experiences restricted rotation with respect to the flavonoid nucleus.

2.5 Chemical Constituents from *Cuscuta* Species

Table 2.3 listed some of the chemical constituents and isolated from *Cuscuta* species. The chemical structures of selected constituents are shown in page 45 - 58.

Table 2.3 Chemical Constituents of *Cuscuta* Spp.

S. No.	Name of Compounds	Molecular Formula	Species	References
1.	Caffeic acid **39**	$C_9H_8O_4$	*C. reflexa, C. platyloba, C. campestris, C. odorata, C. chinensis, C. pedicellata, C. europaea, C. gronovii, C. lupuliformis, C. japonica*	[66, 46, 67, 68, 69]
2.	Chlorogenic acid **40**	$C_{16}H_{18}O_9$	*C. reflexa, C. platyloba, C. campestris, C. odorata, C. chinensis, C. pedicellata, C. europaea, C. gronovii, C. lupuliformis*	[66, 46, 67]
3.	3,5-dicaffeoylquinic acid **41**	$C_{25}H_{24}O_{12}$	*C. reflexa, C. platyloba, C. campestris, C. odorata, C. chinensis, C. pedicellata, C. europaea, C. gronovii, C. lupuliformis, C. reflexa*	[66, 46]
4.	4,5- dicaffeoylquinic acid **42**	$C_{25}H_{24}O_{12}$	*C. reflexa, C. platyloba, C. campestris, C. odorata, C. chinensis, C. pedicellata, C. europaea, C. gronovii, C. lupuliformis, C. reflexa*	[66, 46]
5.	Caffeic-β-D-glucoside **43**	-	*C. australis*	[70]
6.	*p*-coumaric acid **13**	$C_9H_8O_3$	*C. australis, C. europeae, C. campestris, C. japonica*	[70, 67, 71]

7.	5, 6,7- trimethoxycoumarin **44**	$C_{12}H_{12}O_5$	*C. reflexa*	[72]
8.	7-hydroxy-6,8-dimethoxy coumarin **45**	$C_{11}H_{10}O_5$	*C. reflexa*	[20]
9.	6,7,8-trimethoxy coumarin **46**	$C_{12}H_{12}O_5$	*C. reflexa*	[20]
10.	4-*O*-*p*-coumaroyl-*O*-glycoside **47**	$C_{15}H_{18}O_8$	*C. reflexa*	[20]
11.	Methyl caffeate **48**	$C_{10}H_{10}O_4$	*C. japonica*	[73]
12.	Methyl *p*-coumarate **49**	$C_{10}H_{10}O_3$	*C. japonica*	[73]
13.	Kaempferol **30**	$C_{15}H_{10}O_6$	*C. reflexa, C. platyloba, C. campestris, C. odorata, C. chinensis, C. pedicellata, C. europaea, C. gronovii, C. lupuliformis, C. cupulata*	[66, 46, 74, 75, 68]
14.	Astragalin (Kaempferol-3-*O*-glucoside) **50**	$C_{21}H_{20}O_{11}$	*C. reflexa, C. platyloba, C. campestris, C. odorata, C. chinensis, C. pedicellata, C. europaea, C. gronovii, C. lupuliformis, C. micrantha*	[66, 46]
15.	Kaempferol-3-*O*-galactoside **51**	$C_{21}H_{20}O_{11}$	*C. reflexa, C. platyloba, C. campestris, C. odorata, C. chinensis, C. pedicellata, C. europaea, C. gronovii, C. lupuliformis*	[66, 46]
16.	Kaempferol-3-*O*-*α*-rhamnoside **52**	$C_{21}H_{20}O_{11}$	*C. reflexa*	[76]

17.	Quercetin **53**	$C_{15}H_{10}O_8$	*C. reflexa, C. platyloba, C. campestris, C. odorata, C. chinensis, C. pedicellata, C. europaea, C. gronovii, C. lupuliformis*	[66, 46, 75, 68]
18.	Quercetin-3-*O*-glucoside **54**	$C_{21}H_{20}O_{13}$	*C. reflexa, C. platyloba, C. campestris, C. odorata, C. chinensis, C. pedicellata, C. europaea, C. gronovii, C. lupuliformis*	[66, 46]
19.	Quercetin-3-*O*-galactoside (hyperoside) **55**	$C_{21}H_{20}O_{13}$	*C. reflexa, C. platyloba, C. campestris, C. odorata, C. chinensis, C. pedicellata, C. europaea, C. gronovii, C. lupuliformis*	[66, 46, 75, 77]
20.	Quercetin 3-*O*-β-D-galactoside-7-*O*-β-D-glucoside **56**	$C_{27}H_{32}O_{18}$	*C. australis, C. chinensis*	[78, 75]
21.	Quercetin-3-*O*-β-D-apiofuranosyl-(1$\rightarrow$2)-β-D-galactoside **57**	$C_{27}H_{32}O_{18}$	*C. australis, C. chinensis*	[78, 75]
22.	Isorhamnetin **58**	$C_{16}H_{12}O_7$	Semen *Cuscutae, C. europeae*, *C. campestris*, *C. chinensis*	[67, 75]
23.	Isorhamnetin-3-*O*-neohesperidoside **59**	$C_{28}H_{32}O_{16}$	*C. reflexa, C. platyloba*	[46, 79]
24.	Apigenin-7-*O*-glucoside **60**	$C_{21}H_{20}O_{10}$	*C. reflexa*	[76]

25.	Apigenin-7-*O*- Rutinoside **61**	$C_{27}H_{30}O_{14}$	*C. reflexa*	[79]
26.	Astragaloside **62**	$C_{28}H_{32}O_{17}$	*C. australis*	[77]
27.	Myricetin **63**	$C_{15}H_{10}O_8$	*C. japonica*	[73]
28.	Myricetin glucoside **64**	$C_{21}H_{20}O_{13}$	*C. reflexa*	[20]
29.	Myricetin-3-*O*-*α*-rhamnoside **65**	$C_{21}H_{20}O_{12}$	*C. reflexa*	[80]
30.	Taxifolin **66**	$C_{15}H_{12}O_7$	*C. reflexa*	[80]
31.	Coccinoside B **67**	$C_{21}H_{22}O_{11}$	*C. reflexa*	[20]
32.	Taxifolin-7-*O*-β-D-glucopyranoside **68**	$C_{21}H_{22}O_{12}$	*C. reflexa*	[80]
33.	Amarbelin (3',4'-dihydroxy-3,x,y-trimethoxy flavones) **69**	$C_{18}H_{16}O_7.H_2O$	*C. reflexa*	[20]
34.	Azaleatin **70**	$C_{16}H_{12}O_7$	*C. reflexa*	[47]
35.	3',4',6-Trihydroxyaurone **71**	$C_{25}H_{10}O_5$	*C. reflexa*	[20]
36.	Melanettin **72**	$C_{16}H_{12}O_5$	*C. reflexa*	[20]
37.	4,4',6- Trihydroxyaurone **73**	$C_{25}H_{10}O_5$	*C. chinensis*	[81]
38.	3,4',5,7-Tetrahydroxyflavonone **74**	$C_{15}H_{11}O_6$	*C. reflexa*	[82]
39.	Sesamin **75**	$C_{20}H_{18}O_6$	*C. chinensis*	[75]

40.	9(R)-hydroxy-*d*- sesamin **76**	-	*C. chinensis*	[75]
41.	Neosesamin **77**	$C_{20}H_{18}O_7$	*Semen Cuscutae, C. chinensis*	[83]
42.	Luteolin **78**	$C_{15}H_{10}O_6$	*C. australis, C. chinensis*	[84]
43.	Agroclavine **79**	$C_{16}H_{18}N_2$	*C. monogyna, C.chinensis*	[85, 86]
44.	Lupanine **80**	$C_{15}H_{24}N_2O$	*C. reflexa*, *C. platyloba*	[85]
45.	Sparteine **81**	$C_{15}H_{26}N_2$	*C. reflexa*, *C. platyloba*	[85]
46.	Matrine **82**	$C_{15}H_{24}N_2O$	*C. chilensis*	[85]
47.	Sophoranol **83**	$C_{15}H_{24}N_2O$	*C. chilensis*	[85]
48.	Methylcytisine **84**	$C_{12}H_{16}N_2O$	*C. chilensis*	[85]
49.	Cytisine **85**	$C_{11}H_{14}N_2O$	*C. chilensis*	[85]
50.	Cuscutamine **86**	$C_{15}H_{14}N_2O_3$	*C. chinensis*	[87]
51.	β-carotene **87**	$C_{40}H_{56}$	*C. australis*	[88]
52.	α-carotene-5,6-epoxide **88**	$C_{40}H_{56}O$	*C. australis*	[88]
53.	γ-carotene **89**	$C_{40}H_{56}$	*C. australis*	[88]
54.	Taraxanthin **90**	$C_{40}H_{56}O_3$	*C. australis*	[88]
55.	Lutein **91**	$C_{40}H_{56}O_2$	*C. australis*	[88]

56.	Australiside (4-oic acid-7-oxo-kaurene-6-α-*O*-β-D-glucoside) **92**	-	*C. australis*	[70]
57.	Cuscutosides A **93**	$C_{26}H_{25}O_{12}$	*C. chinensis*	[87]
58.	Cuscutosides B **94**	$C_{26}H_{25}O_{12}$	*C. chinensis*	[87]
59.	(+)-pinoresinol **18**	$C_{20}H_{22}O_6$	*C. chinensis*	[87]
60.	(+)-epipinoresinol **95**	$C_{20}H_{22}O_6$	*C. chinensis*	[87]
61.	(+)-pinoresinol 4-*O*-glucoside **96**	$C_{26}H_{34}O_{11}$	*C. chinensis*	[87]
62.	Cholesterol **97**	$C_{27}H_{46}O$	*Semen Cuscutae*	[89]
63.	Campesterol **98**	$C_{29}H_{48}O$	*Semen Cuscutae*	[89]
64.	β-sitosterol **99**	$C_{29}H_{50}O$	*C. australis, C. japonica, C. chinensis, C. reflexa*	[68, 71, 77, 90]
65.	Stigmasterol **100**	$C_{29}H_{48}O$	*Semen Cuscutae, C. reflexa*	[80, 89]
66.	Daucosterol **101**	$C_{35}H_{60}O_6$	*C. japonica, C.chinensis*	[71, 83]
67.	Δ^5-Avenasterol **102**	$C_{29}H_{48}O$	*C. chinensis*	[20]
68.	Lupeol **103**	$C_{30}H_{50}O$	*C. reflexa, C. japonica*	[80]
69.	Stigmast-5-en-3-yl-acetate **104**	$C_{31}H_{52}O_2$	*C. reflexa*	[90]
70.	α-amyrin **105**	$C_{30}H_{50}O$	*C. reflexa*	[80]

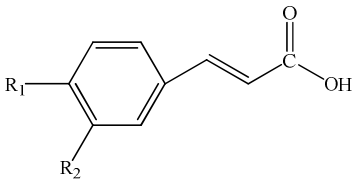
71.	β-amyrin **106**	$C_{30}H_{50}O$	*Semen Cuscutae, C. reflexa*	[80, 89]
72.	α-amyrin acetate **107**	$C_{33}H_{52}O_2$	*C. reflexa*	[80]
73.	β-Amyrin acetate **108**	$C_{33}H_{52}O_2$	*C. reflexa*	[80]
74.	Cuscutic acid A **109**	$C_{38}H_{68}O_{21}$	*C. australis*	[91]
75.	Cuscutic acid B **110**	$C_{37}H_{66}O_{20}$	*C. chinensis*	[91]
76.	Cuscutic acid C **111**	$C_{38}H_{68}O_{20}$	*C. chinensis*	[91]
77.	Cuscutic acid D **112**	$C_{40}H_{72}O_{21}$	*C. chinensis*	[91]
78.	Cuscutic acid A_1 **113**	$C_{34}H_{62}O_{16}$	*C. australis*	[88]
79.	Cuscutic acid A_2 **114**	$C_{32}H_{58}O_{16}$	*C. australis*	[88]
80.	Cuscutic acid A_3 **115**	$C_{32}H_{58}O_{17}$	*C. australis*	[88]
81.	Cus-1 **116**	$C_{40}H_{67}O_{19}$	*C.chinensis*	[92]
82.	Cus-2 **117**	$C_{42}H_{71}O_{19}$	*C.chinensis*	[92]
83.	Palmitic acid **118**	$C_{16}H_{32}O_2$	Semen Cuscutae, *C. chinensis*	[83, 89]
84.	Stearic acid **119**	$C_{18}H_{16}O_2$	*C. japonica, C.chinensis*	[83, 89]
85.	Laceeroic acid **120**	$C_{32}H_{64}O_2$	*C. australis*	[93]
86.	Arbutin **22**	$C_{10}H_{18}O_9$	*C. chinensis*	[70, 87]

87.	Thymidine **121**	$C_{10}H_{14}N_2O_5$	*C. autralis*	[70]
88.	Anthraquinone **122**	$C_{14}H_8O_2$	Semen Cuscutae	[89, 94]
89.	Swarnalin **123**	$C_{21}H_{26}O_{12}$	*C. reflexa*	[94]
90.	Cis-swarnalin **124**	$C_{21}H_{26}O_{12}$	*C.reflexa*	[94]
91.	Cuscutin **125**	$C_{15}H_{12}O_9$	*C. epithymum, C. japonica*	[95]
92.	Tutin **126**	$C_{15}H_{18}O_6$	*C. japonica*	[89]
93.	Neocuscutoside A **127**	$C_{37}H_{46}O_{21}$	*C. chinensis*	[96]
94.	Neocuscutoside B **128**	$C_{36}H_{44}O_{20}$	*C. chinensis*	[96]
95.	Hexadecanoic acid **129**	$C_{16}H_{32}O_2$	*C. australis, C. japonica*	[71, 77]
96.	Eicosanic acid **130**	$C_{54}H_{84}O_{11}$	*C. japonica*	[17]
97.	Coriatin **131**	$C_{15}H_{20}O_6$	*C. japonica*	[17]
98.	Linolenic acid **132**	$C_{18}H_{32}O_2$	*C. reflexa*	[97]
99.	Linoleic acid **133**	$C_{18}H_{30}O_2$	*C. reflexa*	[97]
100.	Oleanolic acid **134**	$C_{30}H_{48}O_2$	*C. reflexa*	[80]
101.	Oleanolic acetate **135**	$C_{30}H_{50}O_4$	*C. reflexa*	[80]
102.	Hydroxyoleanane **136-137**	$C_{47}H_{81}O_2$	*C. reflexa*	[98]

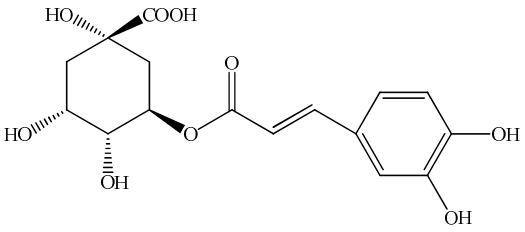
103.	Dulcitol **138**	$C_6H_{14}O_6$	*C. japonica*	[99]
104.	Lycopene **139**	$C_{40}H_{56}$	*C. reflexa*	[100]
105.	Scoparone **140**	$C_{11}H_{10}O_4$	*C. reflexa*	[101]
106.	Triacontanol **141**	$C_{30}H_{62}O$	*C. chinensis*	[81]
107.	Hentriacontane **142**	$C_{31}H_{64}$	*C. chinensis*	[81]
108.	Triacontane **143**	$C_{30}H_{62}$	*C. chinensis*	[81]
109.	Bergenin (3,4,4a, 10-b-tetrahydro-3,4,8,10-tetrahydroxy-2(hydroxymethyl)-9-methoxypyran-6(2*H*)-one **144**	$C_{14}H_{16}O_9$	*C. reflexa*	[102]
110.	Maragenin **145**	$C_{29}H_{46}O_2$	*C. reflexa*	[39]
111.	21-hydroxyodoroside H **146**	$C_{30}H_{46}O_9$	*C. reflexa*	[20]
112.	Odoroside H **147**	$C_{30}H_{46}O_8$	*C. reflexa*	[20]
113.	Neritaloside **148**	$C_{32}H_{48}O_{10}$	*C. reflexa*	[20]
114.	Strospeside **149**	$C_{30}H_{46}O_9$	*C. reflexa*	[20]
115.	Gitoxigenin **150**	$C_{23}H_{34}O_5$	*C. reflexa*	[20]
116.	*N*-trans feruloyl tyramine **151**	$C_{18}H_{19}NO_4$	*C. reflexa*	[20]

117.	*N*-cis feruloyl tyramine **152**	$C_{18}H_{19}NO_4$	*C. reflexa*	[20]
118.	Ursolic acid **153**	$C_{30}H_{48}O_3$	*C. reflexa*	[20]
119.	Methyl cinnamate **154**	$C_{10}H_{10}O_2$	*C. reflexa*	[20]
120.	Ethyl 3-(3',4'-dihydroxy phenyl)-2-propenoate **155**	$C_{11}H_{12}O_4$	*C. reflexa*	[103]
121.	Nonacosane **156**	$C_{29}H_{60}$	*C. reflexa*	[81]
122.	Octacosane **157**	$C_{28}H_{58}$	*C. reflexa*	[81]
123.	Heptacosane **158**	$C_{27}H_{56}$	*C. reflexa*	[81]
124.	Hexacosane **159**	$C_{26}H_{54}$	*C. reflexa*	[20]
125.	Pentacosane **160**	$C_{25}H_{52}$	*C. reflexa*	[81]
126.	Tetracosane **161**	$C_{24}H_{50}$	*C. reflexa*	[20]
127.	Ferulic acid **162**	$C_{10}H_{10}O_4$	*C. reflexa*	[20]
128.	Methyl 3-(4'-hydroxyphenyl)-2-propenoate **163**	$C_{10}H_{10}O_3$	*C. reflexa*	[20]
129.	14, 15-Dihydroajugaptin **164**	$C_{29}H_{44}O_{10}$	*C. reflexa*	[20]
130.	Cuscutalin **165**	$C_{18}H_{10}O_4$	*C. reflexa*	[104]
131.	Calycopteretin **166**	$C_{15}H_{10}O_8$	*C. chinensis*	[68]

132.	α-tocopherol **167**	C_{29} H_{50} O_2	*C. chinensis*	[68]
133.	7'-(3',4'-Dihydroxyphenyl)-*N*-[(4-methoxyphenyl) ethyl] propenamide **168**	$C_{18}H_{19}NO_4$	*C. reflexa*	[103]
134.	7'-(4'-Hydroxyphenyl-3'-methoxyphenyl)-*N*-[(4-butylphenyl) ethyl] propenamide **169**	$C_{22}H_{27}NO_3$	*C. reflexa*	[103]
135.	6, 7-Dimethoxy-*2H*-benzopyran-2-one **170**	$C_{11}H_{10}O_4$	*C. reflexa*	[103]
136.	3-(4-*O*-β-D-glucopyranoside-3,5-dimethoxyphenyl)-2-propenol **171**	$C_{17}H_{24}O_9$	*C. reflexa*	[103]
137.	2-(3-hydroxy-4-methoxyphenyl)-3,5-dihydroxy-7-*O*-β-D-glucopyranoside-*4H*-1-benzopyran-4-one **172**	$C_{22}H_{22}O_{12}$	*C. reflexa*	[103]
138.	Rhamnetol-7-*O*-β-D-glycoside **173**	-	*C. reflexa*	[20]



	R_1	R_2
39	OH	OH
13	OH	OCH_3



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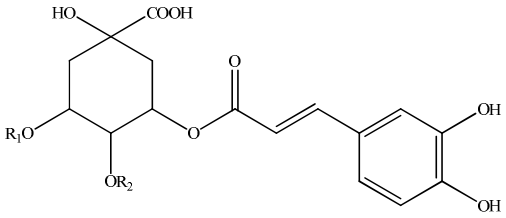
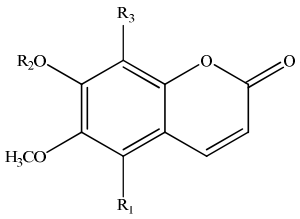
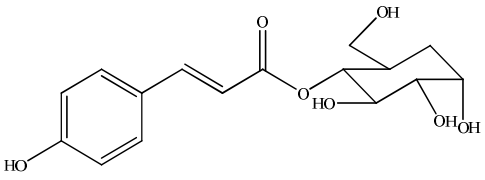


	R_1	R_2
41	caffeoyl	H
42	H	caffeoyl



	R_1	R_2	R_3
44	OCH_3	CH_3	H
45	H	H	OCH_3
46	H	CH_3	OCH_3



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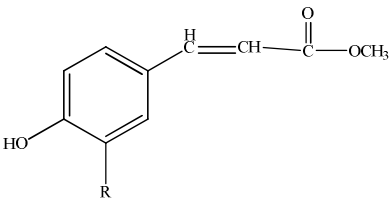
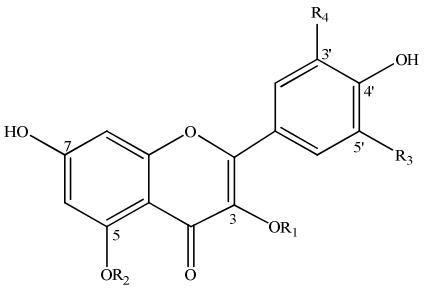
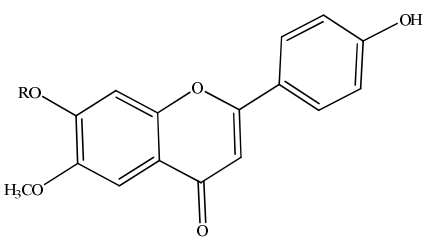


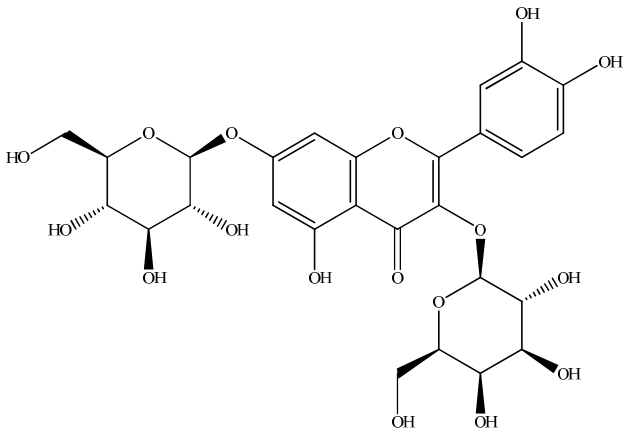
	R
48	OH
49	H



	R_1	R_2	R_3	R_4
30	H	H	H	H
50	β-D-glc	H	H	H
51	β-D-gal	H	H	H
52	α-rham	H	H	H
53	H	H	OH	H
54	β-D-glc	H	OH	H
55	β-D-gal	H	OH	H
57	api(1,2)-gal	H	OH	H
58	H	H	OCH_3	H
59	neohesperidoside	H	OCH_3	H
63	H	H	OH	OH
64	β-D-glc	H	OH	OH
65	α-rham	H	OH	OH
70	H	CH_3	OH	H



	R
60	glucoside
61	rutinoside



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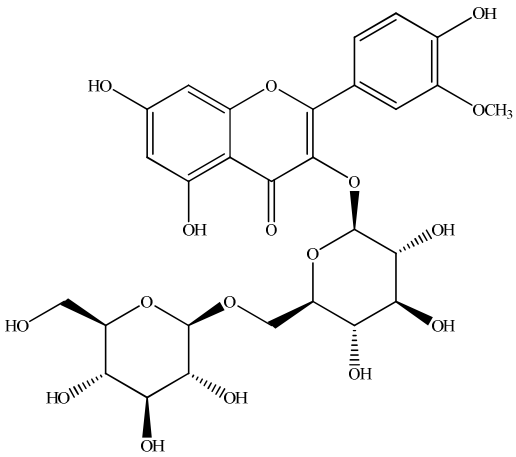

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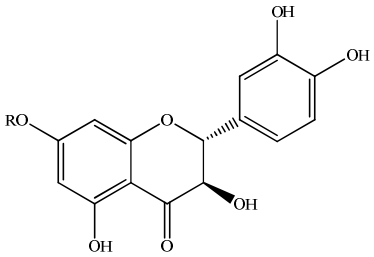
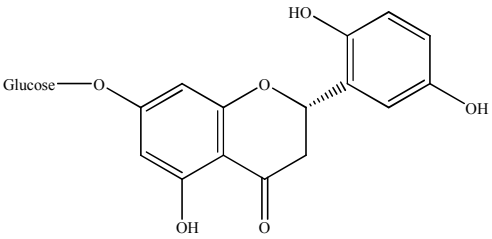


R	
66	H
68	Glu



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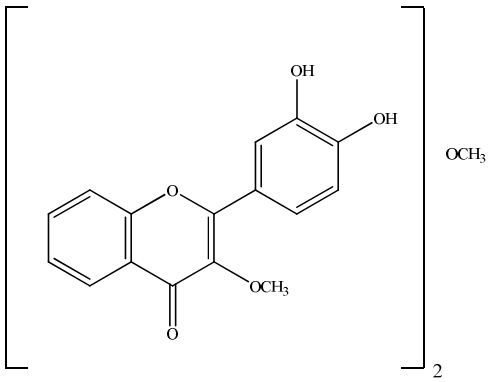

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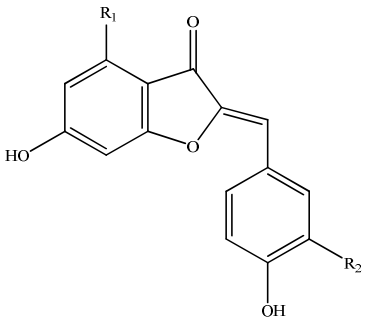
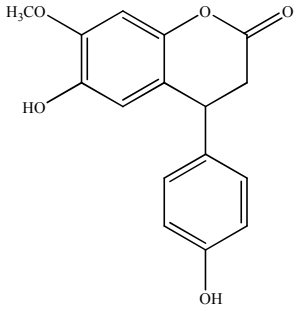


	R_1	R_2
71	H	OH
73	OH	H



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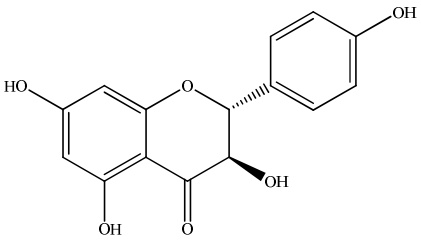

