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وتناقش إمكانات المركبات المعزولة كعلامات chemosystematic داخل جنس Tephrosia وكمؤشرات على الانتماءات النشوء والتطور بين الأنواع الأفريقية والآسيوية.

فحص لنشاط راديكالي الكسح (RSA)من مستخلص الكلوروفورم من الأوراق والجذور والبذور من العاصمة Tepohrosia apollinea (Del.) D.C وكشف متغير مضادة للأكسدة النشاط تتراوح بين 19٪و 32٪.

التوصيات .

وينبغي أن تكون عملية إجراء المزيد من الدراسات على وصلة لتطوير النظم الانزيم من الأنواع من Tephrosiaمع الحمض النووي وتعديل الجينات لدراسة تطور والتنمية بين الأنواع .وعلاوة على ذلك، فإن النشاط المضادة للأكسدة على semiglabrin - (-)وايزوميرو -(-) pseudosemiglabrinالتي تحتاج إلى معالجتها على الأشكال معزولة محض.

University of Khartoum

Faculty of Pharmacy

Department of Pharmacognosy

Isolation and structure elucidation of two diastereoisomeric prenylated flavonoids from *Tephrosia apollinea* root and their Chemotaxonomic Significance

A Thesis is submitted to

THE UNIVERSITY OF KHARTOUM

By

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in fulfilment of the requirements for the degree of

Master of Pharmacy

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August, 2011

Dedication

To my dear parents, who always provide love, care, support and encouraged me. May God give me them the health, joy and happiness in each moment of their life.

To my beloved wife and wonderful son, who make every moment of my life so special and meaningful.

To my lovely sisters who always give a lovely taste for this life and make it colourful.

Anti-oxidant of the crude drug has been test by using the Radical Scavenging Activity assay (RSA), using DPPH as an indicator and Iron-Chelation activity assay using the Ferrous ion-Ferrozin complex as an indicator.

Results and conclusion. Subjecting the chloroform extract of the root for in-depth chromatographic analysis resulted in the isolation of two distereoisomeric prenylated flavonoids which their detailed chemical structures have been elucidated by high resolution nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectroscopy as (-)- semiglabrin and its isomer (-)-pseudosemiglabrin.

The potential of the isolated compounds as chemosystematic markers within the genus *Tephrosia* and as indicators of phylogenetic affinities between the African and Asian taxa are discussed.

Assay of the radical scavenging activity (RSA) of the chloroform extract of the roots, leaves and seeds of *Tephrosia apollinea* (Del.) DC. revealed variable anti-oxidant activity ranging between 19% and 32%.

Recommendations. Further studies should be process on the link of the enzyme development systems of the species of *Tephrosia* with the DNA and Gene modification for studying the evolution and development between the species. Moreover, the anti-oxidant activity on the (-)- semiglabrin and its isomer (-)-pseudosemiglabrin need to be processed on the pure isolated forms.

Acknowledgement

I wish to express my sincere gratitude to my supervisor Prof. Sami Ahmed Khalid for the encouragement, guidance, teaching and criticism throughout my study.

I'm grateful to the University of Khartoum for granting me scholarship to study and do the laboratories work in their laboratories.

I wish to acknowledge the help and support of the head of the Department of Pharmacognosy, Dr. Abdel Khalik Mudather and the technical staff.

I wish also to acknowledge the help received from Dr. Hiba Ali, Phytochemistry Department, The National Research Institute for allowing me to conduct part of the research in their facility.

I wish to acknowledge the assistance of Dr. Assad Khalid, the Biochemistry Department, Medicinal and Aromatic Plants Research Institute in using their facility in the screening of the anti-oxidant activity.

Especial thanks for my wife and son who helped me in their own way to do this work.

ملخص

الخلفية.

نادرا ما يتم إبلاغ Prenylated فلافونيدات بين اجناس النباتات. ويتميز جنس Tephrosia من خلال وجود هذه المركبات التي تخلو من الهيدروكسيل على "C4 ووجود prenylation في C7 و C8. و من بين اجناس ال Tephrosia، Tepohrosia apollinea (Del.) D.C. وهي أقل من درس من بين الأنواع.

الأهداف

لعزل المستقلب الرئيسية الثانوية من جذور العاصمة Tephrosia apollinea (Del) .وتوضيح هيكلها الكيميائية باستخدام تقنية حديثة الطيفية، وتقييم أهمية كيموتصنيفية من المركبات المعزولة وتحديد النشاط المضادة للأكسدة من الأوراق والبذور والجذور استخراج النفط الخام من Tepohrosia apollinea (Del.) D.C

طريقة

تعرض مسحوق المخدرات الخام الى تقنيات التعطين الباردة، تليها TLCإعدادي و LCالعمود لعزل المركب النقي .وقد استخدمت Chromatotronحصول على مزيد من الفصل، مما أدى إلى المركبين crystalisable وقد استخدم الرنين المغناطيسي لتوضيح هيكل. وقد أدرجت كل من الفلافونويد عنها سابقا و isoflavonoidsمن جنس Tephrosia باستخدام النسخة الأخيرة من قاموس المنتجات الطبيعية، ودرس الأهمية كيموتصنيفية.

وكانت مضادة للأكسدة من المادة الخام اختبار باستخدام الراديكالي فحص نشاط الإقتيات (RSA)، وذلك باستخدام DPPHكمؤشر والحديد عملية إزالة معدن ثقيل لفحص النشاط باستخدام الحديدية ايون Ferrozinالمعقد كمؤشر.

النتائج والاستنتاج.

إخضاع مستخلص الكلوروفورم من جذر لتحليل الكروماتوغرافي المتعمق أدت إلى عزلة من الفلافونويد distereoisomeric prenylated 2 والتي تم توضيحها هياكلها الكيميائية مفصل من قبل عالية الدقة بالرنين المغناطيسي النووي (NMR H1 والرنين المغناطيسي النووي C13) الطيفي مثل (-) - semiglabrin ولها ايزومير (-)-pseudosemiglabrin.

Abstract

Background. The Prenylated flavonoids are rarely reported between the genuses. Tephrosia genus is characterised by having such compounds which devoid the hydroxylation on $C_{4'}$ and the presence of prenylation on C_7 and C_8. Among the *Tephrosia* genus, *Tephrosia apollinea* (Del.) DC. is the least studied species.

Objectives. To isolate the major secondary metabolite of the roots of *Tephrosia apollinea* (Del.) DC. and elucidate its chemical structure using a modern spectroscopic technique, evaluate the chemotaxonomic significance of the isolated compounds and identify the anti-oxidant activity of the leaves, seeds and roots crude extracts of *Tephrosia apollinea*.

Method. Powdered crude drug was subjected to cold maceration techniques, followed by preparative TLC and Column LC for the isolation of the pure compound. Chromatotron has used for more separation, resulting in two pure crystallisable compounds. NMR has been used for the structure elucidation.

All of the previously reported flavonoids and isoflavonoids from the genus *Tephrosia* using the recent version Dictionary of Natural Products have been listed and studied for the chemotaxonomic significance.

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Abbreviations

4CL	4-Coumarate:CoA Ligase
ACC	Acetyl CoA: Carbon dioxide Ligase
AChE	Acetyl Cholinesterase
ATP	Adenosine Tri phosphate
BChE	Butyryl Cholinesterase
C4H	Cinnamte 4-hydroxylase
CAM	Complementary and alternative medicine
CHI	Chalcone Isomerase
CHS	Chalcone Synthase
COX	Cyclo-oxygenase
DMSO	Dimethyl Sulfoxide
DPPH	2,2-Di (4-tetraoctylphenyl)-picryl hydrazyl
EDTA	Eythlene diamine tetra-acetic acid
ELISA	Enzyme Immunosorbent Assay
F3H	Flavanone 3β-hydroxylase
IFN	Interferon

LOX	Lipo-oxygenase
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NMR	Nuclear Magnetic Resonance
NPR	Natural Product Reagent
OD	Optical Density
PAL	Phenylalanine Ammonia Lyase
PKR	PolyKetide Reductase
RSA	Radical Scavenging Activity
SD	Standard Deviation
SOD	Super Oxidase
TCA	Tri-Carboxylic Acid
TLC	Thin Layer Chromatography
TM	Traditional medicine
UV-Vis	Ultraviolet- Visible spectra
WHO	World Health Organization

1. Introduction

Medicinal plants are the main source of drugs currently used for treating human and animal diseases. The biological actions of medicinal plants have been recognized by the Egyptians, Greek and Chinese since the early centuries. It is believed that nearly 25% of all available medications were isolated and derived from about 250,000 flowering plants available on the earth surface (Fabricant and Farnsworth, 2001).