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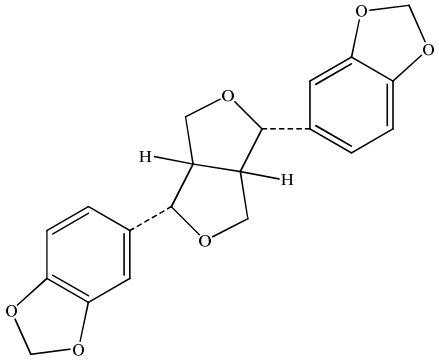

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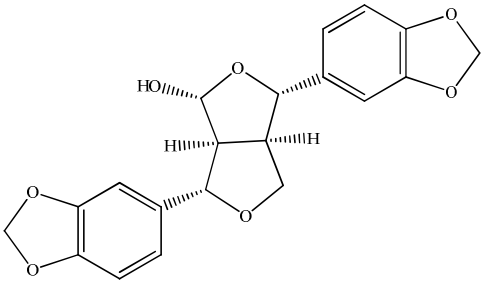

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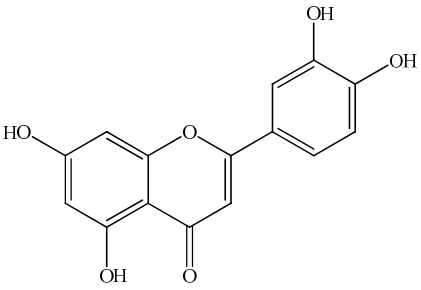

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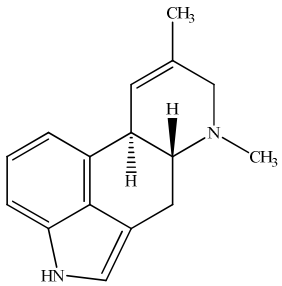
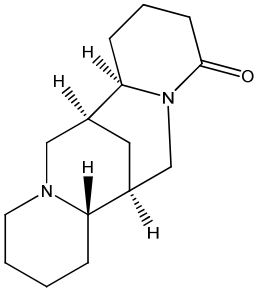
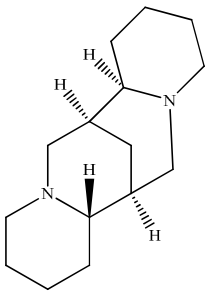


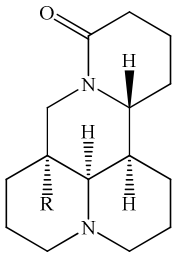
79



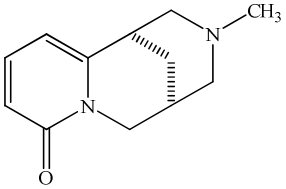
80



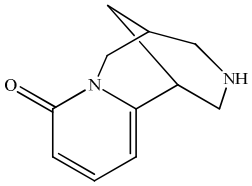
81



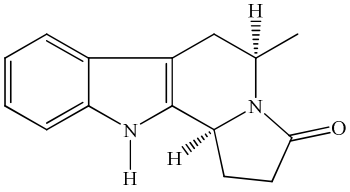
	R
82	**H**
83	**OH**



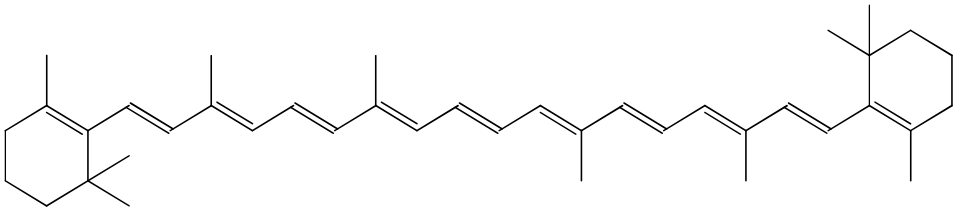
84



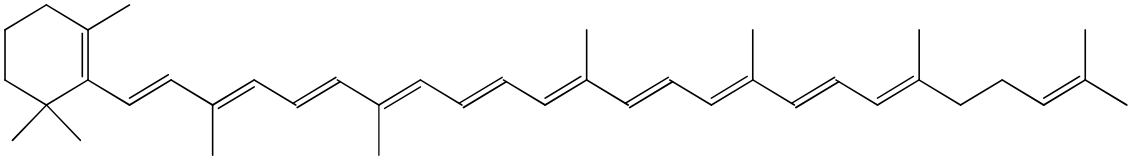
85



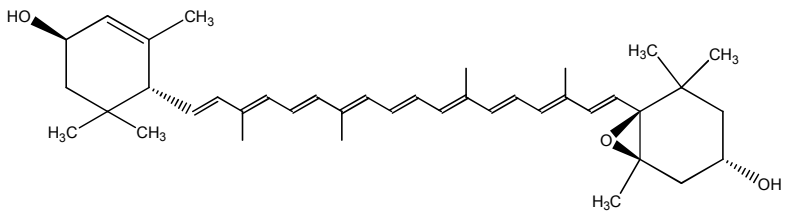
86



87



89



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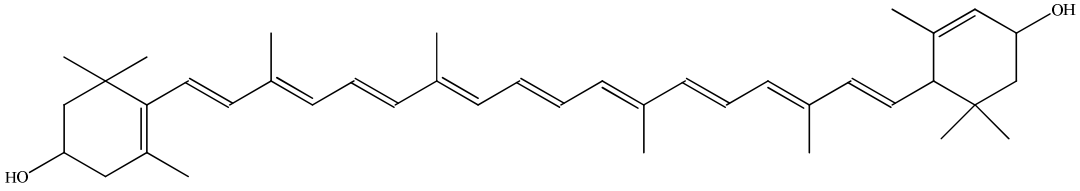
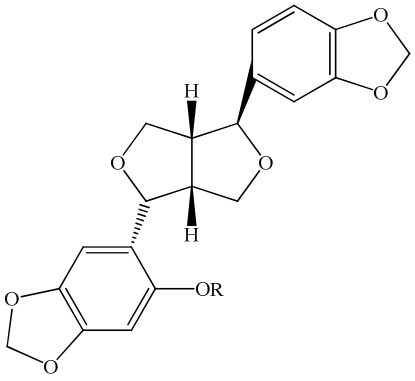
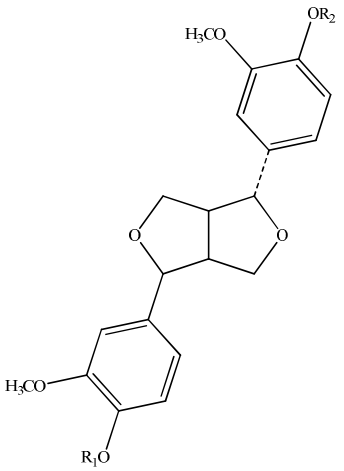


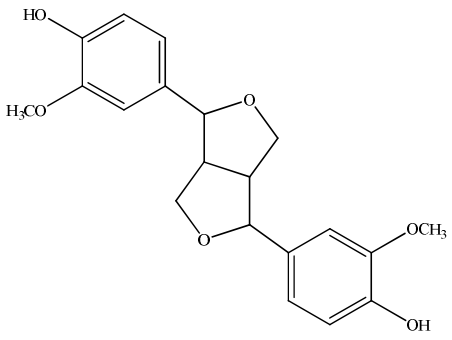
91



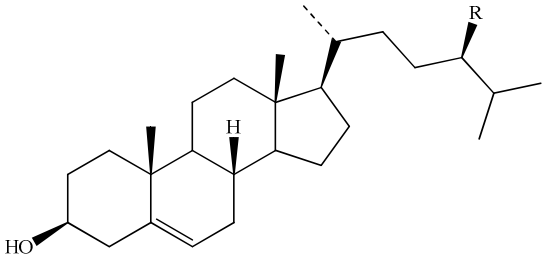
	R
93	Glc^2-Api
94	Glc^6-Xyl



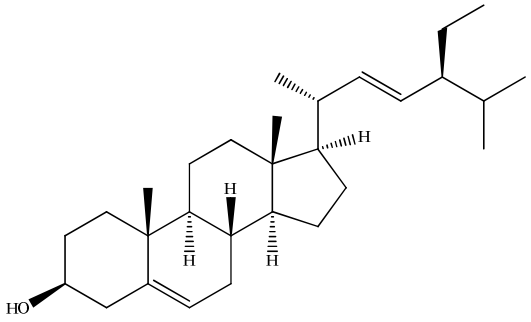
	R_1	**R_2**
95	H	H
96	Glu	H



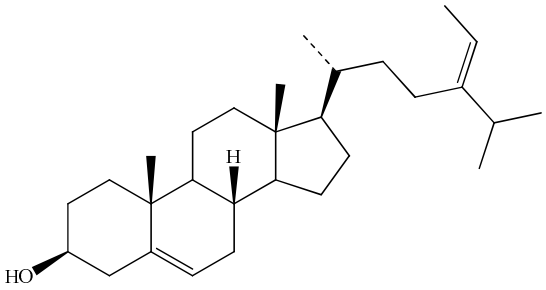
18



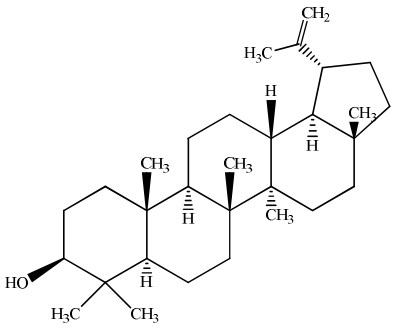
R	
97	H
98	CH_3
99	C_2H_5



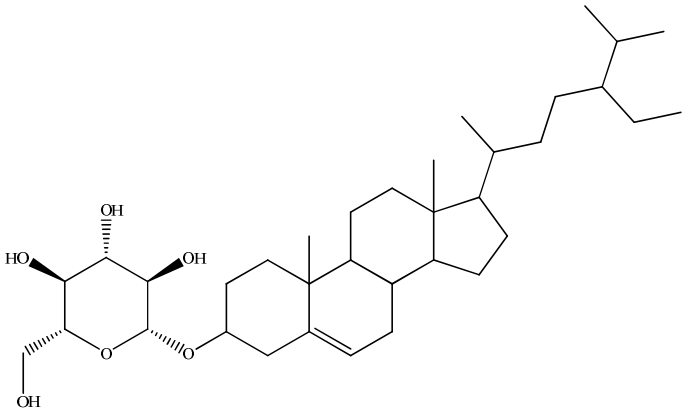
100



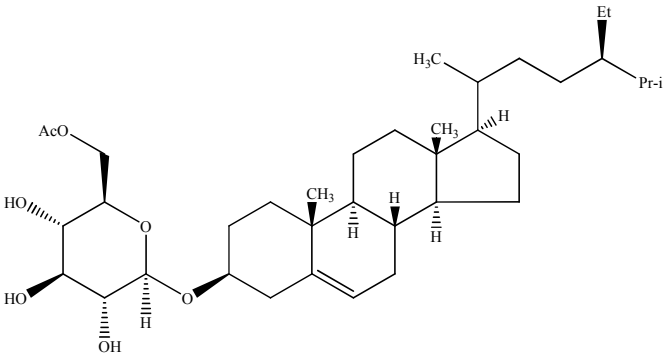
102



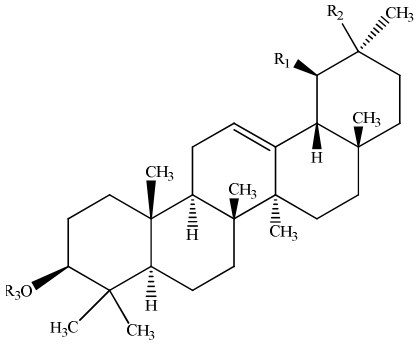
103



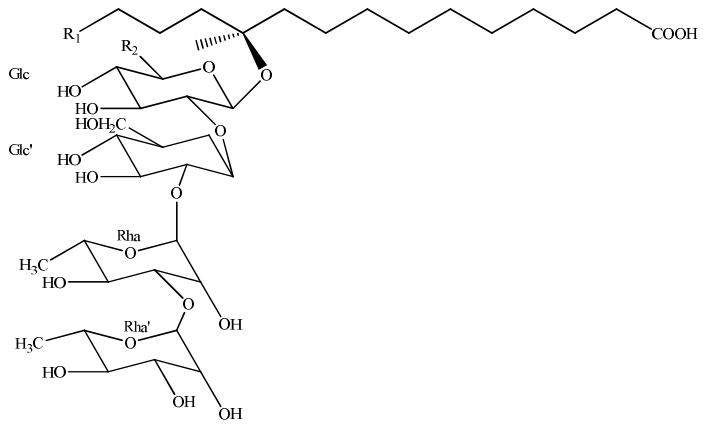
101



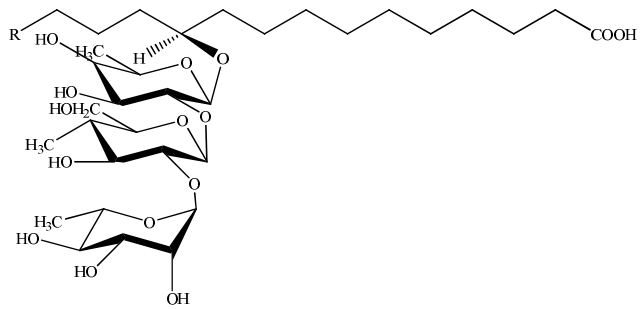
104



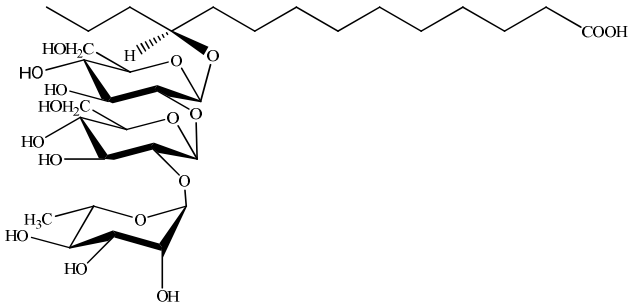
	R_1	R_2	R_3
105	CH_3	H	H
106	CH_3	H	CH_3CO
107	H	CH_3	H
108	H	CH_3	CH_3CO



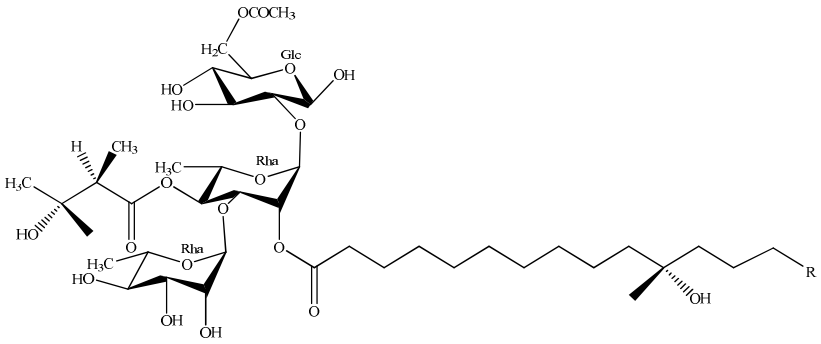
	R_1	R_2
109	H	CH_2OH
110	H	H
111	H	CH_3
112	C_2H_5	CH_2OH



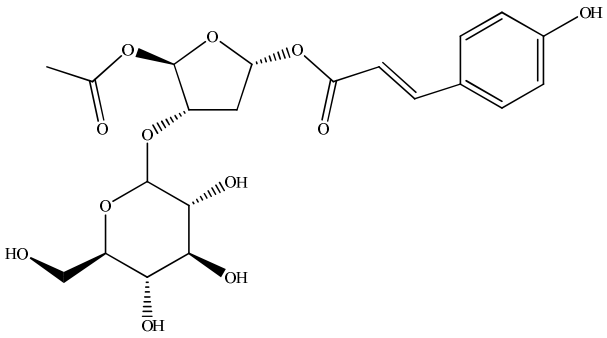
	R
113	C_2H_5
114	H



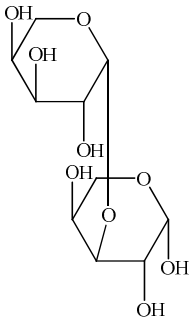
115



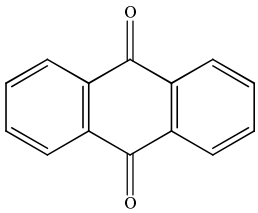
	R
116	H
117	C_2H_5



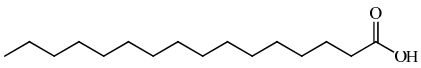
123



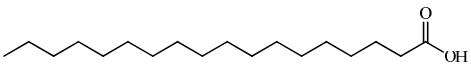
22



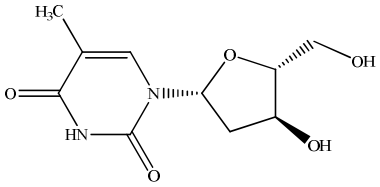
122



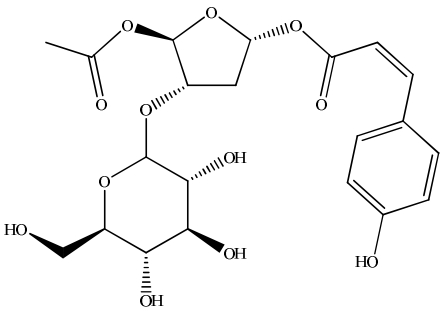
118



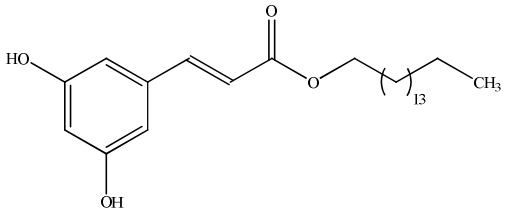
119



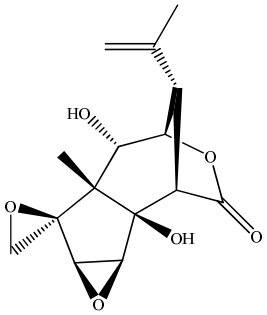
121



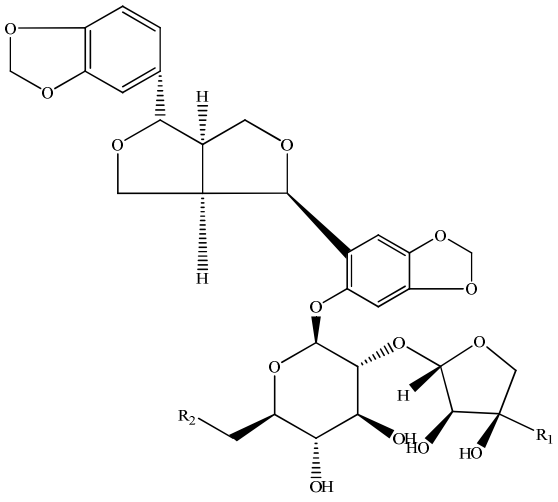
124



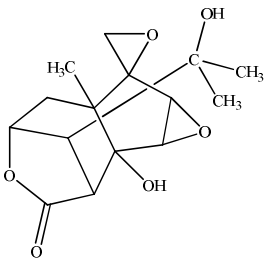
125



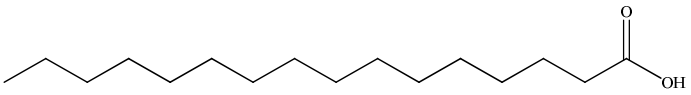
126



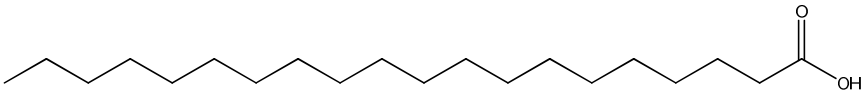
	R_1	R_2
127	Glu	OH
128	OH	Glu



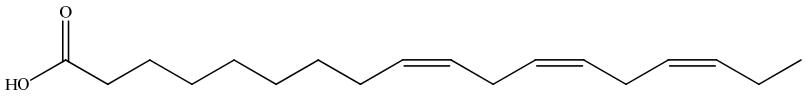
131



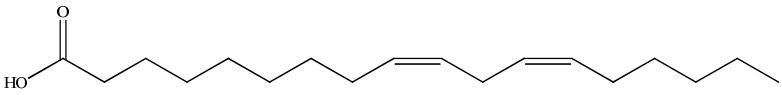
129



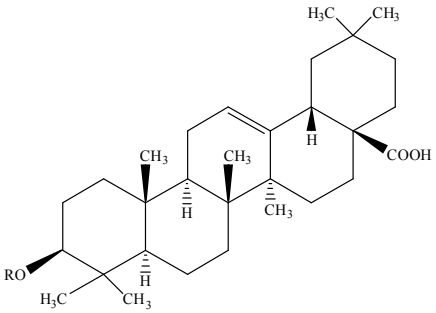
130



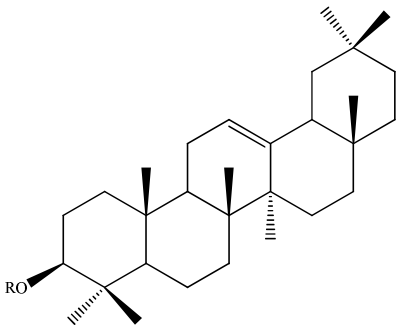
132



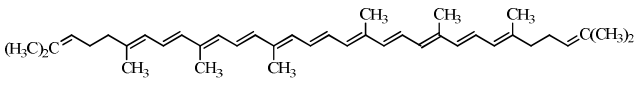
133



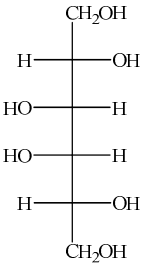
	R
134	H
135	CH_3CO



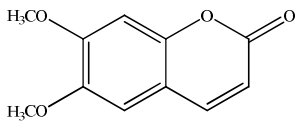
	R
136	$CO(CH_2)_{11}CH_3$
137	$CO(CH_2)_{15}CH_3$



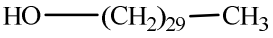
139



138



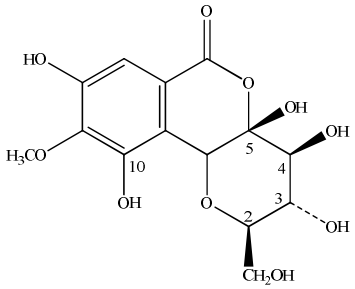
140



141

142

143



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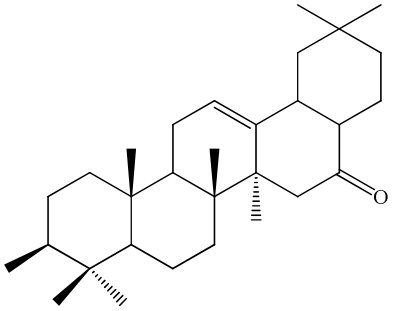
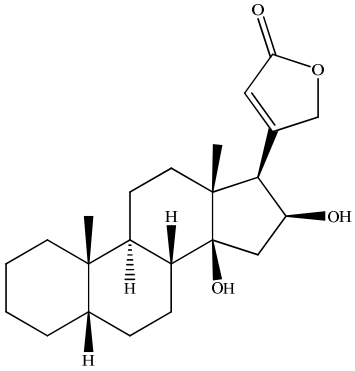
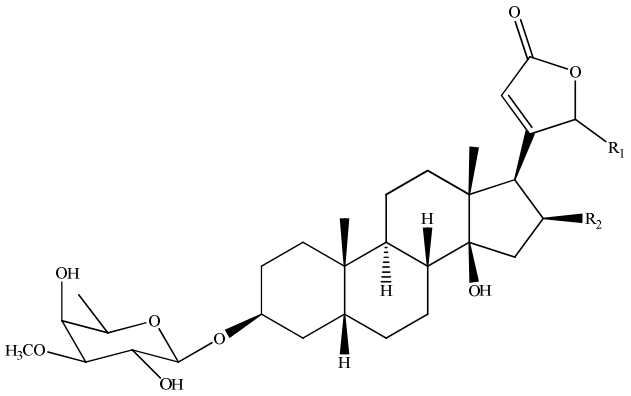


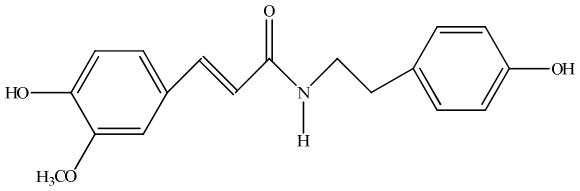
145



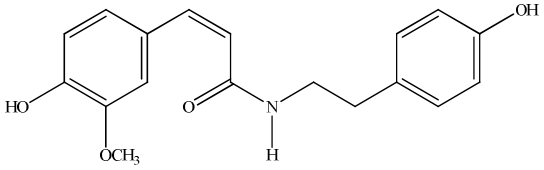
150



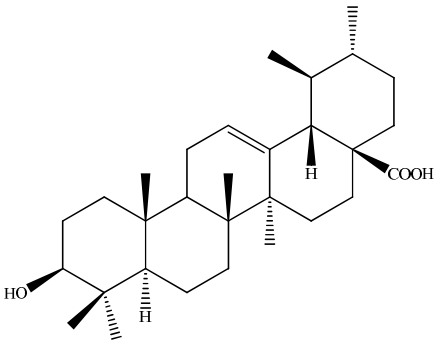
	R_1	R_2
146	OH	H
147	H	H
148	H	$OCOCH_3$
149	H	OH



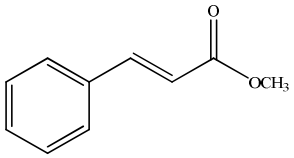
151



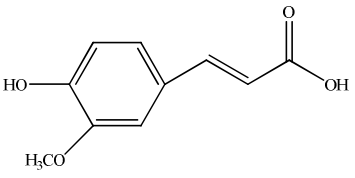
152



153



154



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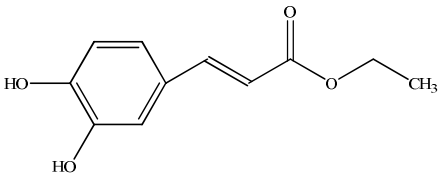
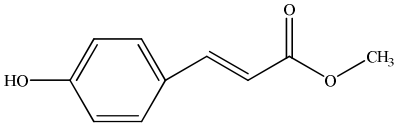
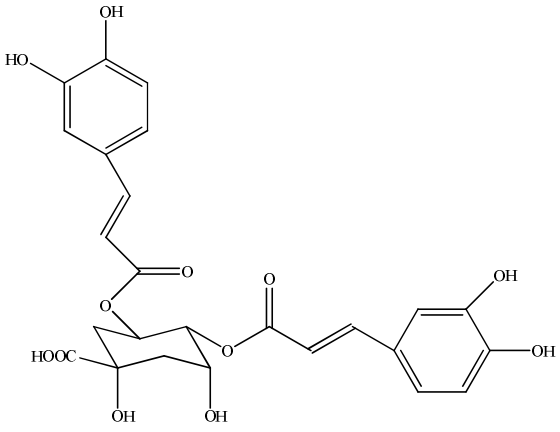


155



163



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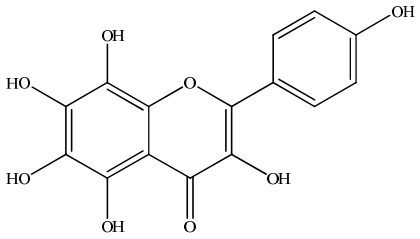

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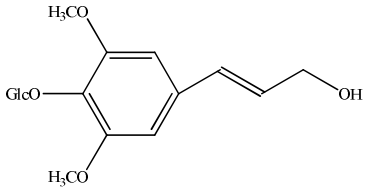

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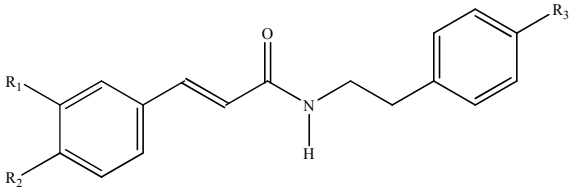
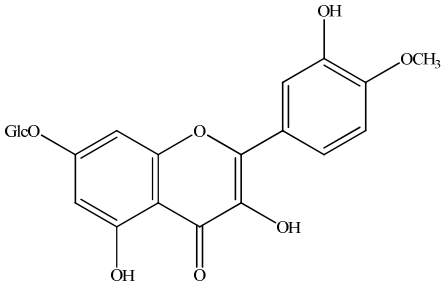


	R_1	R_2	R_3
168	OH	OH	OCH_3
169	OCH_3	OH	$(CH_2)_3CH_3$



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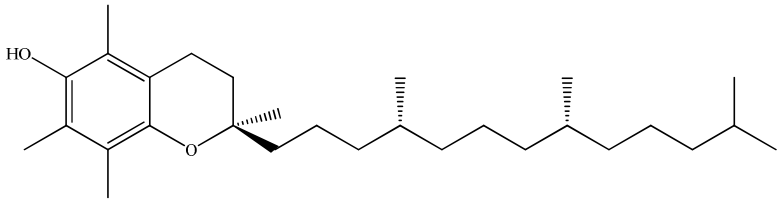

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2.6 Biological Activities of Common Chemical Constituents Isolated from *Cuscuta* Spp.

There are quite a number of reported biological and pharmacological activities of different extracts of *Cuscuta* spp., *viz.* antibacterial activity, antioxidant activity, and enzyme inhibition activity. Although there is no report on the biological activity of isolated compounds from *Cuscuta*, we found that a few of the common compounds (eg. kaempferol, sitosterol and quercetin) have shown some biological and pharmacological activities.

According to Martin *et al.* (2004), kaempferol isolated from *Combretum erythrophyllum* showed good antibacterial activity against *Vibrio cholerae* and *Enterococcus faecalis*, with MIC values in the range of 25–50 μg/ mL[105].

The cytotoxicity of propolis flavonoids was evaluated by Debiaggi *et al*. (1990) too. The isolated kaempferol caused a concentration-dependent reduction of intracellular replication of herpes-virus strains. Quercetin also showed reduction infectivity and intracellular replication at highest concentration.[106]

Kaempferol also exerted significant antioxidant capacity of the estrogenic compounds[107] examined in the ESR system by Mitchell *et al.* (1998), it quenched 2.3 radicals per molecule.

Quercetin is a well known natural mutagenic and induces recombination flavonoid that able to bind DNA causing single-strand DNA breaks, DNA rearrangement and chromosomal damage, and also arrests normal proliferating cells in the G_1 phase of the cell cycle[108-112].

In addition, quercetin is also an antioxidant. Significant inhibition on basal and oxidized low-density lipoproteins-stimulated matrixmetalloproteinase-1 expression by

quercetin showed that it inhibited disruption of atherosclerotic plaques and contributed to plaque stabilization[113]. Quercetin also arrested human leukemic T cells in late G_1 phase of the tumor cell cycle[114].

A lot of researches were done on the anti-inflammatory effects of quercetin, *viz.* inhibition of cytokine and inducible nitric oxide synthase expression[115], inhibition on the activation of the signal transducer and activator of transcription 1[116], and also inhibition of NF-κB activation and on protein concentration of the phosphorylated form of the inhibitor IκBα and of IKK (IκB kinase)α[117]. All these researches showed that quercetin has good anti-inflammatory activity.

According to Lee *et al.* (1981), kaempferol-3-*O*-β-D-glucopyranoside (astragalin) isolated from *Wikstroemia indica* is an antileukimic constituents[118]. Astagalin also showed glycation inhibitory activity which might be used to treat diabetes[119]. Matsuda *et al.* (2001) suggested that astragalin possess potent *in vitro* inhibitory effect on TNF-α, may act as an inflammatory cytokine[120]. Astragalin also showed anti-inflammatory activities by significantly inhibiting cellular nitrite oxide (IC_{50} = 363 μg mL^{-1}), prostaglandin E_2 (IC_{50} = 134 μg mL^{-1}) and interleukin-6 production (IC_{50} = 289 μg mL^{-1}) by lipopolysaccharide-stimulated RAW 264.7 cells[121].

Davis *et al.* (1994) reported that sitosterol showed good anti-inflammatory activity in reducing the croton oil-induced ear swelling. Sitosterol also played a role in decreasing liver cholesterol accumulation in certain conditions[122]. Investigation on the estrogenic effect of sitosterol in fish species, *viz.* goldfish, rainbow trout, and brown trout showed significant result[123].

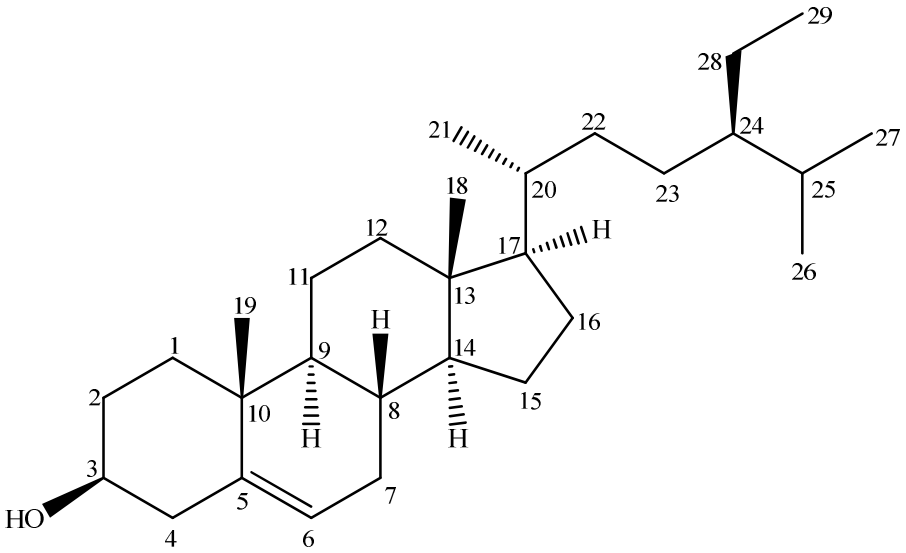
Pinoresinol showed synergistic result when tested on its insecticidal action against houseflies[124] by Haller *at el.* (1942). Besides, pinoresinol also show positive results in anti-feedant activity and moulting inhibition at low dosage on milkweed bug

and haematophagous insect[125]. Páska *et al.* (2010) suggested that pinoresinol isolated from *Ipomoea cairica* showed antioxidant activity and Ca^{2+} antagonist properties[126].

Arbutin is a famous traditional whitening agent (depigmentation) involving melanosomal tyrosinase inhibitory activity[127]. Moreover, Strapkova *et al.* (1991) reported that arbutin also exerted antitussive effect[128].

The above mentioned compounds are common chemicals found in many *Cuscuta* spp. such as *Cuscuta australis*, *Cuscuta reflexa* and *Cuscuta campestris*. Therefore, it is not surprising that *Cuscuta* have intensity biological activities.

3.1 Compound A: Sitosterol



Compound A was obtained as colorless crystalline needle like substance with the melting point of 139 - 142 °C. The mass spectrum showed a parent molecular formula of $C_{29}H_{50}O$.

The ^{1}H NMR (Fig. 3.1) showed proton H-3 resonated as a multiplet at δ3.29. The signal at δ5.32 (m) revealed the presence of olefinic proton. Two singlets corresponding to six protons at δ0.65 and δ0.98 were attributed to C_{18} and C_{19} angular methyl proton respectively.

^{13}C NMR spectrum (Fig. 3.2) showed 29 carbon signals including 9 methyls, 11 methylenes, 6 methane, and 3 quaternary carbons. The signal at δ71.9 indicated the presence of β-hydroxyl group at C-3. The signals at δ11.9 and δ19.5 indicated the presence of angular methyl carbon at C_{18} and C_{19} respectively. The value for C_{18} was lower due to γ-gauche interaction which increased the screening of the C_{18} and lower chemical shift[129, 130]. The detail analysis of NMR data was recorded in Table 3.1.

Compound A was identified as sitosterol **99**.

Table 3.1 **^{1}H and ^{13}C NMR Spectra Data of Compound A in $CDCl_3$ and the Literature Values[131]**

Position	δ_C (ppm)	δ_H (ppm)	δ_C (ppm) Ref.	δ_H (ppm) Ref.
1	37.3	-	37.3	-
2	31.7	-	31.6	-
3	71.9	3.29 (m)	71.8	3.52 (m)
4	42.3	-	42.2	-
5	140.8	-	140.8	-
6	121.8	5.32 (m)	121.7	5.358 (br s)
7	32.0	-	31.9	-
8	32.0	-	31.9	-
9	50.2	-	51.2	-
10	36.6	-	36.5	-
11	21.2	-	21.1	-
12	39.8	-	39.8	-
13	42.4	-	42.3	-
14	56.9	-	56.8	-
15	24.4	-	24.3	-
16	28.3	-	28.3	-
17	56.1	-	56.0	-
18	11.9	0.65 (s)	11.9	0.68 (s)
19	19.5	0.98 (s)	19.4	1.01 (s)
20	36.2	-	36.2	-
21	18.9	0.89 (d, 6.4)	18.8	0.92 (d, 6.4)
22	34.0	-	33.9	-
23	26.1	-	26.1	-
24	45.9	-	45.9	-
25	29.2	-	29.2	-
26	19.9	0.79 (d, 6.9)	19.8	0.814 (d, 6.5)
27	19.1	0.81 (d, 6.9)	19.3	0.833 (d, 6.5)
28	23.1	-	23.1	-
29	12.1	0.84 (s)	12.2	0.84 (t, 7.5)

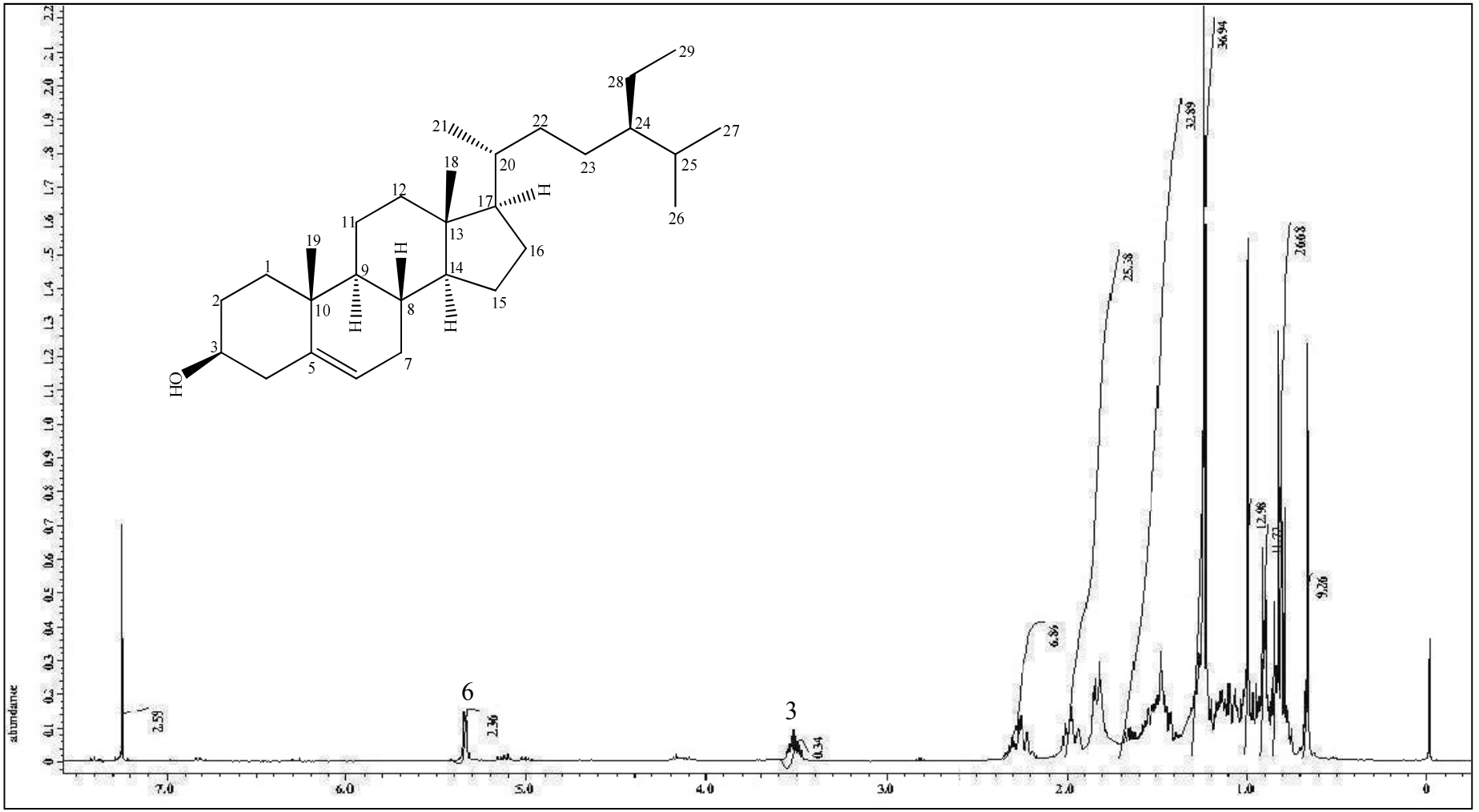


Figure 3.1 **^{1}H NMR Spectrum of Compound A in $CDCl_3$**

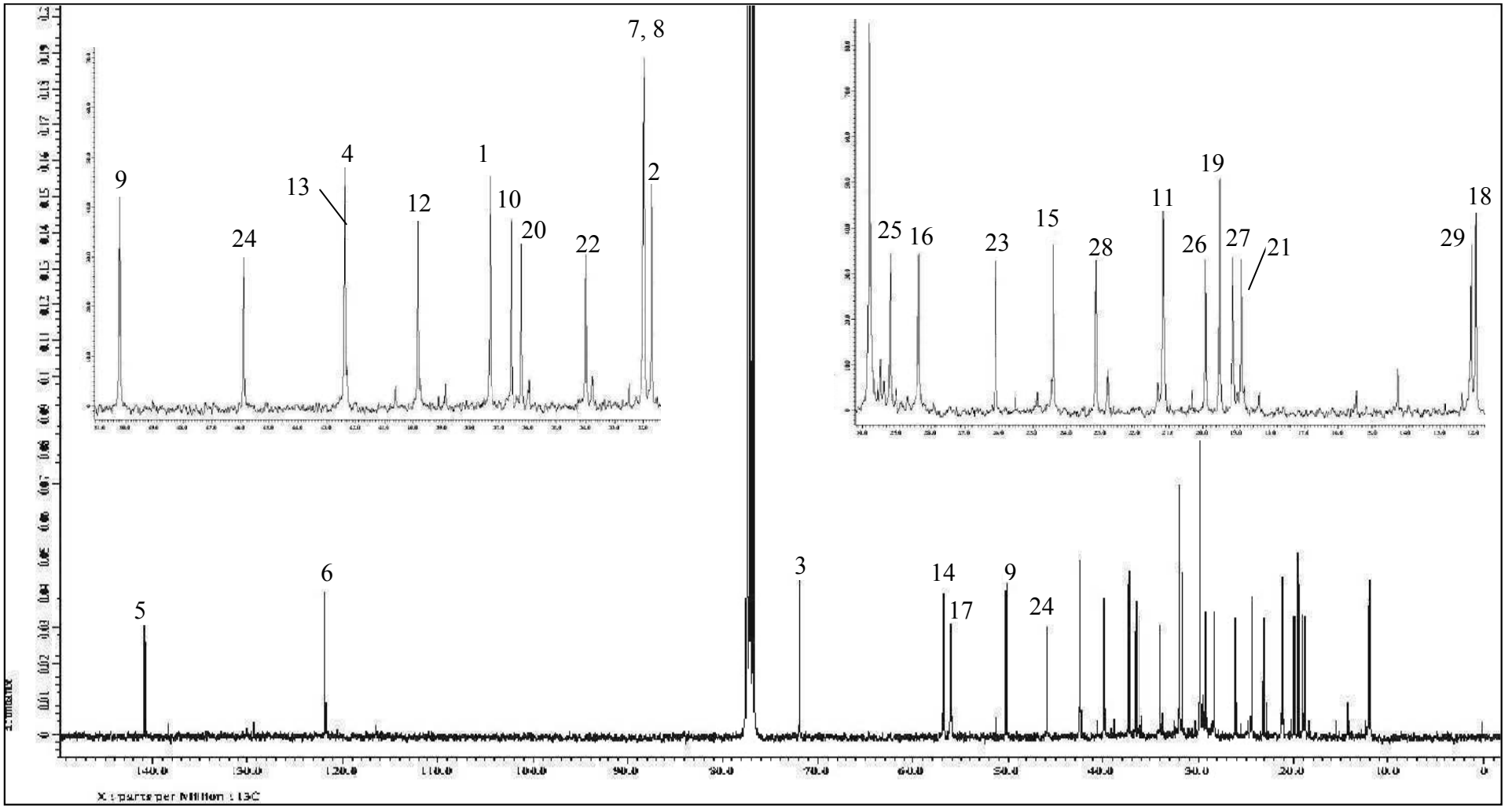
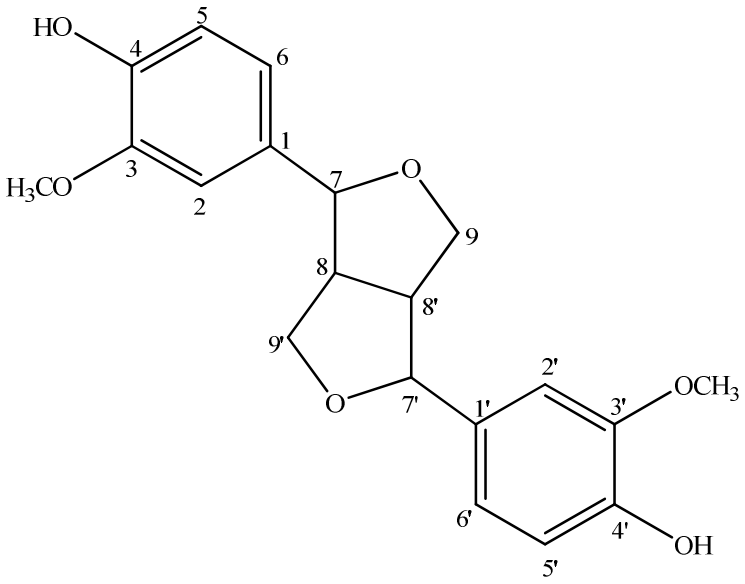


Figure 3.2 **^{13}C NMR Spectrum of Compound A in $CDCl_3$**

3.2 Compound B: Pinoresinol[132, 133]



Compound B was isolated as yellow amorphous powder. The ESI-MS revealed a $[M+H]^+$ peak at *m/z* at 357.12 corresponding to the molecular formula of $C_{20}H_{22}O_6$.

The ^{13}C NMR (Fig. 3.4) and DEPT spectra exhibited 10 signals which corresponded to 20 carbons: 12 aromatic carbons (six methine and six quaternary carbons), two oxymethylenes (δ71.75, C-9 and C-9'), four methines (δ85.97, C-7 and C-7' as well as δ54.23, C-8 and C-8'), and two methoxy carbons (δ56.05).

The ^1H NMR spectrum (Fig. 3.3) showed an ABX type coupling patterns, which indicated the presence of two symmetrical 1,3,4-trisubstituted phenyl groups; δ6.86 (s, H-2 and H-2′), δ6.87 (d, *J*= 8.0 Hz, H-5 and H-5′), and δ6.81 (dd, *J*= 1.84 and 8.00 Hz, H-6 and H-6′). Four proton signals of two oxymethylenes appeared at δ3.85 (m) and δ4.21 (dd, *J*= 6.04 Hz, 9.20 Hz) assignable to H-9 and H-9'. In addition, two benzylic oxymethine protons signals were obtained at δ4.71 (d, *J*= 4.12 Hz, H-7 and H-7'). Moreover, two methyne signals of H-8 and H-8'appeared at δ3.07 as multiplet. Finally, the singlet of the methoxyl protons appeared at δ3.89.

Complete assignments of all ^1H and ^{13}C NMR (Table 3.2) signals were based on HMBC (Fig. 3.6), HSQC (Fig. 3.7), and COSY spectrum data analysis. The COSY spectrum (Fig. 3.5) confirmed the presence of two 1,3,4-trisubstituted phenyl groups

showing connectivity between the hydrogen H-6, H-2, and H-5 of ring A and the hydrogen H-6′, H-2′, and H-5′ of ring B.

Finally, thorough analysis of all spectra data (^{1}H, ^{13}C, COSY, HSQC, and HMBC) of compound B confirmed that it is the known pinoresinol **18**[132, 133].

Table 3.2 ^{1}H and ^{13}C NMR Spectra Data of Compound B in $CDCl_3$ and the Literature Values[133]

Position	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)$^{Ref.}$	δ_H (ppm)$^{Ref.}$
1, 1’	132.95	-	133.3	-
2, 2’	108.67	6.86 (s)	109.0	6.82 (s)
3, 3’	146.78	-	147.1	-
4, 4’	145.31	-	145.6	-
5, 5’	114.35	6.87 (d, 8.00)	114.7	6.80 (d, 8.08)
6, 6’	119.06	6.81 (dd, 1.84, 8.00)	119.4	6.73 (dd, 1.54, 8.08)
7, 7’	85.97	4.71 (d, 4.12)	86.3	4.66 (d, 4.33)
8, 8’	54.23	3.07 (m)	54.5	3.03 (m)
9a, 9’a	71.75	3.85 (m)	72.1	3.79 (dd, 3.69, 9.21)
9b, 9’b		4.21(dd, 6.04, 9.20)		4.17 (dd, 6.88, 9.21)
3-OCH_3, 3’-OCH_3	56.05	3.89 (s)	56.3	3.81 (s)