These medicinal effects of different plants result from the secondary metabolites occurring in these plants. The secondary metabolites are usually unique to a particular genus or species which makes any particular taxon taxonomically distinctive (Wink, 1999).

Unlike the primary metabolites, which are mainly used for building and maintaining the plant cells (Kaufman *et al.*, 1999; Wink 1999). The secondary metabolites are considered to have eco-physiological role as a defence system against the herbivores, pathogen attack, and inter-plant competition and has an attractant role towards pollinators or symbionts (Kaufman *et al.*, 1999; Wink and Schimmer, 1999).

Moreover, the plant secondary metabolites present a protective role for the plant in relation to abiotic stress such as those associated with changes in temperature, water status, light levels, UV exposure and mineral nutrients (Kaufman *et al.*, 1999). Recently, it has been found that they have a potential role in plant growth regulation, modulation of gene expression and in signal transduction (Kaufman *et al.*, 1999).

The secondary metabolites are defined as a non-essential metabolites for the normal growth and development of the plant, although they are important to the organism that produce them such as in defence mechanisms, dispersion of pollen grains, etc. (Herbert, 1994; Kaufman *et al.*, 1999).

Plants secondary metabolites are, conveniently grouped into different chemical classes as saponins, alkaloids, flavonoids, tannins, terpenoids, etc. Their occurrence is sporadic, however they often have certain taxonomic distribution patterns specific for certain taxa such as families, subfamilies, genera and sometimes even for species or subspecies (Fraenkel, 1959).

Most, if not all, secondary metabolites possess characteristic odours or tastes and thus may elicit sensory reactions (Fraenkel, 1959).

1.1. Secondary metabolites Importance for Human Health

Secondary metabolites are responsible for most of the potential pharmacological effects on humans, e.g. secondary metabolites which are responsible of defence system of a plant by being cytotoxic towards pathogens, could be used as anti-microbial if it is not too toxic for humans. It is likely that the secondary metabolites which have a neurotoxic activity against the herbivores could have a beneficial effect on humans (as antidepressant, sedative, muscle relaxant or anaesthetic) through its activity on the central nervous system (Wink and Schimmer, 1999).

1.2. Plants and Traditional medicine

According to Solecki and Shanidar (1975), fossil records dated the human use of plants as medicines from about 60,000 years, since the Middle Paleolithic age. From that time, many ethnopharmacological systems have been developed through consecutive generations, by the continuous practice of dealing with plants as source of remedies. These practices and systems have been diversified by different cultures which eventually resulted in distinctive habits of the various ethnic groups.

Traditional medicine (TM) is defined by World Health Organization (WHO) as a comprehensive term used to refer both to TM systems such as traditional Chinese medicine, Indian ayurveda and Arabic unani medicine, and to various forms of indigenous medicine. TM therapies include medication therapies — if they involve use of herbal medicines. TM is widely used and of rapidly growing health system off economic importance. In Africa up to 80% of the population uses TM to help meet their health care needs. In Asia and Latin America, populations continue to use TM as a result of historical circumstances and cultural beliefs. In China, TM accounts for around 40% of all health care delivered. Meanwhile, in many developed countries, complementary and alternative medicine (CAM) is becoming more and more popular (WHO 2002, Traditional Medicine Strategy 2002-1005, WHO publications)

According to Fabricant and Farnsworth (2001), the goals of using plants nowadays as a source of therapeutic agents are; to isolate bioactive compounds for direct use as a drug, production of bioactive compounds of novel or known structure for semi-synthesis, pharmacological tool, or to use the whole or part of the plant as a herbal medicine i.e. phytomedicines.

It is estimated that the number of the higher plant species on the Earth planet to be about 250,000 (Ayensu and DeFilipps, 1978). To date, only about 6% have been screened for the biological activity and not more than 15% have been evaluated phytochemically (Verpoorte, 2000).

1.3. The Ethnopharmacology of Sudanese Medicinal plants

In Sudan, the main source for the traditional medicine is the herbalist, who inherited his knowledge from his grand and grandfathers.