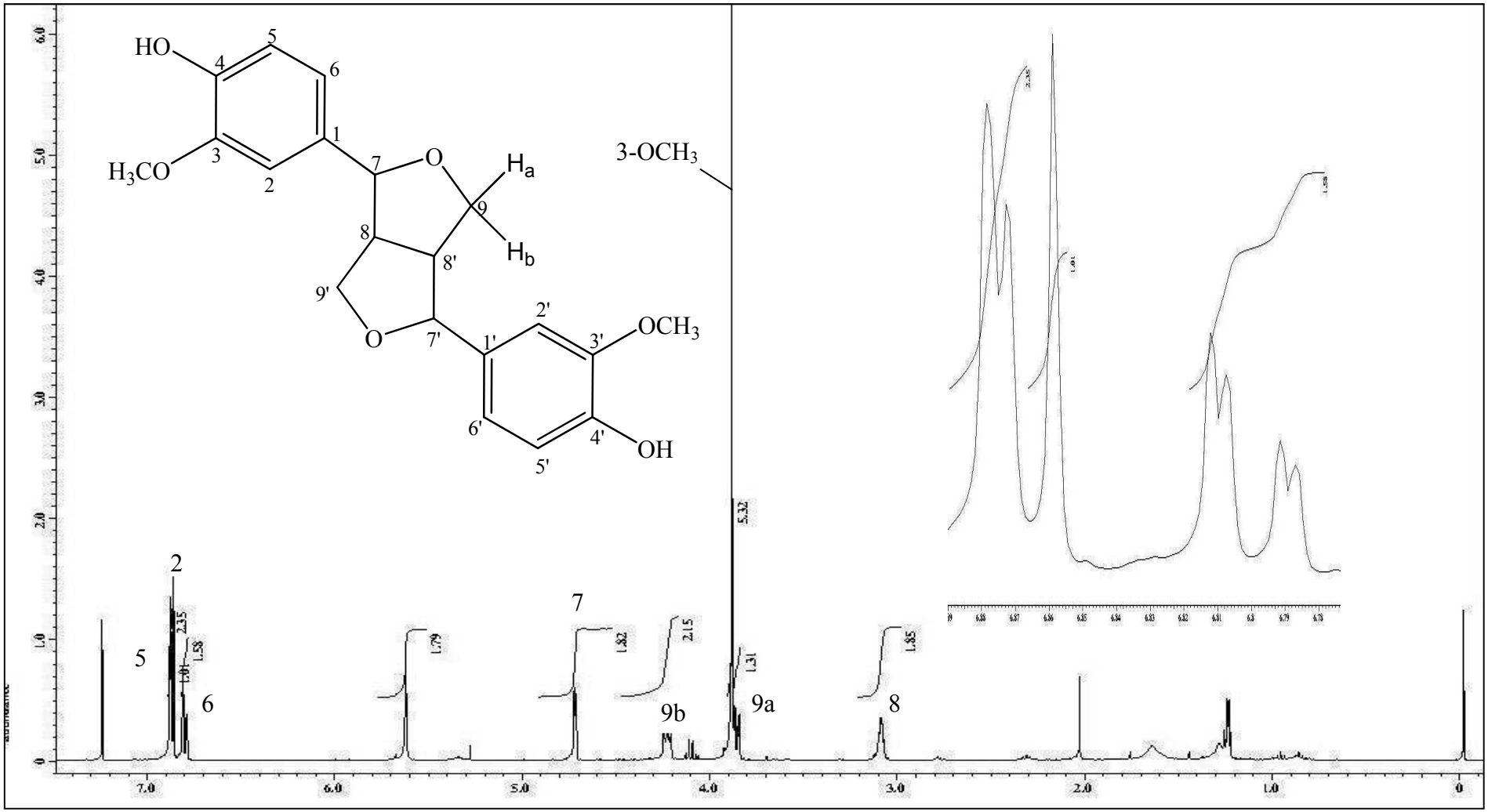


Figure 3.3 ^{1}H NMR Spectrum of Compound B in $CDCl_3$

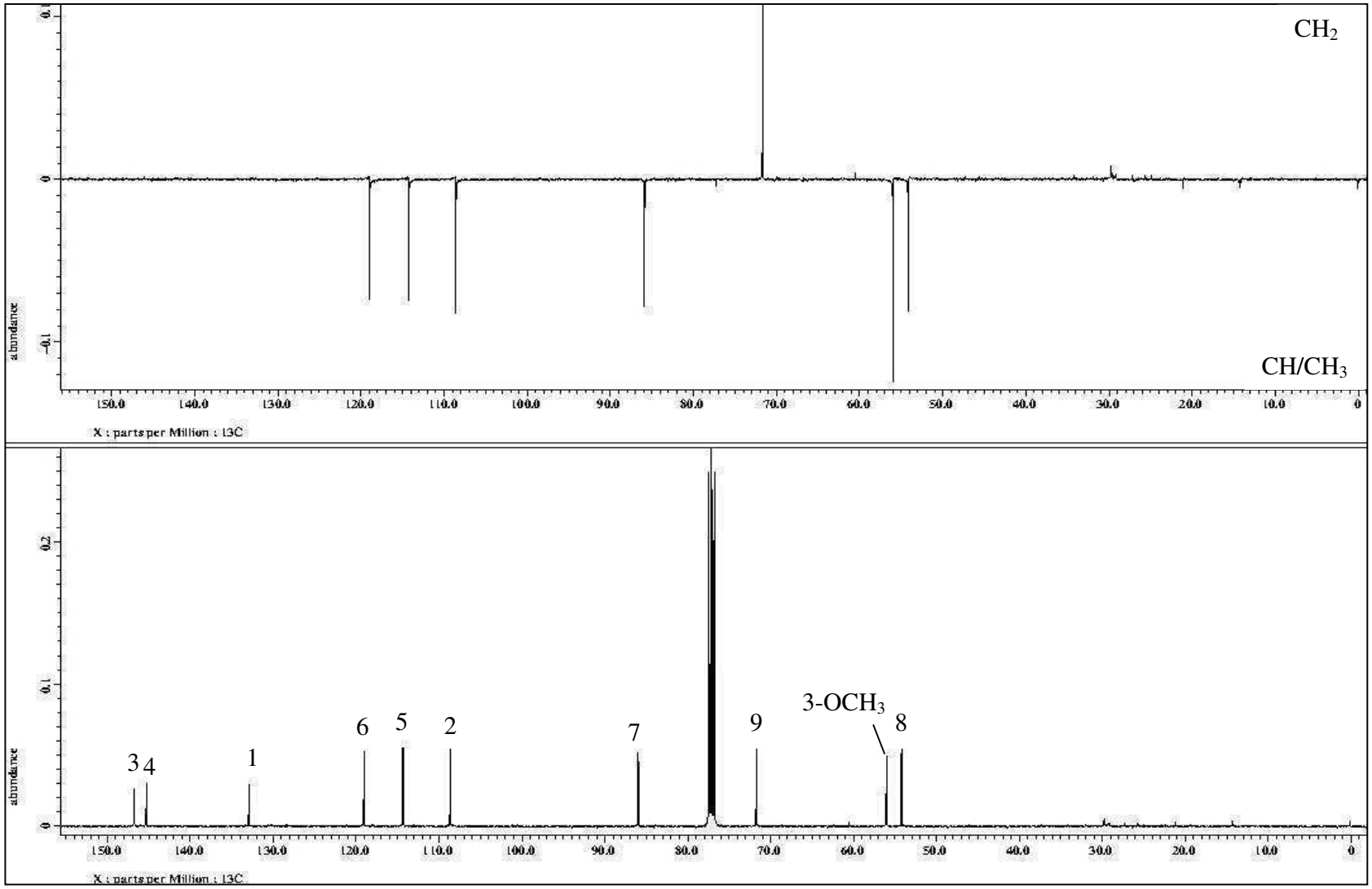


Figure 3.4 ^{13}C **and DEPT Spectra of Compound B in** $CDCl_3$

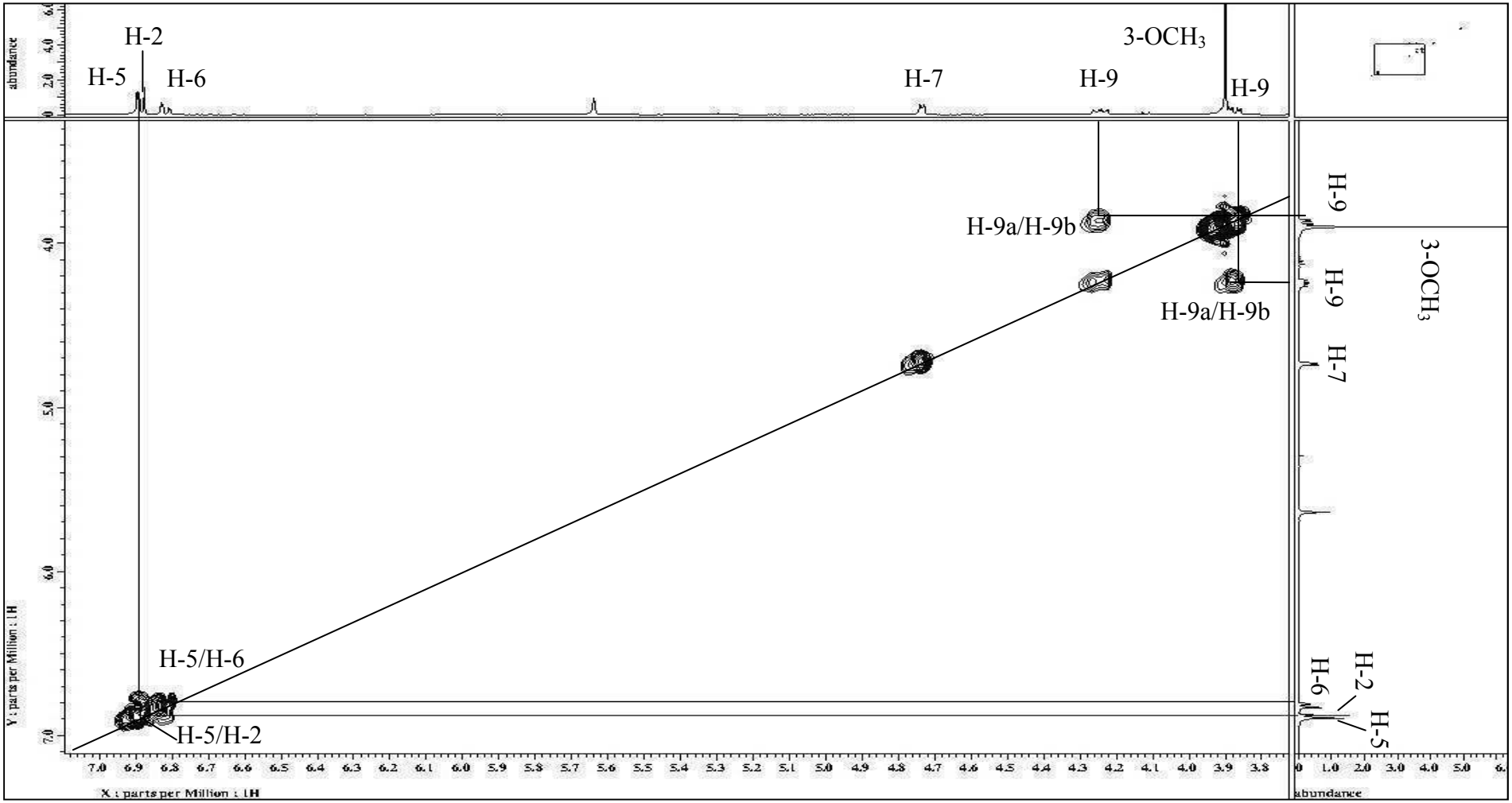


Figure 3.5 COSY NMR Spectrum of Compound B in CDCl3

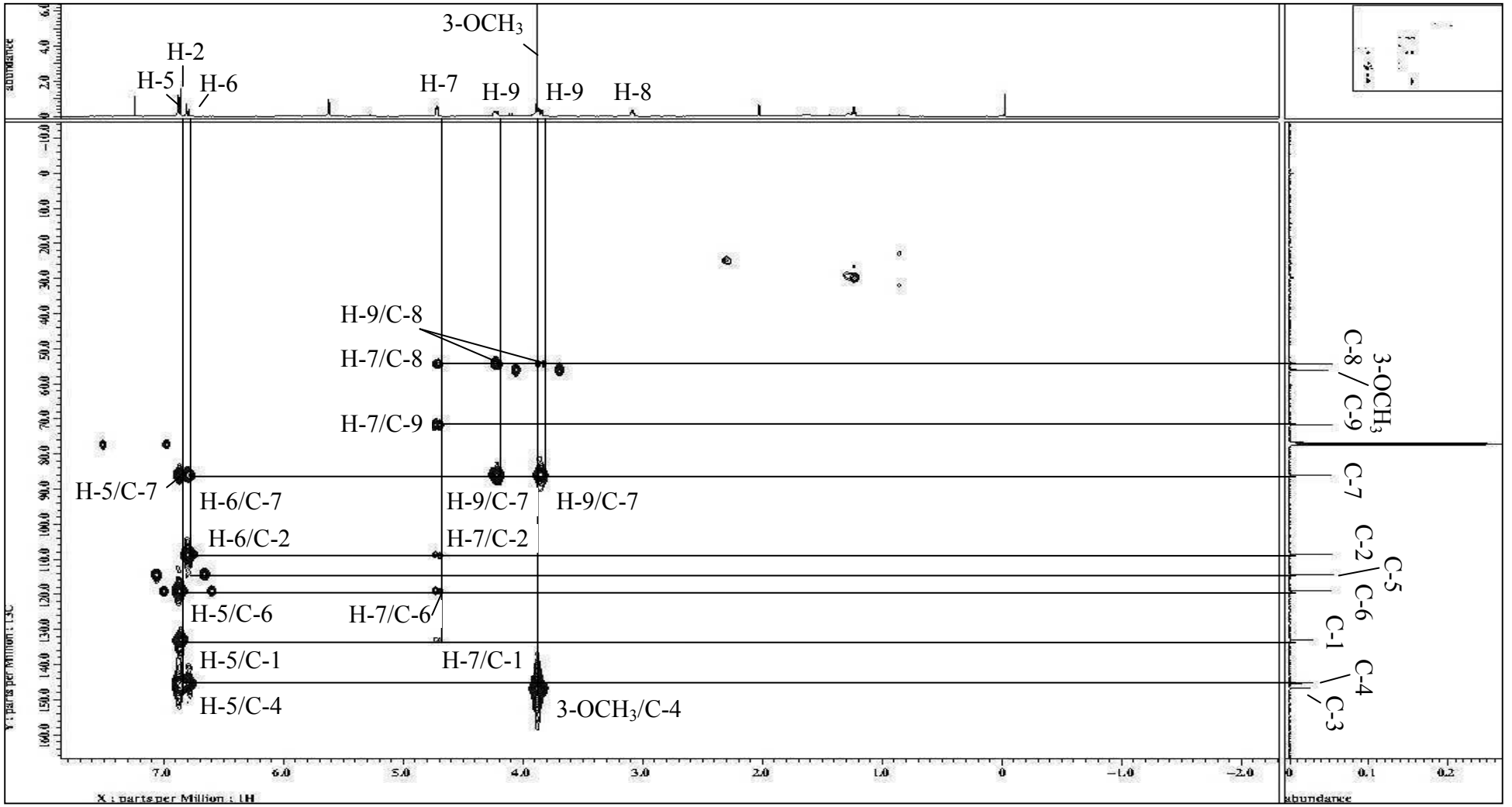


Figure 3.6 HMBC Spectrum of Compound B in $CDCl_3$

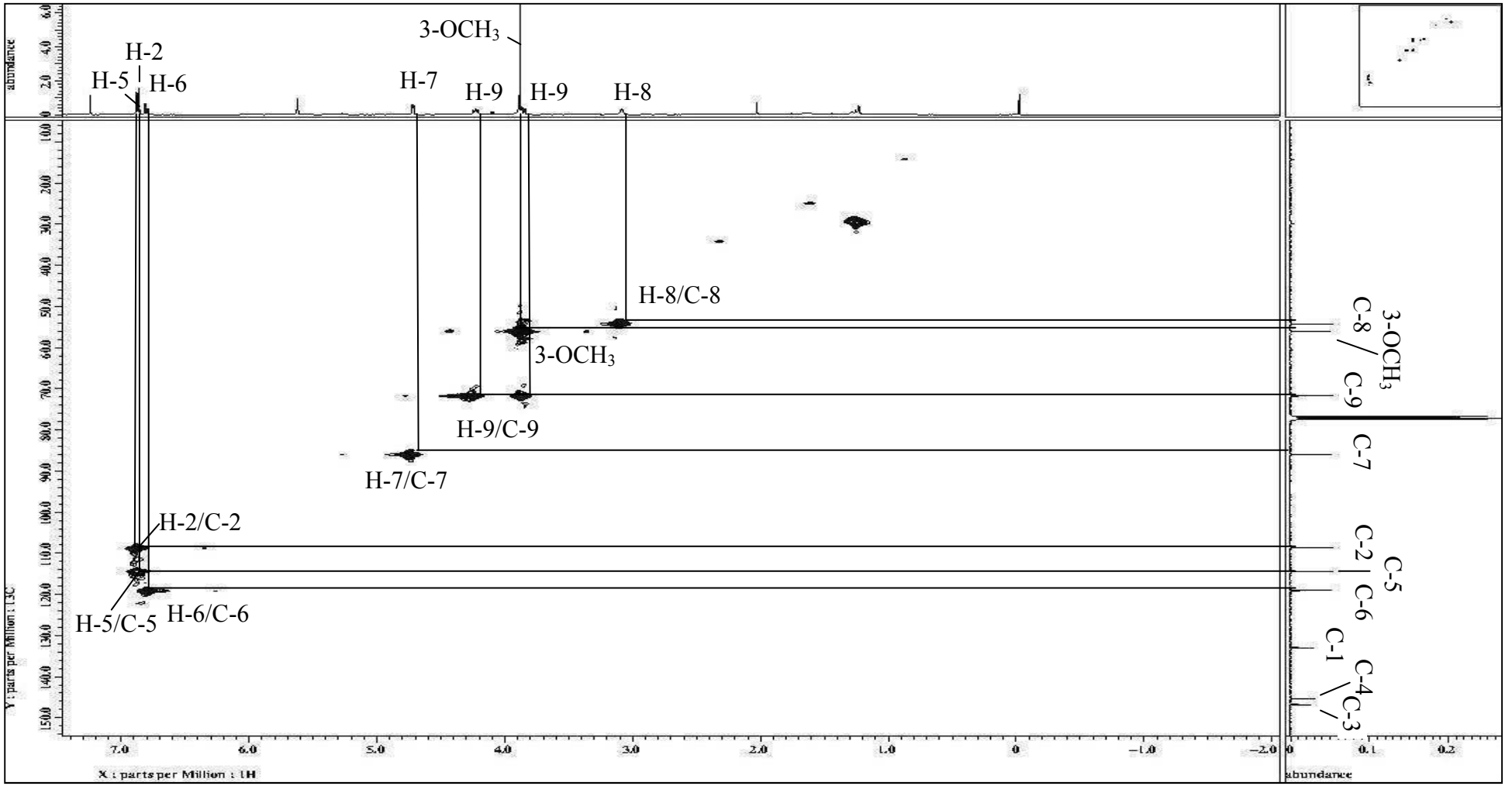
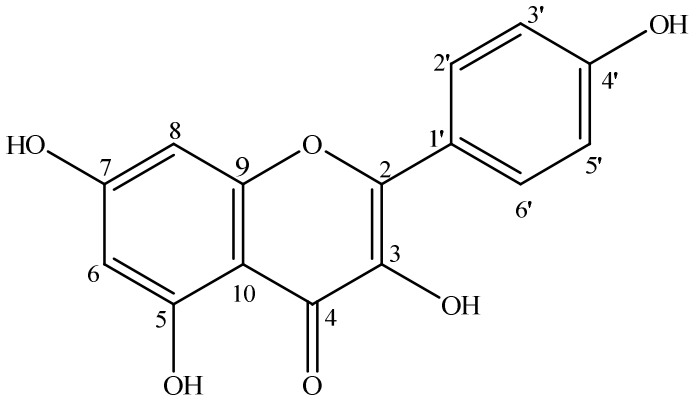


Figure 3.7 HSQC Spectrum of Compound B in $CDCl_3$

3.3 Compound C: Kaempferol[130,134]



Compound C was isolated as yellowish powder. Compound C showed a yellow spot on TLC after spray with vanillin upon heating. The ESI-MS spectrum showed a pseudomolecular ion peak at *m/z* 287.06 $[M+H]^+$ indicating the molecular formula as $C_{15}H_{10}O_6$.

UV spectrum (MeOH) showed maximum absorption at 267 nm which indicated the presence of a benzoyl system and the absorption at 364 nm represented a cinnamoyl system[57].

The ^1H NMR spectrum (Fig. 3.9) of compound C revealed two sets of meta-coupled doublets at δ6.15 (*J*= 2.00 Hz) and 6.37 (*J*= 2.00 Hz) implying the existence of a 1,2,3,5-tetrasubstitution ring A. These peaks were assignable to H-6 and H-8 respectively. The presence of a set of A_2B_2 doublets at δ6.87 and δ8.05 each integrating for two protons may be assigned to H-2', H-6' and H-3', H-5' respectively.

^{13}C NMR spectrum (Fig. 3.10) showed six oxygenated sp^2 quaternary carbons at δ146.7, δ135.8, δ161.2, δ164.2, δ156.9, δ159.2 assignable to C-2, C-3, C-5, C-7, C-9, C-4' respectively. The signal at δ176.0 indicated the presence of a conjugated ketone at C-4, further suggested the presence of flavonol type of skeleton for compound C[109].

The HMBC (Fig. 3.8) spectrum of compound C showed the correlations of H-6/C-5, H-6/C-7, H-6/C-8, H-6/C-10, and H-8/C-9, which established the substructure of

ring A. The HMBC correlations of H-2' with C-3', C-4', and C-6' was observed corresponding to the ring B substructure.

Thus, thorough analysis of the HMBC (Fig. 3.11), COSY and HSQC (Fig. 3.12) spectra, together with comparison of the spectral data with literature values[134, 135] confirmed that compound C is kaempferol **30**. The details of NMR data of compound C is shown in Table 3.3.

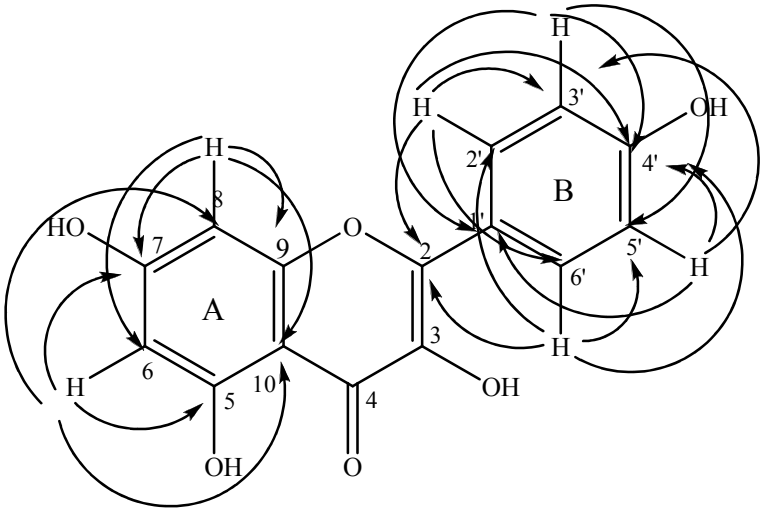


Figure 3.8 The HMBC Correlation of Compound C

Table 3.3 **^{1}H, ^{13}C and HMBC NMR Spectra Data of Compound C in CD_3OD and the Literature Value**[134]

Position	δ_C (ppm)	δ_H (ppm)	HMBC (H→C)	δ_C (ppm)$^{Ref.}$
2	146.7	-	-	146.2
3	135.8	-	-	134.2
4	176.0	-	-	176.9
5	161.2	-	-	160.7
6	97.9	6.15 (d, 2.00)	5, 7, 8, 10	98.8
7	164.2	-	-	163.7
8	93.1	6.37 (d, 2.00)	6, 7, 9, 10	93.6
9	156.9	-	-	157.7
10	103.2	-	-	103.2
1’	122.4	-	-	121.7
2’	129.3	8.05 (d, 9.16)	2, 4’, 3’, 6’	130.5
3’	115.0	6.87 (d, 9.16)	1’, 4’, 5’	115.9
4’	159.2	-	-	160.1
5’	115.0	6.87 (d, 9.16)	1’, 3’, 4’	115.9
6’	129.3	8.05 (d, 9.16)	2, 2’, 4’, 5’	130.5