Being the biggest land in Africa by area (1 million sq. mile), Sudan has diversified medicinal plants which are widely distributed throughout the land, cultivated or wild. The diversification in ethnic and tribal groups among the population of Sudan resulting in a variety of different cultures which provided different habits and ways for using a single plant in treating different diseases from place to another within the country. Example of this is the use of *Tephrosia spp.* in the Sudanese traditional medicine may vary in its indication and uses. As a case in point *T. obcordata* (lam.) Bak. Is commonly used in the White Nile as anthelemtic while is used in Northern Kordofan for treating gonorrhoea.

1.4. Objectives of the study

The main objectives of the present study on *T. apollinea* (Del.) DC. are:

- To isolate the major secondary metabolites from the roots of *T. apollinea* and elucidate their chemical structures by modern spectroscopic techniques.
- To evaluate the chemotaxonomic significance of the isolated compounds.
- To identify the anti-oxidant and activity of the crude extracts of the seeds, leaves and roots of *T. apollinea*.

2. Literature Review

2.1. Flavonoids

Flavonoids are defined as bioactive phenols (Hollman and Katan, 1999) of a low molecular weight (Fernandez *et al.*, 2006; Heim *et al.*, 2002) which display a variety of biological activities (Cushnie and Lamb, 2005).

The basic structure of flavonoids is composed of 2 benzene rings (A and B) linked together by a carbon chain of 3 carbon atoms (Figure 1). They are widely distributed among the plant kingdom (Cushnie and Lamb, 2005). They are available in vegetables, seed, fruits, leaves, roots, etc. The estimated dairy intake of flavonoids is 1-2 g/day (Fernandez *et al.*, 2006). Flavones and flavonols average intake is 23 mg/ day, 16 mg of which is quercetin (Heim *et al.*, 2002).

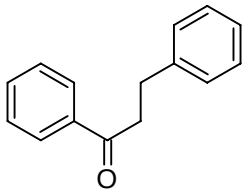


Figure 1. Basic chemical skeleton of flavonoids

2.1.1. Flavonoids Biosynthesis

The main precursors are the phenylalanine and malonyl-CoA. The modification of the phenylalanine to 4-coumaroyl-CoA forming the B-ring while malonyl-CoA used as an extending units forming the A-ring of the chalcone, the first flavonoid in the biosysnthetic pathway, (Figure 2) (Anderson and Markham, 2006).

The main precursors for building of the C_{15} backbone of flavonoids (chalcone) are 4-coumaryl CoA and malonyl CoA.

The 4-coumaryl CoA is formed from phenylalanine though three consecutive catalytic steps. In which, phenylalanine is converted to *trans*-cinnamate (*E*-cinnamate) by *trans* elimination of ammonia and pro-S proton in a reaction catalysed by Phenylalanine Ammonia Lyase (PAL).

The *trans*-cinnamate is then hydroxylated by cinnamate 4-hydroxylase (C4H) to *trans*-4-coumarate by introducing the 4′ hydroxyl group (B-ring), which is common in most of the flavonoids.