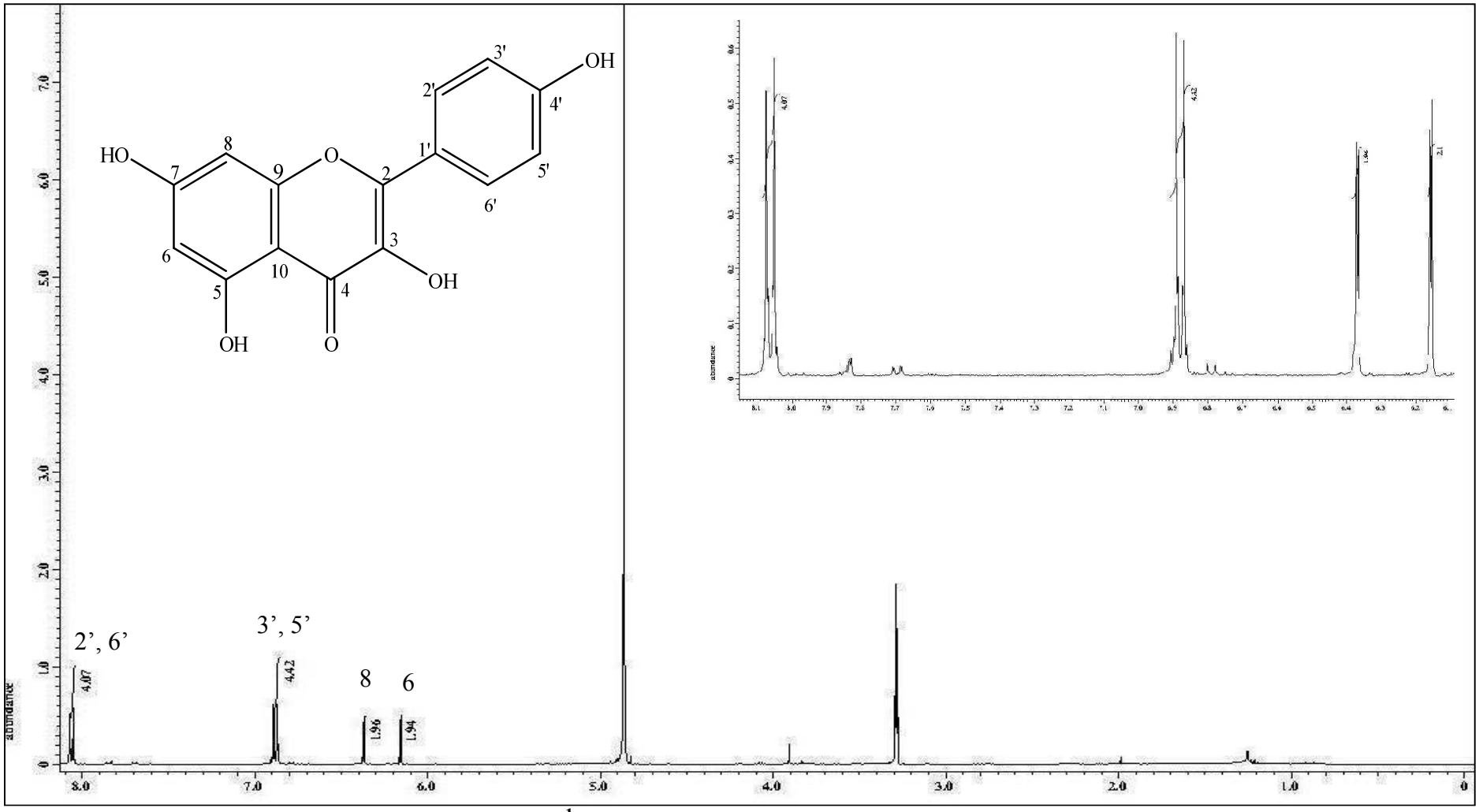


Figure 3.9 ^{1}**H NMR Spectrum of Compound C in CD_3OD**

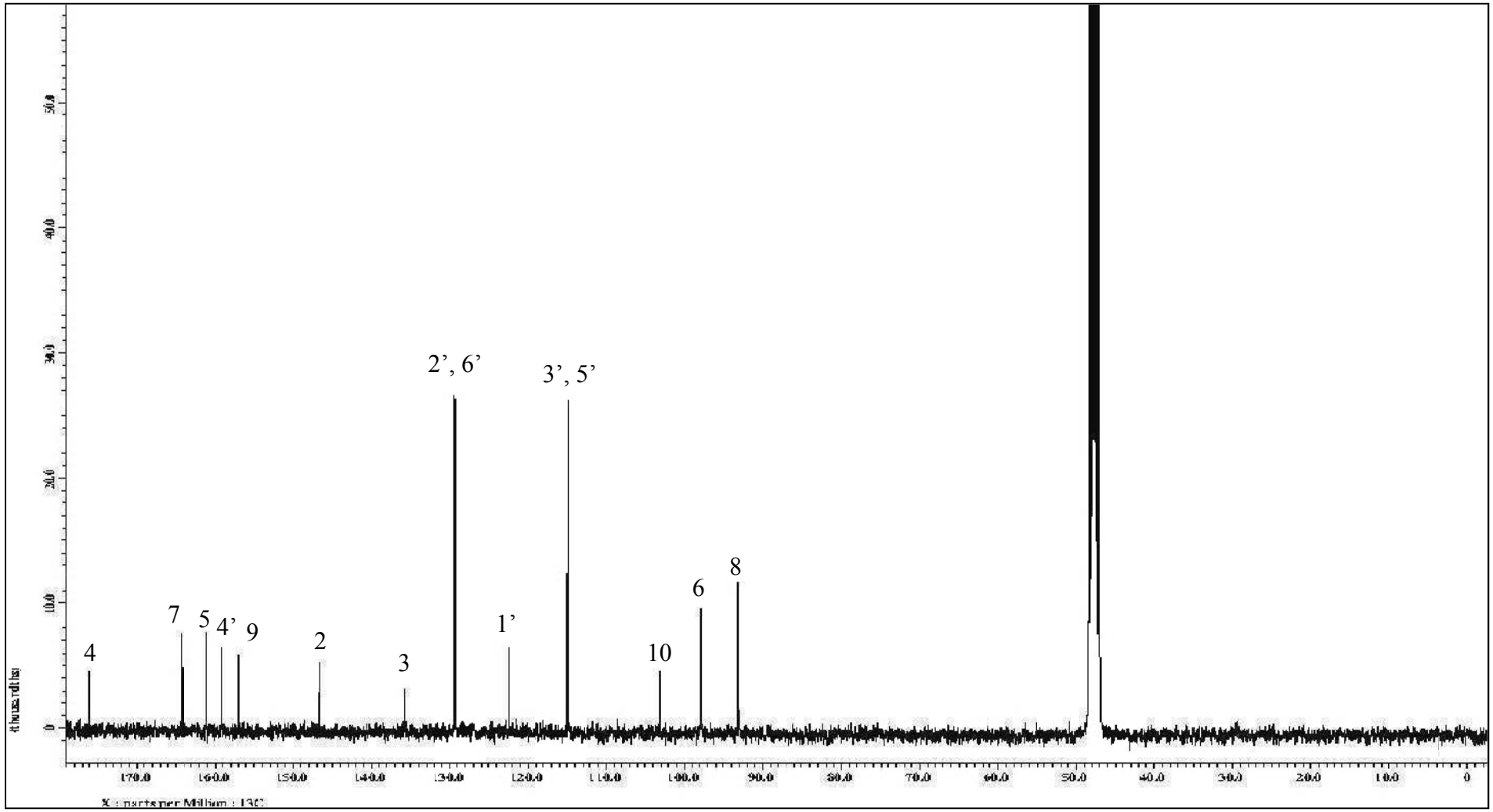


Figure 3.10 ^{13}C NMR Spectrum of Compound C in CD_3OD

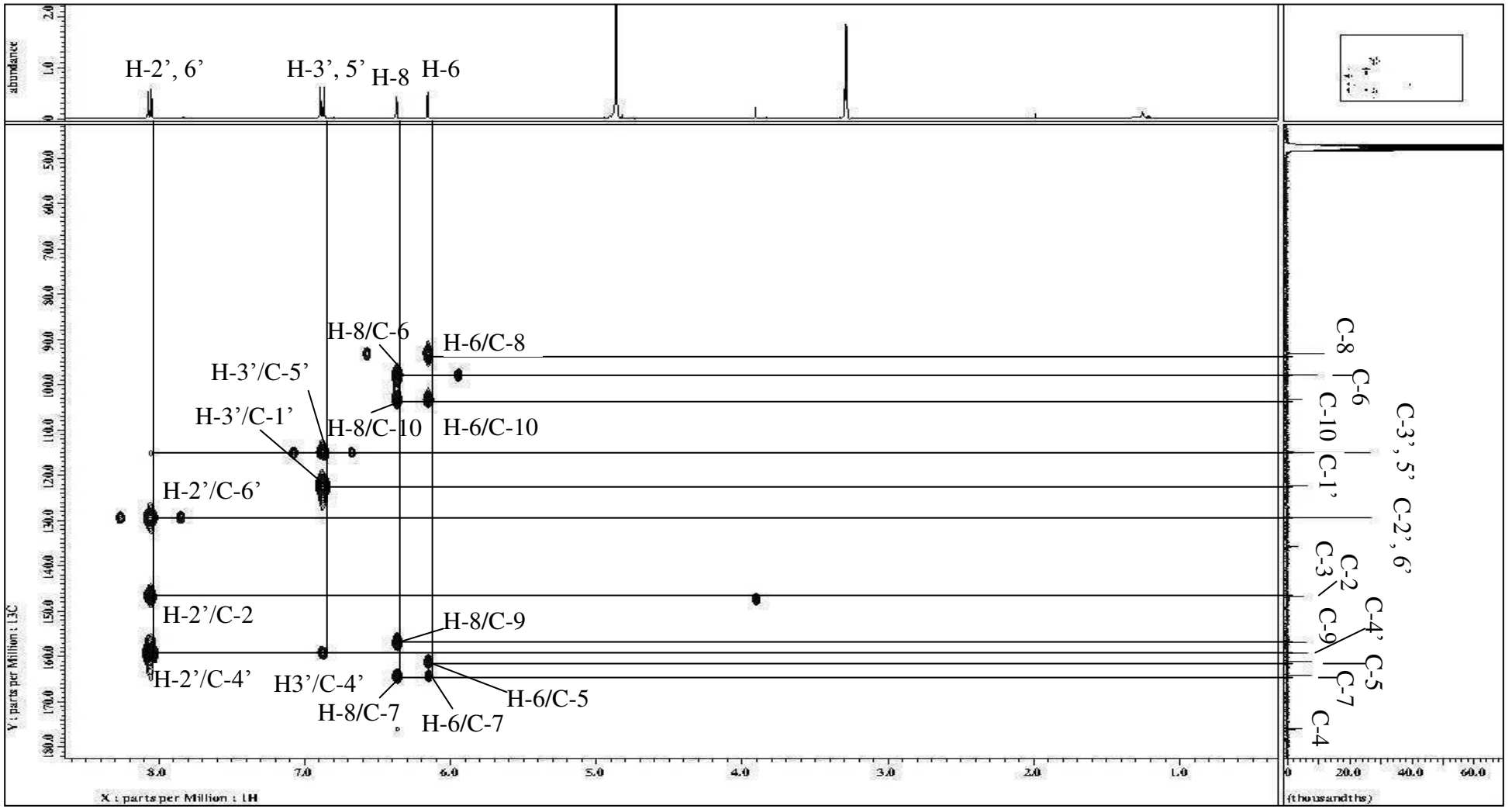


Figure 3.11 HMBC Spectrum of Compound C in CD_3OD

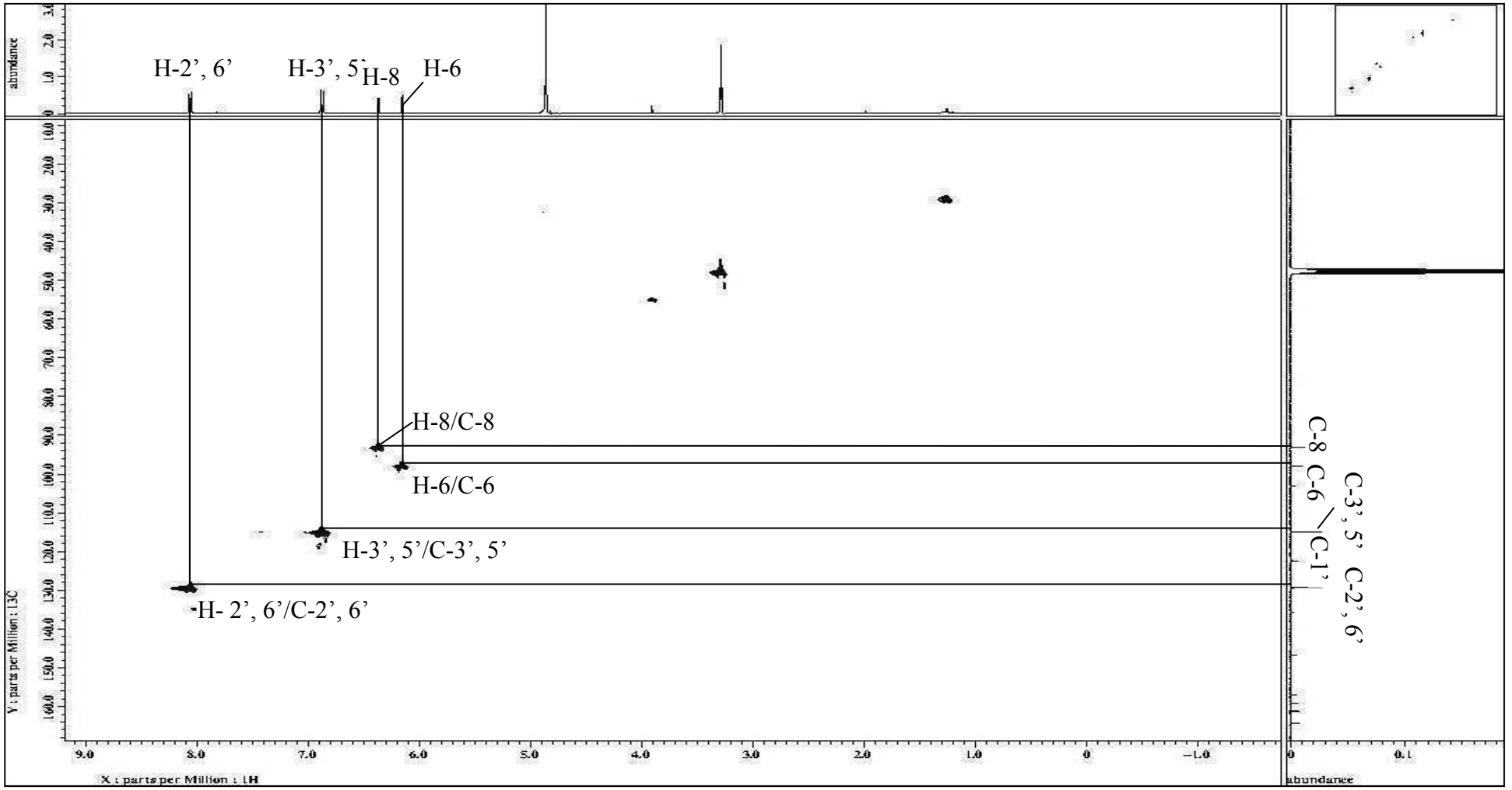
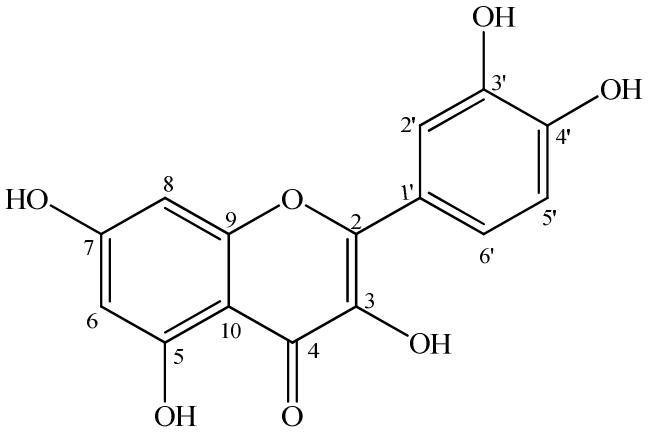


Figure 3.12 HSQC Spectrum of Compound C in CD_3OD

3.4 Compound D: Quercetin[130, 136]



Compound D was isolated as a yellow amorphous powder. The negative ESI-MS of compound D was observed at *m/z* 301 $[M-H]^-$, corresponding to the molecular formula of $C_{15}H_{10}O_7$. The UV spectra (MeOH) showed the presence of a benzoyl system at 257 nm and a cinnamoyl system at 372 nm[57].

The ^1H and ^{13}C NMR spectra of compound D (Fig. 3.13 and Fig. 3.14) exhibited resonance due to aromatic systems. Two typical meta coupling doublets revealed at δ6.17 and δ6.38 in ^1H NMR were due to 1,2,3,5-tetrasubstitution at ring A. These peaks were assigned to H-6 and H-8 respectively. An ABX system existed at δ7.72 (d, *J*= 2.1 Hz, H-2'), δ6.87 (d, *J*= 7.56 Hz, H-5') and δ7.61 (dd, *J*= 2.1, 7.56 Hz, H-6'). This was due to the 3',4'-disubstitution of ring B.

There were 15 aromatic carbon signals revealed in the ^{13}C NMR spectrum of compound D. Seven sp^2 oxygenated quaternary carbon were assigned to C-2, C-3,C- 5, C-7, C-9, C-4', C-5' respectively. The presence of conjugated ketone at C-4 (δ176.1) further suggested the flavonol type skeleton for compound D.

Hence, compound D was confirmed as quercetin **53** and the detail analyzed NMR data of compound D was shown in Table 3.4.

Table 3.4 **^{1}H and ^{13}C NMR Spectra Data of Compound D in CD_3OD and the Literature Values**[136]

Position	δ_C (ppm)	δ_H (ppm)	δ_C (ppm) Ref.	δ_H (ppm) Ref.
2	148.1	-	147.9	-
3	137.4	-	137.2	-
4	177.6	-	177.3	-
5	162.7	-	162.5	-
6	99.3	6.17 (d, 2.0)	99.3	6.17 (d, 2.0)
7	165.7	-	165.7	-
8	94.5	6.38 (d, 2.0)	94.4	6.37 (d, 2.0)
9	158.3	-	158.2	-
10	104.5	-	104.4	-
1’	121.8	-	122.1	-
2’	116.0	7.72 (d, 2.1)	116.0	7.73 (d, 2.0)
3’	146.3	-	146.2	-
4’	148.7	-	148.7	-
5’	116.3	6.87 (d, 7.56)	116.2	6.87 (d, 8.0)
6’	124.2	7.61 (dd, 2.1, 7.56)	124.1	7.62 (dd, 2.0, 7.5)

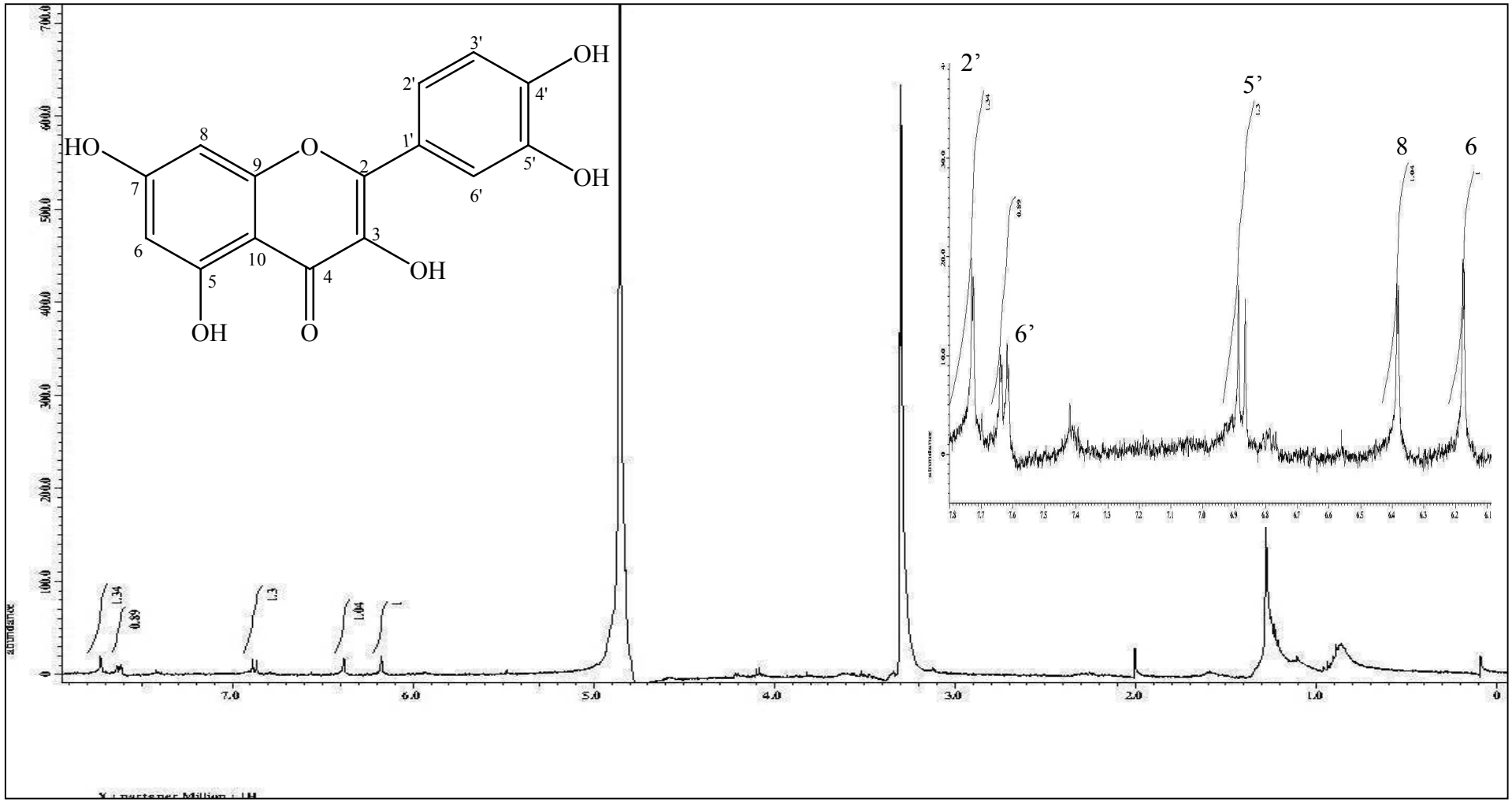


Figure 3.13 ^{1}H NMR Spectrum of Compound D in CD_3OD

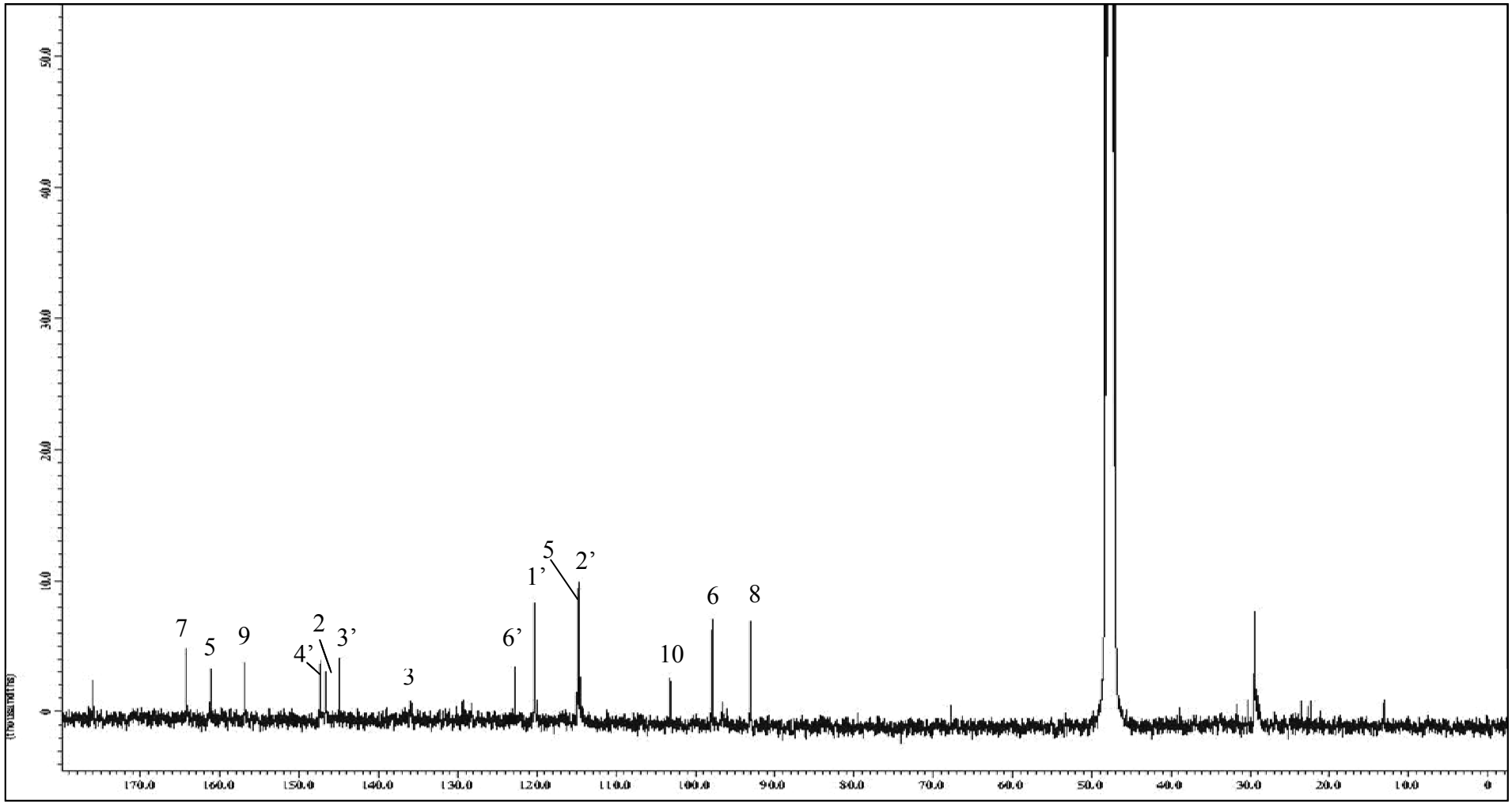
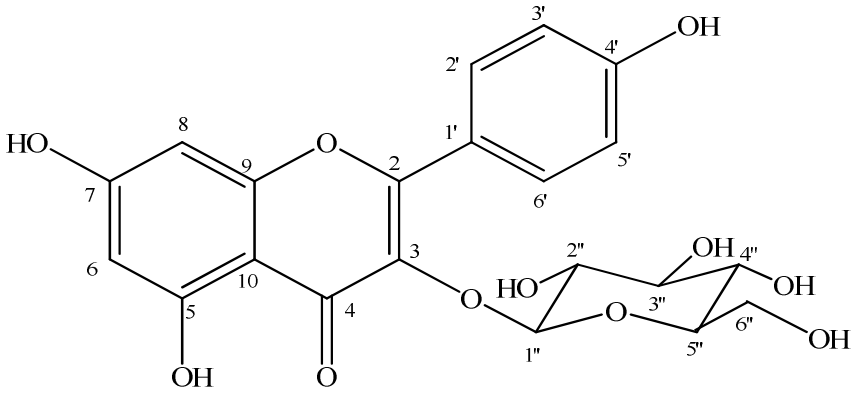


Figure 3.14 ^{13}C NMR Spectrum of Compound D in CD_3OD

3.5 Compound E: Astragalin[137-139]



Compound E was isolated using HPLC with methanol: deionized water (30: 70, 1 % of 0.25 M formic acid) as mobile phase. The negative EI-MS of compound E gave two ion peak $[M-H]^-$ at *m/z* 448 and 284, which was compatible with the molecular formula $C_{21}H_{20}O_{11}$. The fragmented peak corresponding to the sugar moiety was also observed at *m/z* 284.

The NMR spectra of compound E were shown in Fig. 3.15 and Fig. 3.16 respectively. The doublet that exist at δ5.25 was assignable to the anomeric proton (H-1'') with the corresponding carbon signal at δ104.2. The peaks at δ3.33 - 3.96 (m) showed the typical signals of glucose. Other glucosidic carbon signals were observed at δ104.2 (C-1''), 75.8 (C-2''), 78.1 (C-3''), 71.5 (C-4''), 78.5 (C-5''), 72.7 (C-6''). The attachment of glucose unit to the aglycon unit at C-3 was verified by the HMBC spectrum. The cross peak between δ5.25 (d, *J*= 7.1 Hz, H-1'') and δ135.5 (C-3) was observed. Hence, this compound was identified as kaempferol-3-*O*-*β*-D-glucopyranoside **50** and confirmed by comparing with literature values[137-139].

The complete assignment of compound E was summarized in Table 3.5.

Table 3.5 **^{1}H and ^{13}C NMR Spectra Data of Compound E in CD_3OD and the Literature Values**[138]

Position	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)$^{Ref.}$	δ_H (ppm)$^{Ref.}$
2	157.2	-	158.3	-
3	134.0	-	135.3	-
4	178.1	-	178.3	-
5	161.6	-	162.8	-
6	98.7	6.20 (d, 1.96)	99.7	6.19 (br s)
7	165.1	-	165.7	-
8	93.5	6.39 (d, 1.96)	94.6	6.38 (br s)
9	157.7	-	158.8	-
10	104.2	-	104.9	-
1’	121.4	-	122.6	-
2’	131.2	8.05 (d, 8.8)	132.1	8.04 (d, 8.4)
3’	114.7	6.88 (d, 8.8)	116.0	6.87 (d, 8.4)
4’	160.2	-	161.4	-
5’	114.7	6.88 (d, 8.8)	116.0	6.87 (d, 8.4)
6’	130.9	8.05 (d, 8.8)	132.1	8.04 (d, 8.4)
1’’	102.7	5.25 (d, 7.1)	104.0	5.23 (d, 7.2)
2’’	74.3	3.33-3.96 (m)	75.6	3.18- 3.70 (m)
3’’	76.6	3.33-3.96 (m)	78.3	3.18- 3.70 (m)
4’’	71.2	3.33-3.96 (m)	71.2	3.18- 3.70 (m)
5’’	77.0	3.33-3.96 (m)	77.9	3.18- 3.70 (m)
6’’	61.5	3.67 (d, 2.2)	62.5	3.58 (dd, 12.0, 5.2)
		3.70 (d, 2.2)		3.71 (dd, 12.0, 2.4)

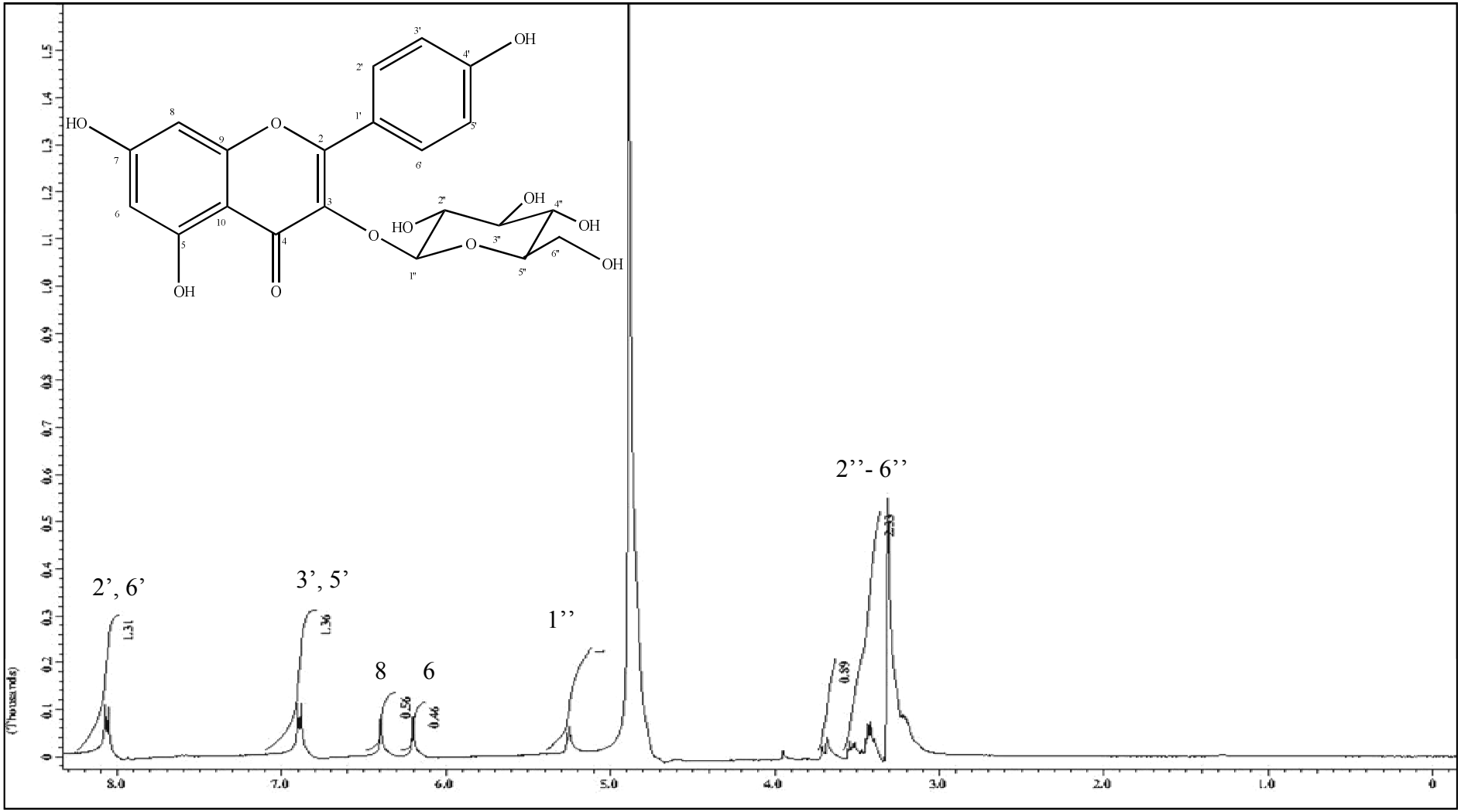


Figure 3.15 ^{1}H NMR Spectrum of Compound E in CD_3OD

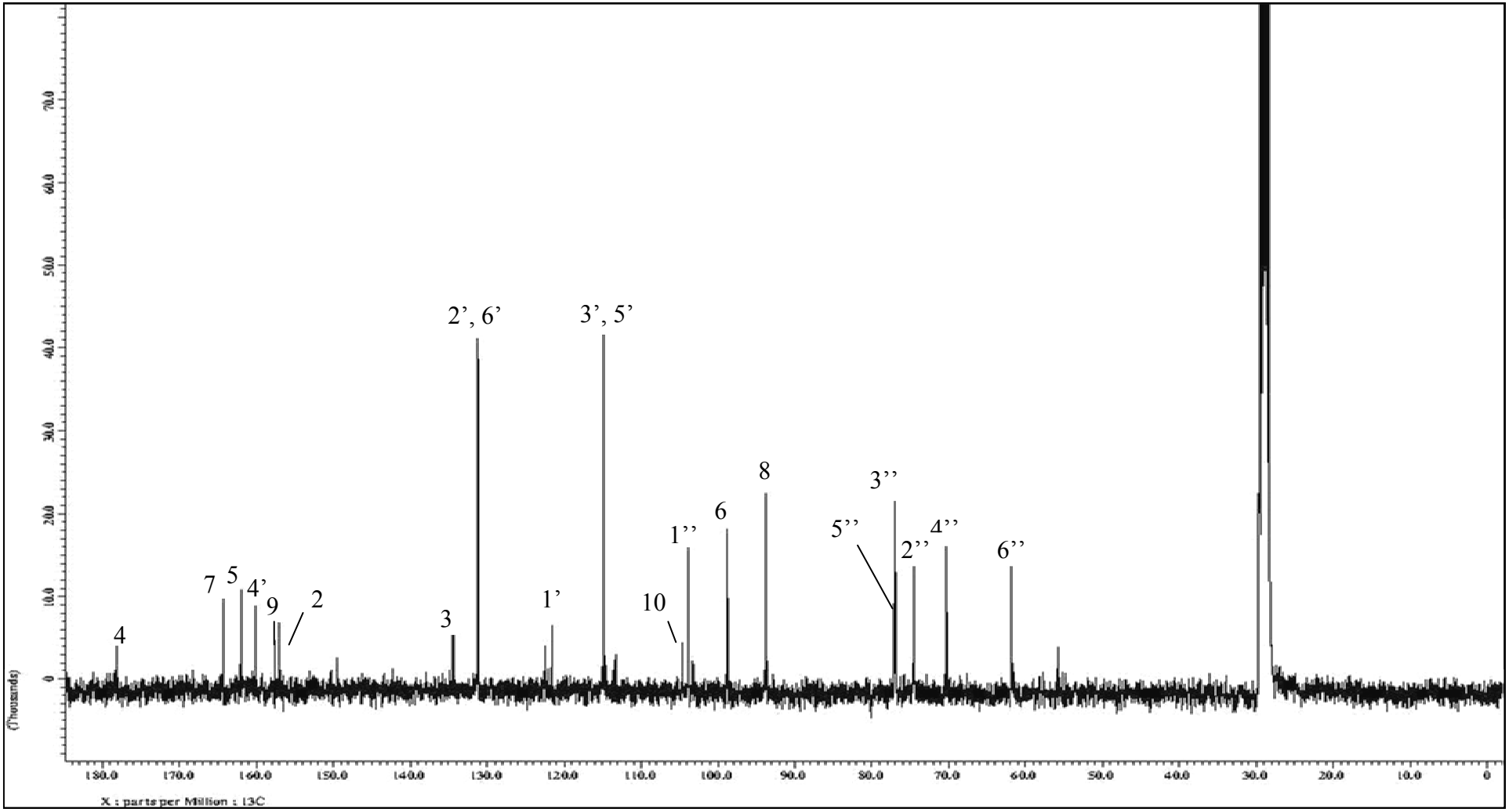
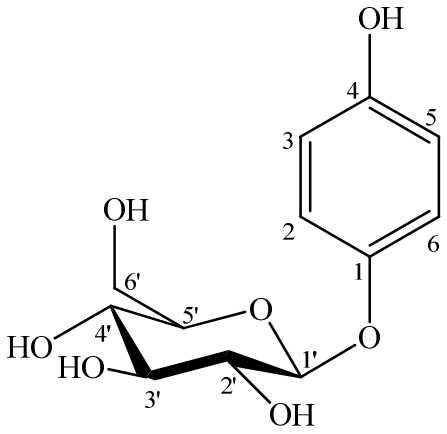


Figure 3.16 ^{13}C NMR Spectrum of Compound E in CD_3OD

3.6 Compound F: Arbutin[140]



Compound F was isolated as colorless amorphous. The EIMS gave an ion peak at *m/z* 284 and a base at *m/z* 117, consistence with the molecular formula $C_{12}H_{16}O_7$.

The ^{1}H NMR spectra (Fig. 3.18) showed typical signals of a glucose moiety at δ3.25 - 3.88 with the anomeric proton signal appearing at δ4.70 (1H, d, *J*= 7.2 Hz). Two signals at δ6.66 (H-3 and H-5, d, *J*= 8.8 Hz) and δ6.93 (H-2, and H-6, d, *J*= 8.8 Hz) were assignable to four aromatic protons of the hydroquinone residue.

The ^{13}C NMR spectrum (Fig. 3.19) showed the appearance of 12 carbon signals; two oxygenated quaternary carbons [C-1 (δ153.9) and C-4 (δ152.5)], four aromatic methines (C-2, C-3, C-5, C-6), five benzylic oxymethine (C-1', C-2', C3', C-4', and C-5'), one oxymethylene (C-6'). An anomeric carbon signal was observed at δ103.8 which implied the existence of glucose moiety.

The HMBC correlation of compound F was shown in Fig. 3.17 and the 2D spectra data (HMBC and HSQC) was shown in Fig. 3.20 and Fig. 3.21.

The physical property and spectroscopic data of this compound (Table 3.6) were identical to arbutin **22**, namely 1,4-dihydroxyl-1-β-D-glucopuranoside.

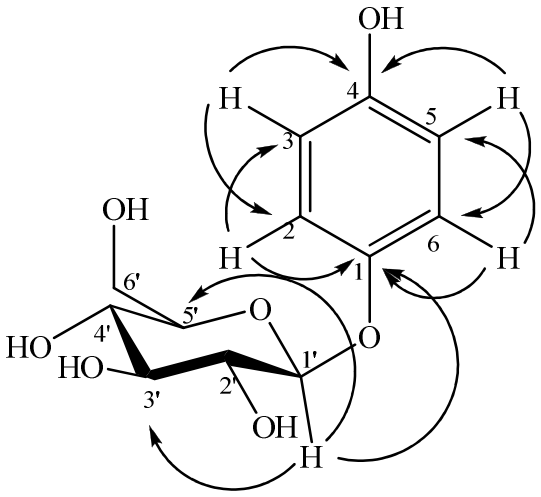


Figure 3.17 The HMBC Correlation of Compound F

Table 3.6 ^{1}H, ^{13}C and HMBC NMR Spectra Data of Compound F in CD_3OD and the Literature Values[140]

Position	δ_C (ppm)	δ_H (ppm)	HMBC (H→C)	δ_C (ppm)$^{Ref.}$	δ_H (ppm)$^{Ref.}$
1	153.9	-	-	152.36	-
2	119.5	6.93 (d, 8.8)	1, 3	118.61	6.85 (d, 8.7)
3	116.7	6.66 (d, 8.8)	2, 4	115.64	6.65 (d, 8.7)
4	152.5	-	-	150.54	-
5	116.7	6.66 (d, 8.8)	4, 6	117.81	6.65 (d, 8.7)
6	119.5	6.93 (d, 8.8)	1, 5	115.64	6.85 (d, 8.7)
1’	103.8	4.70 (d, 7.2)	1, 3’, 5’	101.90	4.62 (d, 7.0)
2’	75.1	3.25-3.88 (m)	-	73.46	3.38-3.86 (m)
3’	78.0	3.25-3.88 (m)	-	76.80	3.38-3.86 (m)
4’	71.5	3.25-3.88 (m)	-	69.93	3.38-3.86 (m)
5’	78.1	3.25-3.88 (m)	-	77.13	3.38-3.86 (m)
6’	62.7	3.25-3.88 (m)	-	60.93	3.38-3.86 (m)

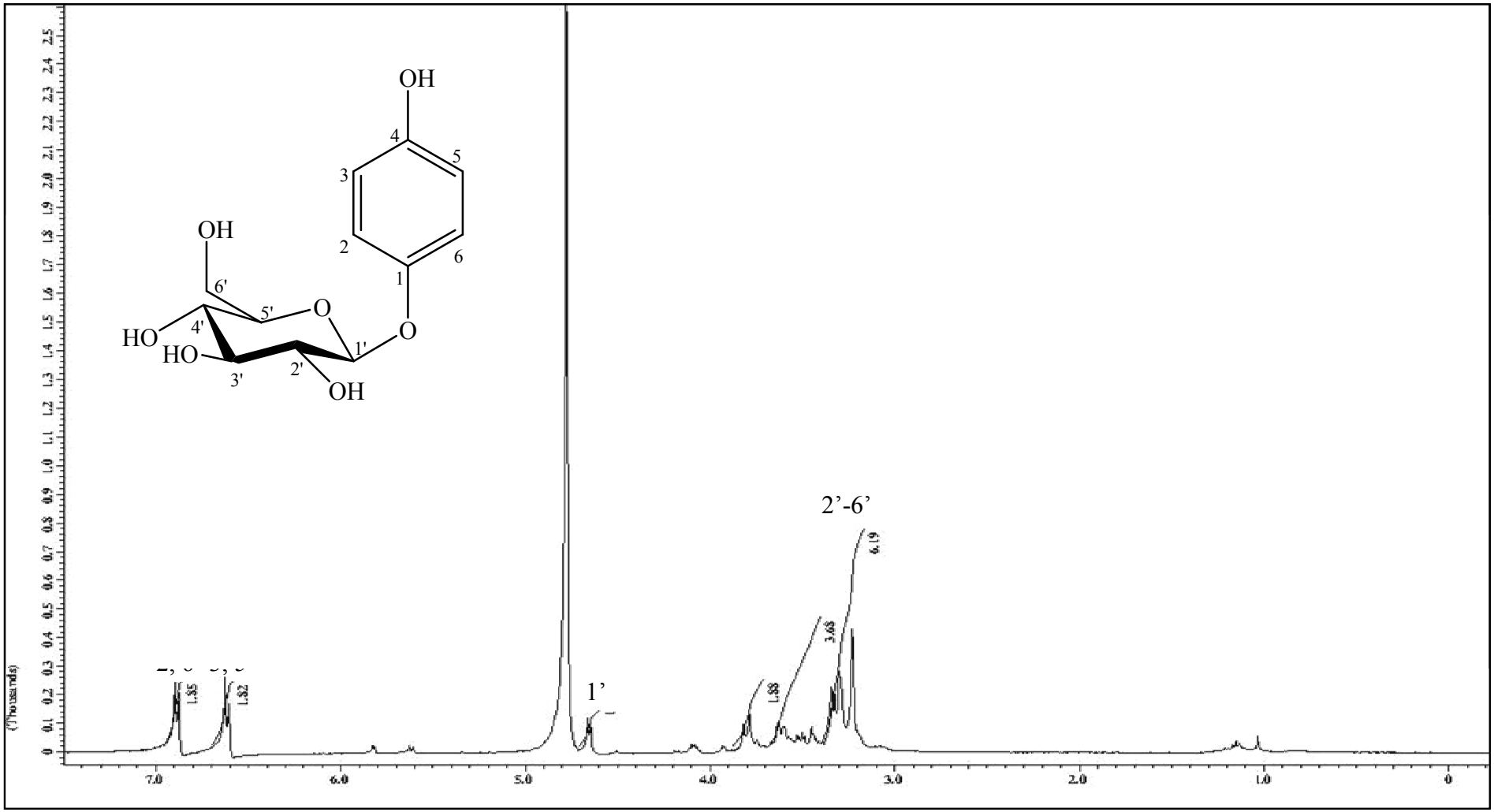


Figure 3.18 ^{1}H NMR Spectrum of Compound F in CD_3OD

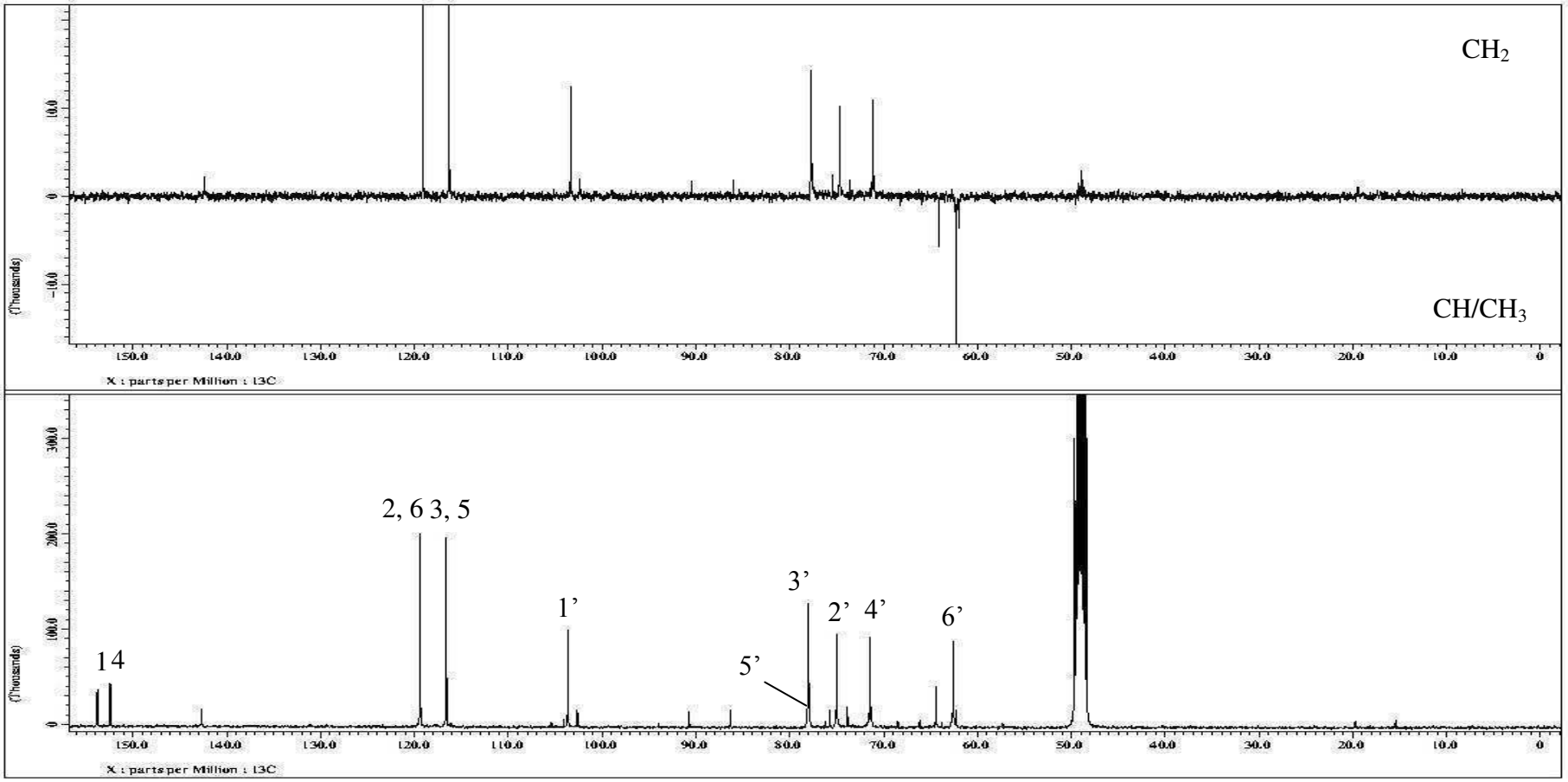


Figure 3.19 ^{13}C and DEPT Spectra of Compound F in CD_3OD

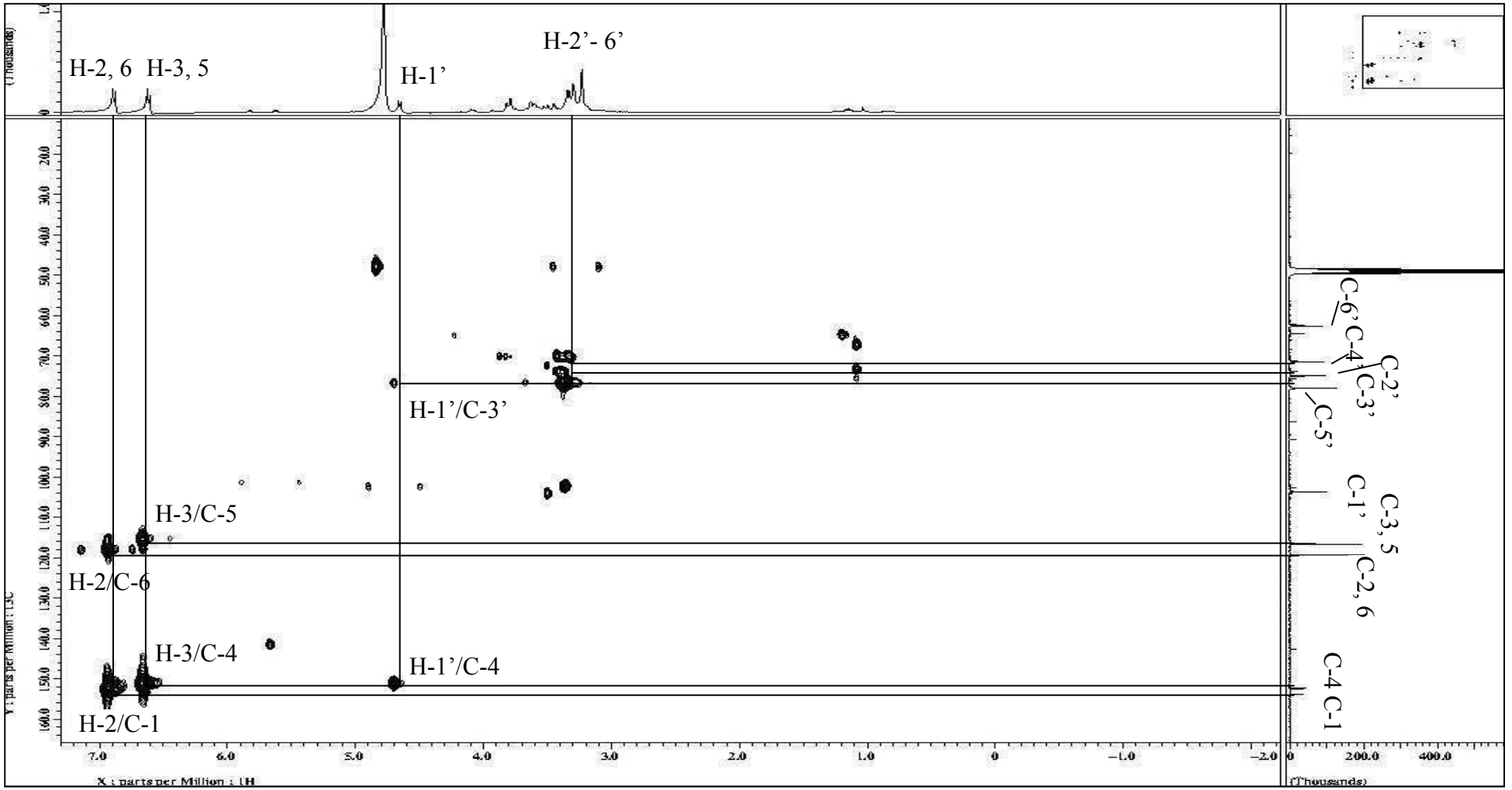


Figure 3.20 HMBC Spectrum of Compound F in CD_3OD

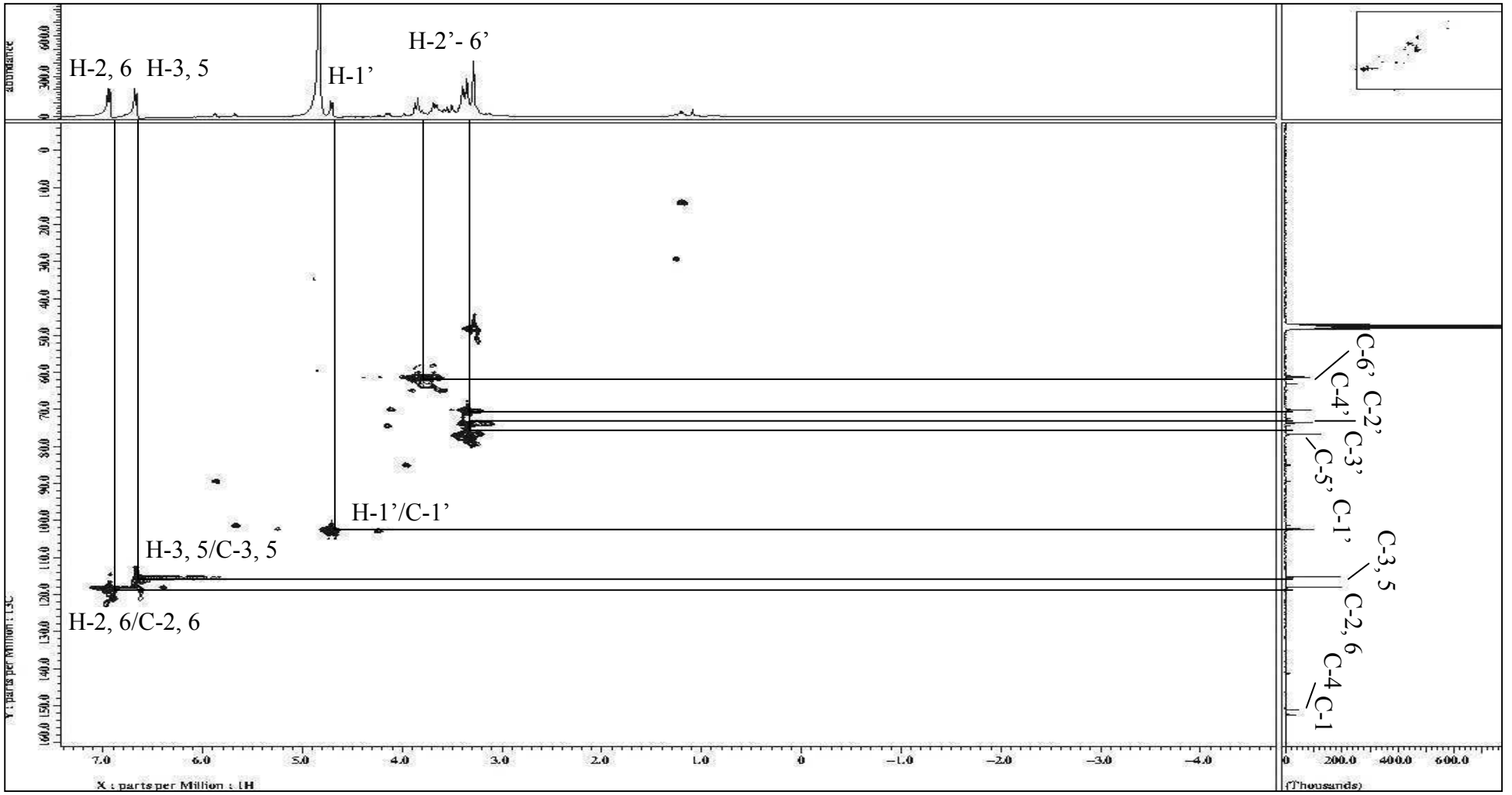


Figure 3.21 HSQC Spectrum of Compound F in CD_3OD

CHAPTER 4

ALLELOPATHIC POTENTIAL OF *CUSCUTA CAMPESTRIS*

4.1 Bioassay on Ethanol Extract of *Cuscuta campestris*

The work was conducted to investigate any possible allelopathic activity of *C. campestris* Yuncker. Results of the effect of different concentrations of ethanol extract of *Cuscuta* on three different crops are shown in Table 4.1. There were no significant effects on the germination rate of radish and weedy rice but *Cuscuta* inhibited lettuce seed germination. However, the growth of shoot and root of all three assayed species were severely affected. The efficacy of the *Cuscuta* extract was higher on the roots than shoots of the lettuce and weedy rice seedlings. The responses of all assayed species were dose-dependent (Fig. 4.1).

At low concentration (100 -1000 ppm), there was negligible and non significant reduction in germination of lettuce. At higher dose (5000 ppm), lettuce germination was markedly decreased (45 % inhibition). There was a significant inhibition (89 % inhibition) on shoot growth of lettuce by 5000 ppm of *Cuscuta*, whereas synergistic effects showed on the other applied doses. Lettuce root displayed higher sensitivity to the effect of *Cuscuta* extract than the shoot, a significant synergistic effect (14 - 47 % stimulation) on root growth obtained when low concentration applied (100 - 200 ppm). However, when treated with higher dosage, root growth was obviously decreased (43 - 95 %). (Table 4.1)

The ethanol extract of *Cuscuta* did not show any significant effect on radish seed germination. However, the roots appeared greatly affected compared with the control in terms of total length. With parallel increase in extract concentration, root growth was getting inhibited (27 - 84 %), although there was no obvious inhibition at low concentration (100 - 200 ppm). The effect of *Cuscuta* extract on radish shoot was not as great as the effect on root. In fact, growth of radish shoot was stimulated when low dosage (100 - 200 ppm) of *Cuscuta* treated on it. The situation change when

Table 4.1 Effect of Ethanol Extract of *Cuscuta campestris* on the Germination and Growth of Lettuce, Radish, and Weedy Rice Seedlings

Concentration (ppm)	Germination (%)	Shoot Length (mm)	Root Length (mm)	Dry Weight (g)
Lettuce				
0	100.0b (0.0)	10.0b (0.0)	39.3d (0.0)	0.010a (0.0)
100	100.0b (0.0)	14.0c (-39.0)	57.8f (-47.1)	0.011a (-10.0)
200	100.0b (0.0)	13.0c (-30.0)	44.7e (-13.8)	0.012a (-20.0)
500	98.3b (1.7)	12.4bc (-23.1)	22.4c (43.0)	0.013 a (-30.0)
1000	100.0 b (0.0)	12.5bc (-24.1)	11.2b (71.6)	0.011a (-10.0)
5000	55.0a (45.0)	1.1a (88.9)	1.9a (95.3)	0.017b (-70.0)
Radish				
0	100.0a (0.0)	32.6b (0.0)	71.9c (0.0)	0.15a (0.0)
100	98.3a (1.7)	41.2c (-26.5)	71.0c (1.2)	0.17a (-13.3)
200	98.3a (1.7)	40.4c (-23.9)	62.2bc (13.5)	0.15a (0.0)
500	100.0a (0.0)	29.6b (9.2)	52.2b (27.4)	0.16a(-6.7)
1000	100.0a (0.0)	15.9a (51.1)	16.1a (77.6)	0.16a (-6.7)
5000	95.0a (5.0)	11.4a (65.1)	11.6a (83.8)	0.18a (-20.0)
Weedy rice				
0	100.0a (0.0)	52.4d (0.0)	51.2c (0.0)	0.34a (0.0)
100	100.0a (0.0)	59.3e (-13.1)	50.3c (1.8)	0.33a (2.9)
200	100.0a (0.0)	49.8cd (5.0)	43.2b (15.6)	0.35a (-2.9)
500	98.3a (1.7)	45.6bc (12.9)	43.6b (14.8)	0.36ab (-5.9)
1000	98.3a (1.7)	41.7b (20.4)	40.6b (20.8)	0.36ab (-5.9)
5000	100.0a (0.0)	19.4a (63.0)	3.5a (93.1)	0.39b (-14.7)

Values in the column with the same letter are not significantly different at $p<0.05$.
Values in the parentheses are inhibition percentages over control.
Values in the parentheses with (-) are promotion percentages over control.

higher dosage (1000 - 5000 ppm) used, the shoot growth of radish was strongly inhibited (Table 4.1).

There were negligible effects of *Cuscuta* extract on weedy rice germination. The shoot and root growths were inhibited when higher dosage was applied. The shoot growth was inhibited about 13 – 63 % (500 – 5000 ppm), and the root growth was inhibited about 15 – 93 % (200 - 5000 ppm) (Table 4.1).

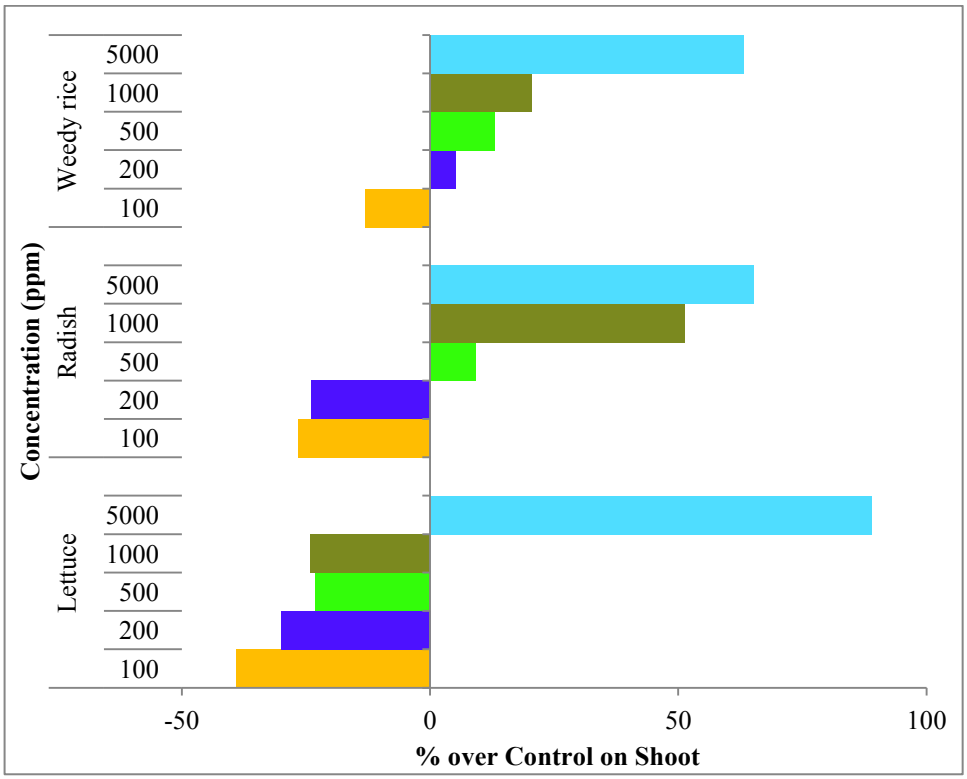


Figure 4.1 **Effect of *Cuscuta campestris* on the Shoot Growths of Lettuce, Radish and Weedy Rice Seedlings.**

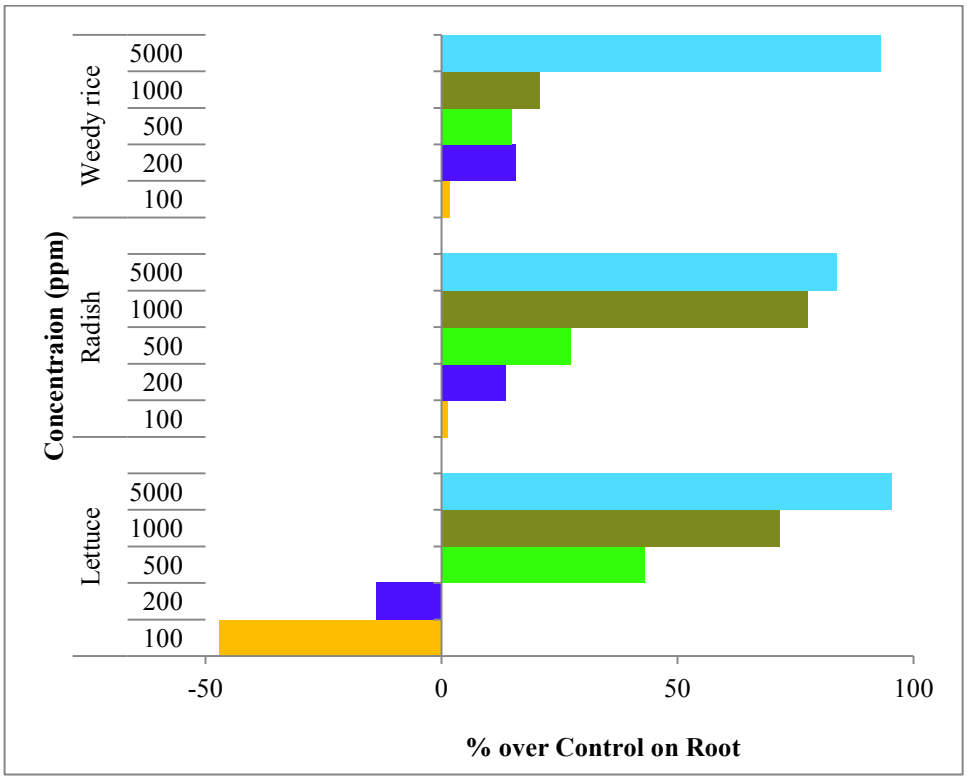


Figure 4.2 Effect of *Cuscuta campestris* on the Root Growths of Lettuce, Radish and Weedy rice Seedlings.

4.2 Allelopathic Potential of Constituents from *Cuscuta campestris* on Lettuce

Three compounds, *viz*. kaempferol, pinoresinol and sitosterol were selected to determine the allelopathic potentials. Overall, the germination of lettuce was not affected by all three constituents. However, the growth of shoots and roots of lettuce showed synergistic effect. The growth was seen to be reversely affected when the concentration increased. This shows that the response of lettuce was dose-dependent with the roots displaying greater sensitivity than the shoots.

Pinoresinol showed the greatest stimulatory effect on the growth of lettuce's root and shoot. It stimulated lettuce's root for 60 – 76 % and shoot for 21 – 64 %. Lettuce's shoot growth was increased by kaempferol for 20 – 60 % and the root growth was increased for 58 – 67 % (Table 4.2).

Table 4.2 Effect of Three Constituents from *C. campestris* on the Germination and Growth of Lettuce (*Lactuca sativa*) Seedlings.

Concentration (µM)	Germination (%)	Shoot Length (mm)	Root Length (mm)	Dry Weight (g)
Kaempferol				
0	96.67a (0.0)	8.52a (0.0)	11.85a (0.0)	0.032a (0.0)
1	100.00a (-3.44)	13.66b (-60.27)	19.76b (-66.70)	0.010a (69.29)
10	98.33a (-1.72)	12.56b (-47.43)	18.94b (-59.83)	0.010a (68.97)
100	96.67a (0.0)	10.25a (-20.31)	18.71b (-57.89)	0.009a (73.04)
Pinoresinol				
0	96.67a (0.0)	8.52a (0.0)	11.85a (0.0)	0.032a (0.0)
1	100.00a (-3.44)	13.99c (-64.18)	20.91b (-76.48)	0.011a (64.58)
10	98.33a (-1.72)	12.78c (-49.93)	22.48b (-89.69)	0.010a (67.40)
100	100.00a (-3.44)	10.33b (-21.18)	18.97b (-60.06)	0.010a (69.59)
Sitosterol				
0	96.67a (0.0)	8.52a (0.0)	11.85a (0.0)	0.032a (0.0)
1	98.33a (-1.72)	12.74b (-49.48)	19.36b (-63.36)	0.010a (68.97)
10	100.00a (-3.44)	12.34b (-44.85)	20.67b (-74.40)	0.010a (70.22)
100	96.67a (0.0)	9.62a (-12.83)	17.30b (-45.98)	0.009a (70.85)

Values in the column with the same letter are not significantly different at $p<0.05$.
Values in the parentheses are inhibition percentages over control.
Values in the parentheses with (-) are promotion percentages over control.

Sitosterol showed the least stimulatory effect on lettuce seedling growths. It increased 13 – 49 % of shoot growth and 46 – 63 % of root growth at the concentration of 1 – 100 µM (Table 4.2). However, the beneficial effects of three constituents on seedling growths of lettuce were reduced when concentration treated increased.

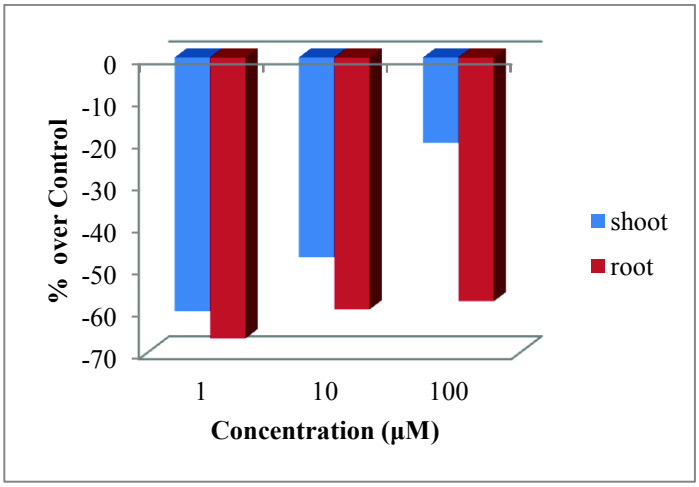


Figure 4.3 Effect of Kaempferol from *Cuscuta campestris* on the Growth of Lettuce Seedlings

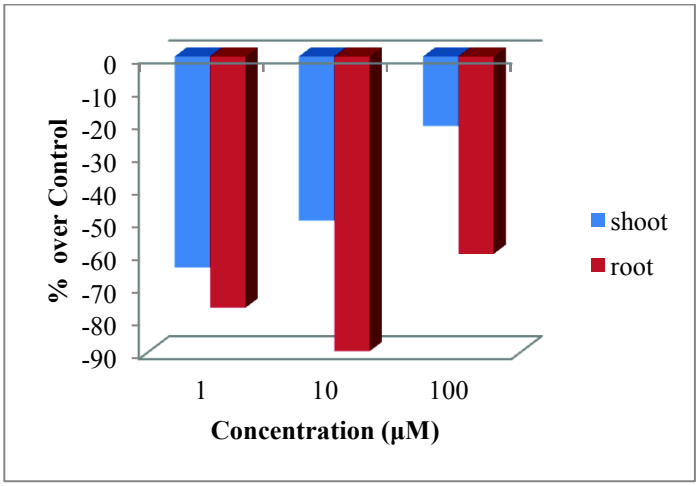


Figure 4.4 Effect of Pinoresinol from *Cuscuta campestris* on the Growth of Lettuce Seedlings

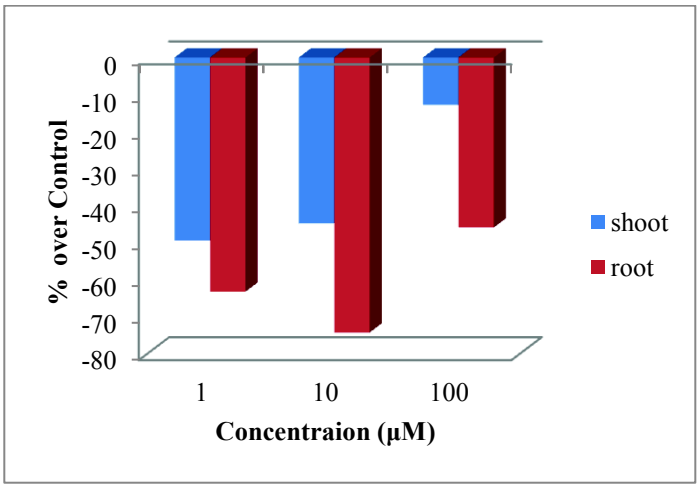


Figure 4.5 Effect of Sitosterol from *Cuscuta campestris* on the Growth of Lettuce Seedlings

CHAPTER 5

CONCLUSIONS

5.1 Conclusions

There were six constituents isolated from the ethanol extract of stem of *C. campestris* Yuncker. After elucidation through the ^{1}H and ^{13}C NMR spectra, these compounds were identified as kaempferol **30**, quercetin **53**, and kaempferol-3-*O*-glucoside **50**, sitosterol **99**, pinoresinol **18** and arbutin **22**.

Cuscuta campestris can be found easily growing on weeds such as *Melastoma malabathricum*, *Mimosa pudica*, *Mikania micrantha*, and *Asystasia intrusa*. Nevertheless, not much research was done on the allelopathic potential of the weed against other weed species and crops. So, in this study, the bioassay showed that the ethanol extract of *C. campestris* Yuncker is a good inhibitor of plant growth at 500 ppm and above although the effect of *C. campestris* on the three types of seeds were not significant at low concentration. The bioassay of the compounds, *viz.* kaempferol **30**, sitosterol **99**, and pinoresinol **18** did not inhibit measurably allelopathic activity against the test plant. Intriguingly, these compounds from *C. campestris* showed synergistic effect on shoot and root growth of lettuce probably due to hormesis effect at concentration 1 – 100 μM. This study has shown that *C. campestris* has allelopathic potential on weeds (weedy rice) and crops (lettuce and radish). Further investigation on a broader range of doses and application times of *C. campestris* extract and the pure compounds in petri dish and soil on different plants is commendable to carry out to understand better its allelopathic potential and herbicidal potential.

CHAPTER 6

EXPERIMENTAL

6.1 Plant Material

Wild *Cuscuta campestris* was collected from Pontian, Johor on May 25, 2009. The major hosts of *Cuscuta* that we collected are *Asystasia gangetica* and *Mikania micrantha*.

6.2 Instrumentation

The Mass spectra were obtained from Shimadzu Liquid Chromatography Mass Spectrometer (LCMS-IT-TOF).

Nuclear Magnetic Resonance spectra (^{1}H and ^{13}C-NMR) were run in $CDCl_3$, CD_3OD, or Acetone-D (specified with each compound) on Jeol Lambda-400 or Jeol ECA-400. 2D-NMR experiments (COSY, HMBC, and HSQC) were performed on the same instrument using the same solvents. Chemical shifts were reported in ppm, while coupling constants were recorded in Hz.

The Ultra-Violet (UV) was recorded in HPLC Grade Methanol on a UV-VIS NIR Scanning Spectrophotometer (Shidmazu UV-310 IPG).

The Infra Red (IR) spectrum was recorded in chloroform or methanol on the Perkin Elmer 1600 Series FT-IR spectrometer.

The petri dishes of bioassay were put in Precision Plant Growth Chamber Model 818 (230 V, 860 watts) with the temperature 25 °C, 12 hours with light and 12 hours in dark.

Heto centrifugal vacuum sample concentrator/ freeze dryer (Maxi Dry Lyo) was used to free ethanol extract of *C. campestris* from solvent.

6.3 Chromatography

Preparative column chromatographic separation was performed on silica gel $60GF_{254}$ and 60G as the stationary phase for column chromatography.

Merck silica gel, 60 F_{254} thin layer chromatography was used to check the purity of the compounds.

Waters HPLC that equipped with Binary Gradient Module, System Fluidics Organizer and UV detector set at 200 – 400 nm. Zorbax Eclipse Plus C18 (4.6 mm i. d. x 150 mm x 3.5 μm) was used as HPLC column. MassLynx Software was used to analyze the data being collected.

6.4 Extraction

After the sample was collected from Johor, the hosts were removed from the parasite (*Cuscuta*). After cleaning with tap water and then followed by distilled water, the *Cuscuta* was dried with temperature around 40 °C. About 200 g of the dry *Cuscuta* was used for extraction while the rest of the sample was kept in herbarium. The dried *Cuscuta* was extracted with ethanol at room temperature for three times. The ethanol extract was dried by using rotary evaporator and freeze dryer.

6.5 Isolation and Separation

About 5 g of the crude was subjected to column chromatography with silica gel as the adsorbents. Gradient elution was used to complete the isolation. Three solvents that were used as the mobile phase were hexane, ethyl acetate, and methanol. Hexane-

ethyl acetate and ethyl acetate-methanol were the solvent systems used. Every fraction was collected in 200 mL of eluents.

Each fraction was tested on TLC to check for the purity. Further column chromatography or HPLC was done until single spot was shown on TLC (Figure 6.1). The chromatographic solvent systems and yield of constituents were shown in Table 6.1.

Table 6.1 Chromatographic Solvent Systems and Yield of Constituents of *C. campestris.*

Solvent System	**Ratio**	**Compound**	**Weight (mg)**
Hexane : Ethyl Acetate	30 : 70	Kaempferol **30**	5.6
Dimethyl chloride : Ethyl Acetate	50: 50	Quercetin **53**	0.5
MeOH: Deionized H_2O	30:70	Astragalin **50**	1.3
MeOH: Deionized H_2O	30:70	Arbutin **22**	2.1
Hexane : Ethyl Acetate	50 : 50	Pinoresinol **18**	3.5
Hexane : Ethyl Acetate	94 : 6	Sitosterol **99**	1.3

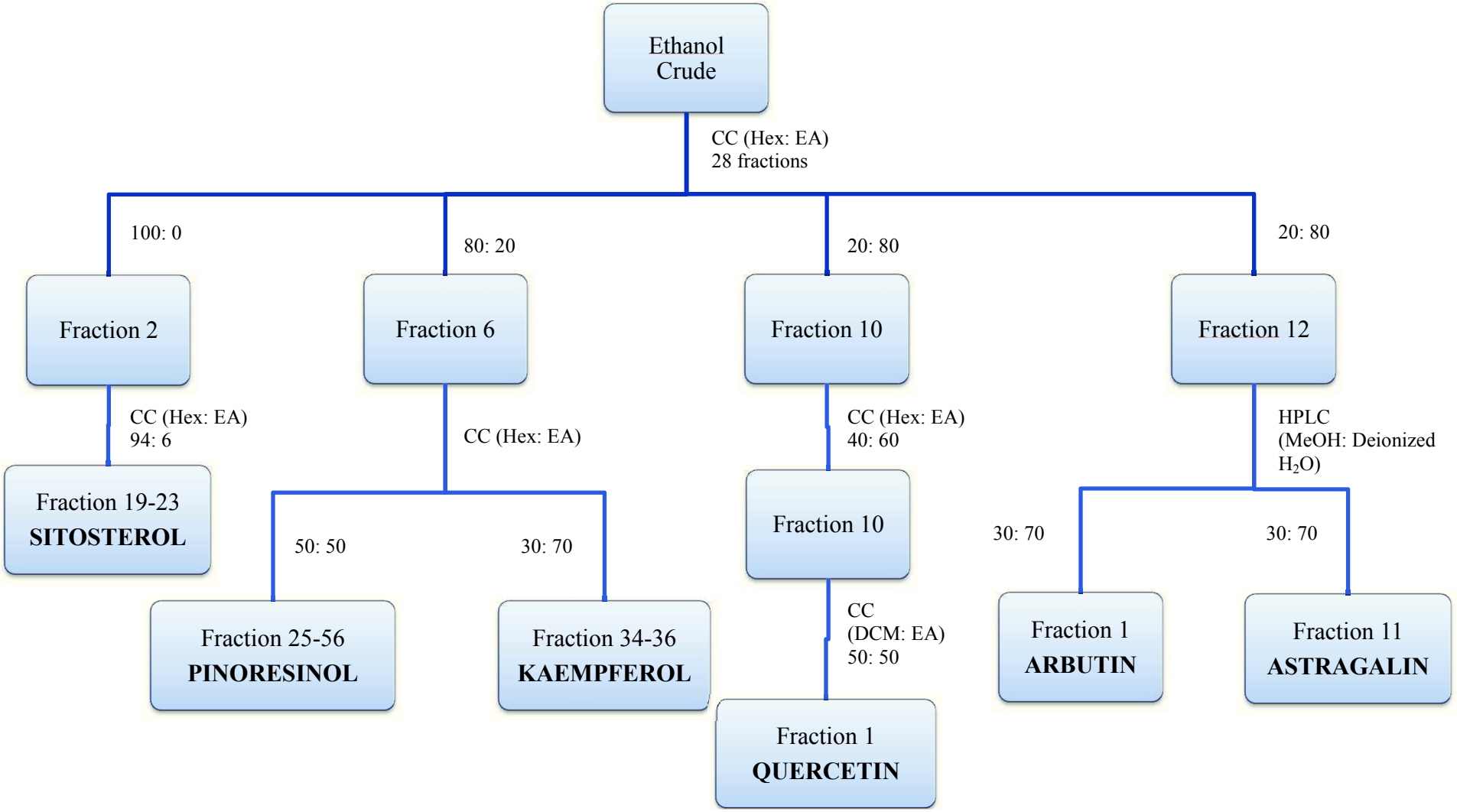


Figure 6.1 Flow of the Isolation of the Chemical Constituents from Ethanol Extract of *Cuscuta campestris*

6.6 Bioassay on Seed Germination and Seedling Growths

About 1 g of ethanolic extract of dry *Cuscuta campestris* was dissolved in 200 mL of deionized water with 1 % of methanol. This extract was set as original concentration and diluted into 100 ppm, 200 ppm, 500 ppm, and 1,000 ppm. An 8 mL aliquot of the extract was pipette in petri dish that lined with filter paper and sowed with 20 seeds of radish. The control used was deionized water with 1 % of methanol. These treatments were place in a growth chamber for 7 days. Three replicates were prepared for each treatment. The seed germination, shoot length, root length, and dry mass were recorded 7 days after treatment.

The same experiment was repeated by using lettuce and weedy rice seeds.

6.7 Allelopathic Potential of Allelochemicals from *Cuscuta campestris* Yuncker

100 μM of three chemical constituents (kaempferol, sitosterol, and pinoresinol) that isolated from ethanol extract of *C. campestris* Yuncker was prepared by dissolving in water with 1 % of methanol. The samples were then diluted to 10 μM and 1 μM. The same bioassay method was prepared by using lettuce seeds.

6.8 Statistical Analysis

The statistical analyses were performed using analysis of variance (ANOVA) from SPSS 15.0 to analyze treatment differences with the Tukey's at the 0.05 significance level.

6.9 Physical and Spectra Data of the Isolated Constituents

Characterization of Kaempferol

Molecular formula: $C_{15}H_{10}O_6$

IR V_{max}^{KBr}: 3245 cm^{-1}, 1610 cm^{-1}

UV λ_{max}^{MeOH}: 204 nm, 267 nm and 364 nm

MS *m/z:* 287.06

^{1}H and ^{13}C-NMR Data in Methanol-D4 at 400 MHz (Table-3.3)

Characterization of Astragalin

Molecular formula: $C_{21}H_{20}O_{11}$

IR V_{max}^{KBr}: 3420 cm^{-1}, 1680 cm^{-1}

UV λ_{max}^{MeOH}: 207 nm, 267 nm and 350 nm

MS *m/z*: 447.04 and 284.02

^{1}H and ^{13}C-NMR Data in Methanol-D4 at 400 MHz (Table-3.5)

Characterization of Quercetin

Molecular formula: $C_{15}H_{10}O_7$

IR V_{max}^{KBr}: 3294 cm^{-1}, 1617 cm^{-1}, 1513 cm^{-1}

UV λ_{max}^{MeOH}: 206 nm, 257 nm and 372 nm

MS *m/z:* 301.03

^{1}H and ^{13}C-NMR Data in Methanol-D4 at 400 MHz (Table-3.4)

Characterization of Pinoresinol

Molecular formula: $C_{20}H_{22}O_6$

IR V_{max}^{KBr}: 2936 cm^{-1}, 1604 cm^{-1}, 1523 cm^{-1}

UV λ_{max}^{MeOH}: 209 nm, 232 nm and 281 nm

MS *m/z:* 357.12

^{1}H and ^{13}C-NMR Data in Chloroform-D at 400 MHz (Table-3.2)

Characterization of Sitosterol

Molecular formula: $C_{29}H_{50}O$

IR V_{max}^{KBr}: 3545 cm^{-1}, 2951 cm^{-1}, 1736 cm^{-1}, 1466 cm^{-1}, 1172 cm^{-1}, 717 cm^{-1}

UV λ_{max}^{MeOH}: 202 nm

MS *m/z*: 414.38

^{1}H and ^{13}C-NMR Data in Chloroform-D at 400 MHz (Table-3.1)

Characterization of Arbutin

Molecular formula: $C_{10}H_{18}O_9$

IR V_{max}^{KBr}: 3412 cm^{-1}, 2891 cm^{-1}, 1514 cm^{-1}, 1222 cm^{-1}, 1069 cm^{-1}, 835 cm^{-1}

UV λ_{max}^{MeOH} : 224 nm

MS *m/z*: 284.07 with a base peak 117.01

^{1}H and ^{13}C-NMR Data in Methanol-D4 at 400 MHz (Table-3.6)

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APPENDIX

Journal of Agricultural Science; Vol. 4, No. 9; 2012
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Allelopathic Potentials of *Cuscuta campestris* Yuncker Extracts on Germination and Growth of Radish (*Raphanus sativus* L.) and Lettuce (*Lactuca sativa* L.)

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Received: April 9, 2012 Accepted: April 23, 2012 Online Published: July 26, 2012

doi:10.5539/jas.v4n9p57 URL: http://dx.doi.org/10.5539/jas.v4n9p57

Abstract

Cuscuta campestris Yuncker, commonly known as Golden dodder is an annual problematic parasitic weed in abandoned and derelict areas in Malaysia. The weed is leafless plant, glabrous, yellow-white in colour, with haustoria, sucker-like attachments to the aerial parts of a wide range of host plants. This study was instituted to assess the allelopathic potentials of *C. campestris* on lettuce and radish as test plants. Three types of treatment using aqueous extract of fresh (fc), and dried *s* (dc) and ethanol extract of dried *C. campestris* (ec) were assayed for their allelopathic effects on radish (*Raphanus sativus*) and lettuce (*Lactuca sativa*) seeds. These extracts reduced seed germination, root and shoot lengths of both radish and lettuce. The roots of radish were more sensitive *vis-à-vis* the shoots when exposed to fc, while shoots were more sensitive than roots when exposed to dc. Dose-mediated differences in shoot and root lengths of radish were registered when treated with ec. The roots of lettuce were more sensitive compared with the shoots when exposed to ec, while no measurable effect was observed when roots and shoots of lettuce were exposed to fc and dc. The results demonstrated the allelopathic effects of dodder on the tested host plants. The potentials of these extracts and their chemical constituents as bioactive ingredients for new herbicides are implied.

Keywords: Allelopathy, *Cuscuta campestris*, natural herbicides, germination, *Raphanus sativus*, *Lactuca sativa*

1. Introduction

The term “allelopathy” was first coined by Molisch (1937). Allelopathy involves the release of bioactive compounds or chemicals into the environment by plants or organisms, and their ensuing biochemical activities may affect the growth of other plant species or organism presence in the immediate environment (Rice, 1974 & 1984), or impacting predators, fungi or bacteria from growing in the area (Putnam, 1988; Rice, 1974). Tesio and Ferrero (2010) showed that allelopathic activity was present in the annual and seasonal weeds, having an impact on agricultural crops especially giving effect by inhibiting the growth and proliferation of plants. Theoretically this allelopathic effect reduced seed germination. The implication of this finding would be extended toits application in commercial agriculture, principally in reducing seed germination of weeds (Singh et al., 2003).

Plant allelopathy is a breakthrough in the field of agricultural science. Allelopathy serves as secondary metabolites, which result from the adaptation process of plants in relation to the hosts. Allelopathy evolution resulting from changes in the plant environment factors such as competition for oxygen, sources of nutrients, space and light has led to the production of secondary metabolites that serve as allelopathy (Inderjit et al., 2011). In addition, there are several types of allelopathy in the form of chemical compounds such as alkaloids, sesqui-and terpenoids, which may serve as protective materials from the animals’ herbivora (Macías, 2007). Leslie and Stephen (2003) defined that these activities involved chemical mechanisms. The presence of this mechanism is evident that internal activity also plays a role in the protection of weedy plants.

Weeds population dynamics can be influenced by several inherent factors such as seed dormancy and the prevailing agro-edaphic factors in the habitat (Baki, 2007; Baki et al., 2009). Another pertinent factor that affects plant growth include the presence of allelochemicals and the associated allelopathic activities, and this in turn

may affect growth in the immediate environment principally in agricultural areas (Parker & Riches, 1993; Qasem, 2011).

Cuscuta campestris Yuncker, a parasitic weed, has its own functional system of the haustoria to take nutrients from the host plants. The haustoria act as roots transferring nutrients from the host plants to *C. campestris.* The intricacy of nutrient transfer mechanism via the haustoria in *C. campestris* from the host plants has led to various studies being conducted to assess and evaluate these special traits (Press & Graves, 1995; Parker & Riches, 1993; Press & Pheonix, 2005).

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The principal objective of this study was to assess the allelopathic potentials of water aqueous extracts of fresh and dried *C. campestris*, and the ethanol extract of dried *C. campestris* on seed germination and growth of radish (*Raphanus sativus* L.) and lettuce (*Lactuca sativa* L.) seedlings.

2. Materials and Methods

Plant samples of *Cuscuta campestris* Yuncker was collected from Pekan Nanas, Johor, Malaysia (N 1°20' to N 2° 35' and E 102° 28' 59.9" to E 104° 33' 52.9"). These samples were cleaned of any attached host plant materials followed by washing them with tap water for several times prior to storage in the refrigerator below 20°C until use. Three types of treatment were instituted for the purpose of samples preparation and bioassays: (i) water extract of fresh *C. campestris* (fc), (ii) water extract of dried *C. campestris* (dc) and (iii) ethanol extract of dried *C. campestris* (ec). The test plants for the bioassay were radish (*Raphanus sativus* L.) and lettuce (*Lactuca sativa*).

2.1 Preparation of Water Extract of Fresh C. campestris

A total of 40g of fresh *C. campestris* was cut into small pieces 1-2 cm and then macerated with a blender. These materials were then soaked in 8L of distilled water for 24h at 25 °C in a shaker, and placed on a shaker (Faravani et al. 2008). The mixture was then filtered through four layers of cheesecloth. The supernatant was filtered again using paper "Whatman no. 42" as a stock solution for water extract of fresh *C. campestris* (40g/8L= 5000ppm).

2.2 Preparation of Water Extract of Dried C. campestris

About 200g of fresh *C. campestris* was dried in an oven at 40°C for 48h, after dried, take 40g of dried were pounded into powdered form, and about 8L of distilled water were then added in a conical flask, and left to soak for 24h. The mixture was then filtered through four layers of cheesecloth. The supernatant was filtered again using paper "Whatman no. 42" as a stock solution for water extract of dried *C. campestris* (40g /8L= 5000ppm).

2.3 Preparation of Ethanol Extract of C. campestris

About 200g of dried *C. campestris* samples were placed into a conical flask and was soaked with 95%of ethanol. The mixture was left to shake for 8 hours, and then left to soak for 3 days. The mixture was filtered with a "Whatman No. 42" filter paper. The filtrate was poured into the smaller round conical flask, and this was used for freed from the solvent under reduced pressure using rotary evaporator with 40°C on speed 5 to 6. The crude was placed in the bottle and was left to evaporate at room temperature. About 5g from the crude was adding 1000ml to become 5000ppm.

2.4 Bioassays

Dilutions were made with distilled water to concentrations of 1000ppm, 500ppm, 200ppm and 100ppm. Respective controls of 0ppm were prepared likewise for each type of extract. An 8ml aliquot of the extract was pipetted into each petri-dish that previously lined with a filter paper and sowed with 20 seeds of radish or lettuce. These treatments were place in a growth chamber model 818(230V, 860 watts) for 7 days. These routines were repeated 3 times for each type of extract. The percentage of seed germination, shoot and root lengths were recorded 7 days after treatment.

2.5 Analysis

The data on seed germination, shoot and root lengths of radish and lettuce were subjected to analysis of Variance (ANOVA) and any difference in treatment means were tested with LSD tests at $p< 0.05$ (Zar 2009; Ilori et al. 2010; Omezzine et al. 2011; Shahbaba 2012).

The shoot and root lengths of treated radish and lettuce seedlings as percentages of the control were tested for growth promotion and inhibition was based on the formula:

$$\%\ growth\ inhibition = 100(pc - pt)/pc$$

Where *pc* and *pt* are the shoot or root lengths of the control and the treated sample, respectively.

3. Results

3.1 Allelopathic Effect of 3 Types of Extracts of Cuscuta campestris on Radish

The fresh plant extracts of *C. campestris* did not reduce seed germination of radish (Table 1) while, root and shoot lengths were also not affected despite the increase in dose from 100ppm to 5000ppm (Figure 2). In the same vein, exposure to dried plant and ethanol extracts of dodder also failed to register meaningful reductions in seed germination of radish. Albeit differences in the quanta of dose-mediated reductions in shoot growth following exposures to extracts of dried plant samples of *C. campestris,* these reductions were not significant. In contrast, similar exposures to ethanol extracts registered significant dose-mediated reductions in shoot and root growth of radish seedlings, with roots being more sensitive than shoots. Shoots and roots of radish were measurably more sensitive when exposed to ethanol extracts of dried sample with parallel increase in concentrations from 1000ppmto 5000ppm.For example, at the extreme dose of 5000ppm, the shoot length of 11.38mm compared with 32.60mm of control seedlings of radish. Similarly, the parallel figures for roots were 11.64mm (control) against 71.89mm (exposed to 5000ppm), fortifying the argument that the roots were more sensitive than shoots. Exposures to dried plant extracts of *C. campestris* did not inflict any significant reductions in shoot lengths of radish, even at the extreme does of 5000ppm. On the contrary, a similar exposure has led to erratic dose-mediated reduction, albeit small, in root lengths of radish.

Table 1. Effect of extracts *Cuscuta campestris* on the germination and growth of radish (*Raphanus sativus*) seedlings

Concentration (ppm)	Germination (%)	Shoot Length (mm)	Root Length (mm)
Ethanol extract of dried *C. campestris* (ec)			
0	100.00a (0.00)	32.60b (0.00)	71.89c (0.00)
100	98.33a (-1.67)	41.24c (+8.63)	71.02c (-0.88)
200	98.33a (-1.67)	40.39c (+7.78)	62.17bc (-9.72)
500	100.00a (0.00)	29.61b (-3.00)	52.20b (-19.69)
1000	100.00a (0.00)	15.93a (-16.67)	16.09a (-55.80)
5000	95.00a (-5.00)	11.38a (-21.22)	11.64a (-60.25)
Water extract of dried *C. campestris* (dc)			
0	100.00a (0.00)	28.19abc (0.00)	68.49b (0.00)
100	100.00a (0.00)	24.67a (-3.52)	52.87a (-15.63)
200	98.33a (-1.67)	28.64bc (+0.46)	67.16b (-1.33)
500	96.67a (-3.33)	30.15bc (+1.96)	63.52b (-4.97)
1000	96.67a (-3.33)	27.47ab (-0.71)	60.84ab (-7.65)
5000	98.33a (-1.67)	31.61c (+3.42)	52.28a (-16.21)
Water extract of fresh *C. campestris* (fc)			
0	100.00a (0.00)	28.19ab (0.00)	68.49a (0.00)
100	96.67a (-3.33)	26.86a (-1.33)	62.67a (-5.82)
200	100.00a (0.00)	29.27ab (+1.08)	69.85a (+1.36)
500	98.33a (-1.67)	27.09a (-1.10)	69.19a (+0.70)
1000	100.00a (0.00)	27.51ab (-0.68)	67.70a (-0.79)
5000	100.00a (0.00)	30.33b (+2.14)	66.24a (-2.25)

Values in a column with the same lowercase letters are not significantly different at $p<0.05$.(LSD tests)

Values in parentheses indicate growth synergism (positive values) or antagonism (negative values) *vis-à-vis* the control.

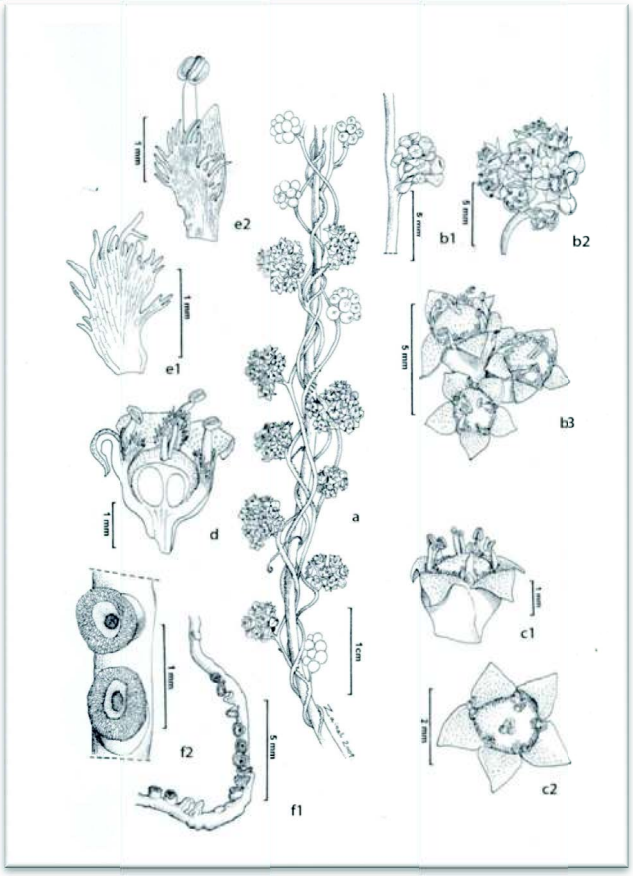

Figure 1. *Cuscuta campestris* Yuncker. (a) flowering branch, (b1) detail of inflorescence, (b2) floral cluster, (b3) top-view of a cluster of three flowers. (c1) side-view of a flower, (c2) top-view of a flower showing exposed stigmas and stamens, (d) flower, longitudinal section, (e1, e2), appendage enveloping pollen tube, (f1) branch with haustoria, and (f2) closer-view of haustoria

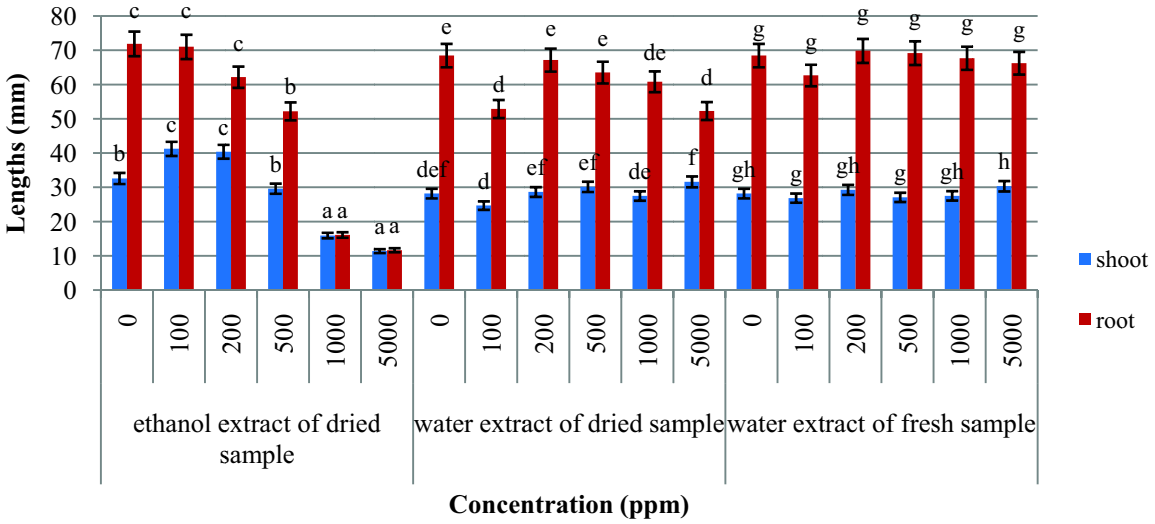

Figure 2. Shoot and root lengths of radish when exposed to different concentrations of 3 types extracts of *C. campestris*. Values with different lowercase letters denote significant difference as determined by LSD test at $p<0.05$

3.2 Allelopathic Effect of 3 Types of Extracts of Cuscuta campestris on Lettuce

The fresh and dried plant, and ethanol extracts of *C. campestris* failed to reduce seed germination of lettuce. The only exception was the exposure to 5000ppm of ethanol extracts of *C. campestris* which registered measurable and significant reduction in seed germination compared to the control (Table 2, Figure 3). The roots and shoots of lettuce showed a significant reduction in lengths following exposure to ethanol extracts of dodder starting at 200ppm similar to those registered in radish. The lengths of lettuce shoots were very much affected by the ethanol extracts of *C. campestris* at 5000ppm concentration of but showed reduction in root lengths at 500ppm and beyond. Invariably, the roots of lettuce displayed enhanced growth after being exposed to ethanol plant extracts of dodder ranging from 100ppm to 200ppm. Thereafter, meaningful dose-dependent reductions in root lengths were observed. There were erratic responses following exposures to various doses of fresh and dried plant extracts of dodder in the root lengths of lettuce. The shoots of lettuce were more sensitive to exposures to fresh, dried or ethanol plant extracts of dodder compared to roots. However, the dose-mediated responses following exposures to those extracts were not clearly displayed.

Table 2. Effect of extracts *Cuscuta campestris* on the germination and growth of lettuce (*Lactuca sativa*) seedlings

Concentration (ppm)	Germination (%)	Shoot Length (mm)	Root Length (mm)
Ethanol extract of dried *C. campestris* (ec)			
0	100.00 b (0.0)	10.04 b (0.0)	39.28 d (0.00)
100	100.00 b (0.0)	13.96 c (+3.91)	57.81 f (+18.53)
200	100.00 b (0.0)	13.02 c (+2.98)	44.70 e (+5.41)
500	98.33 b (-1.67)	12.36 bc (+2.32)	22.37 c (-16.91)
1000	100.00 b (0.0)	12.46 bc (+2.42)	11.16 b (-28.12)
5000	55.00 a (-45.00)	1.11 a (-8.94)	1.86 a (-37.42)
Water extract of dried *C. campestris* (dc)			
0	100.00a (0.0)	4.869ab (0.0)	26.339bc (0.0)
100	100.00a (0.0)	5.08ab (+0.21)	23.591bc (-2.75)
200	100.00a (0.0)	4.728a (-0.14)	29.073bc (+2.73)
500	98.33a (-1.67)	4.583a (-0.29)	30.203c (+3.86)
1000	98.33a (-1.67)	4.557a (-0.31)	22.392b (-3.95)
5000	96.67a (-3.33)	5.507b (+0.64)	10.031a (-16.31)
Water extract of fresh *C. campestris* (fc)			
0	100.00a (0.0)	5.90ab (0.0)	17.90ab (0.0)
100	98.33a (-1.67)	4.90a (-1.00)	23.34c (+5.44)
200	98.33a (-1.67)	5.46ab (+0.438)	17.88ab (-0.02)
500	100.00a (0.0)	6.06b (+0.165)	20.22bc (+2.32)
1000	100.00a (0.0)	5.82ab (-0.08)	21.27bc (+3.365)
5000	98.33a (1.67)	7.90c (+2.003)	13.85a (-4.047)

Values in a column with the same lowercase letters are not significantly different at $p<0.05$. (LSD tests)

Values in parentheses indicate growth synergism (positive values) or antagonism (negative values) *vis-à-vis* the control.

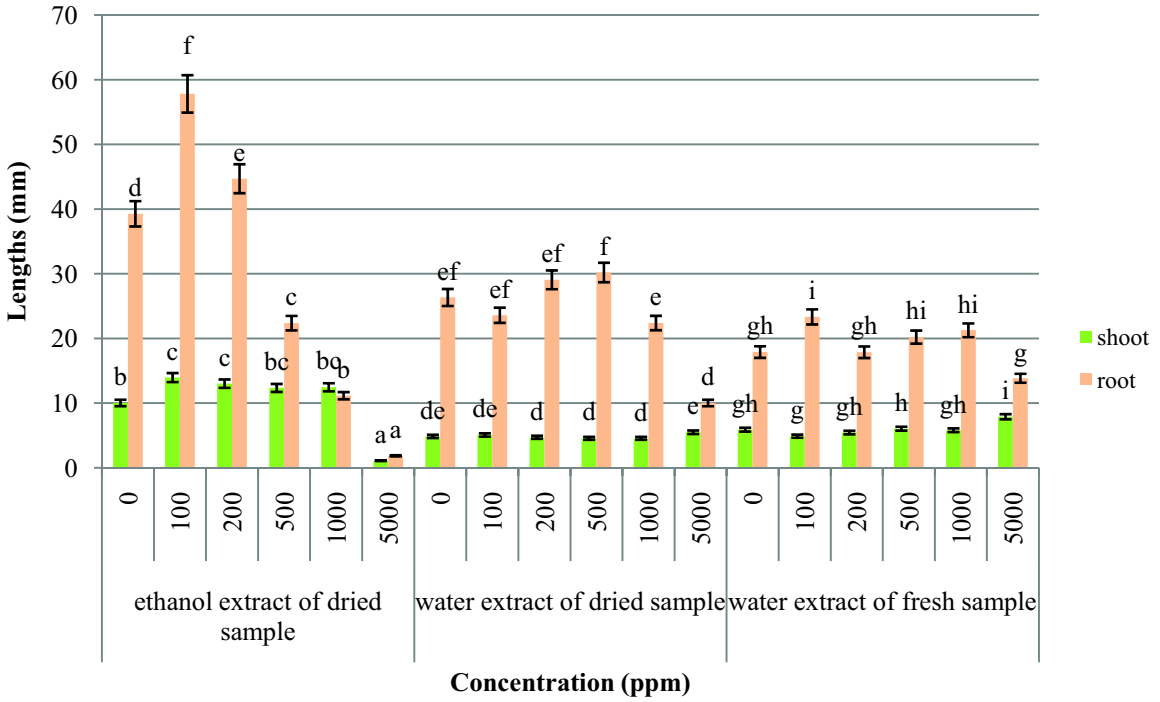

Figure 3. Shoot and root lengths of lettuce when exposed to different concentrations of 3 types extracts of *C. campestris*. Values with different lowercase letters denote significant difference as determined by LSD test at $p< 0.05$

4. Conclusions

This study revealed the potential of dodder's extract as a potential bioherbicide. Despite erratic responses to the three different types of extracts of *C. campestris*, there was a general trend of dose-mediated deleterious effects on the growth of seedlings of the test plants, and in this case of lettuce and radish. However, growth enhancement of shoots of radish seedlings following exposures to 100-200ppm of ethanol extracts of dodder, fortifying the argument that these extracts can act as growth-promoting substances. There is a promotion or encouragement over the control of elongation occurs (refer Tables 1 and 2). The extracts may contain a lot of water, as can be seen in the non-deleterious effects of the extracts of fresh and dried plant materials of dodder on germination of radish and lettuce, registering almost100% germination. Seed germination of radish was inhibited by the ethanol extracts of dodder in excess of 5000ppm, while the growth of radish and lettuce seedlings as test plants were inhibited by dodder's ethanol extracts in excess of 200ppm.In fact, several previous studies have been carried out had proved that certain types of weeds can be slowed or inhibited the growth by using aqueous extracts allelopathic plant samples (Khanh et al.2008; Macías 2007; Omezzine et al. 2011; Yu et al. 2011). Khanh (2008) suggested that allelochemicals from parasitic plants, including *Cuscuta* spp. might be useful for the development of bioactive pesticides in the future. Further, this study also proves that there are allelochemicals in *Cuscuta campestris* and many *Cuscuta* spp. inhibited the growth and the germination of seeds, including weeds that can influence plant population density (Yu et al. 2011).

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