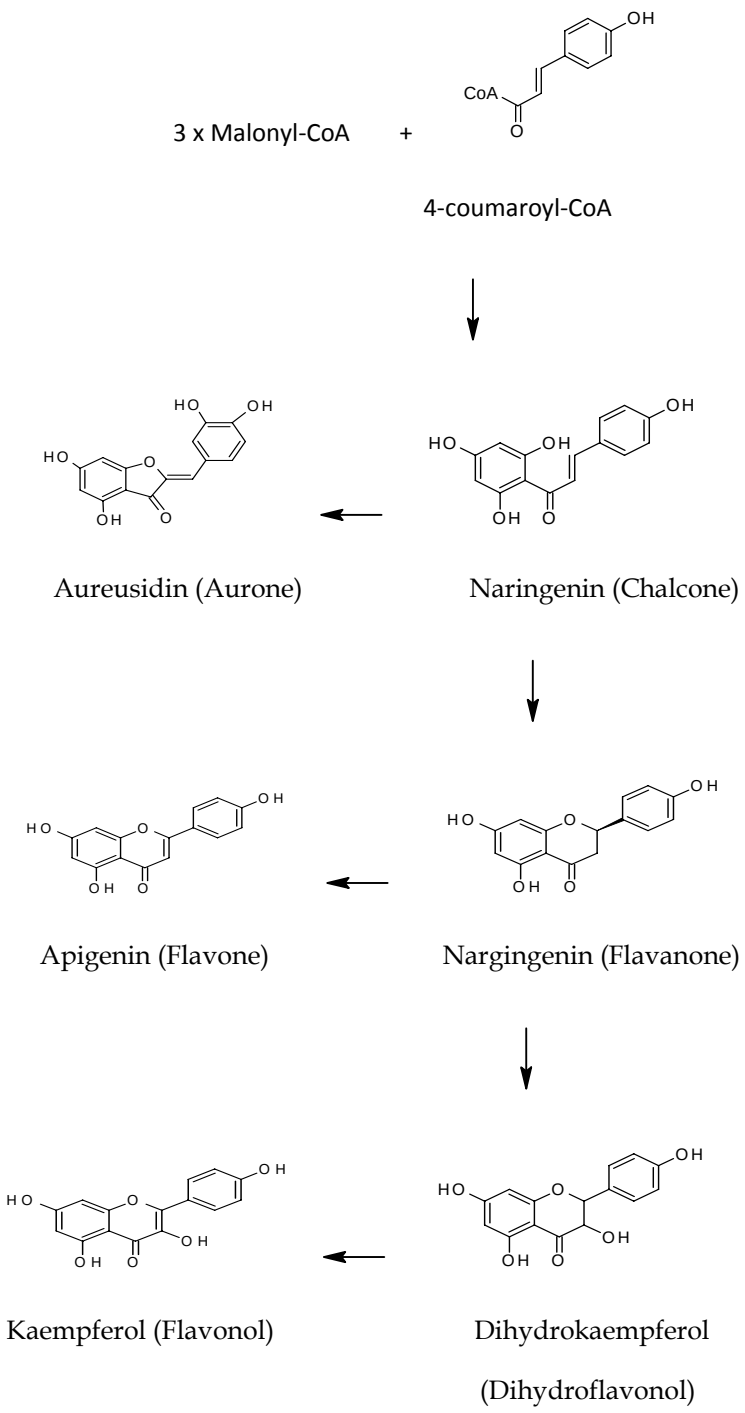


Figure 2. Flavonoids biosynthetic pathway

The 4-coumarate is then activated by 4-coumarate:CoA ligase (4CL) to form 4-coumaryl CoA which is the required precursor to form the C_{15} backbone of flavonoids together with the malonyl CoA formed by the catalysed carboxylation of acetyl CoA in the presence of Mg^{2+} as a co-factor in a ATP-dependent reaction by Acetyl CoA:Carbon dioxide ligase (ACC).

One molecule of 4-coumaryl CoA with 3 molecules of malonyl CoA, undergoes a decarboxylation followed by a consecutive condensation reactions catalysed by chalcone Synthase. During the process, polyketide intermediate is formed which undergoes cyclization and aromatization forming the A-ring, thus results in the formation of chalcone.

In the presence of the chalcone isomerase (CHI), chalcone undergoes a stereospecfic isomerization which results in the formation of the heterocyclic C-ring to form its corresponding (2S)-flavanone.

(2S)-Flavanone is converted by stereospecific hydroxylation to the respective (2R, 2S)-dihydroflvanols by the flavanone 3β-hydroxylase (F3H).

Flavones and flavonols are formed by the desaturation reaction forming double bond between C_2 and C_3 of the C-

ring of flavanone and dihydroflavonol respectively as shown on Figure 2.

In parallel to the flavonoid biosynthetic pathway, there is the isoflavonoids biosynthetic pathway (Figure 3). The latter is common in legumes and starts by the same precursor, 4-coumaryl CoA, which will react with three molecules of malonyl-CoA together in the presence of chalcone synthase (CHS) and PolyKetide Reductase (PKR). The main function of the PKR is to remove the hydroxyl group from $C_{6'}$ forming 6'-deoxychalcone. The latter enters to several modifications, one of this is the hydroxylation of the C6' followed by condensation and cyclization forming pterocapans, cumestans and rotenoids (Figure 3) (Anderson and Markham, 2006).

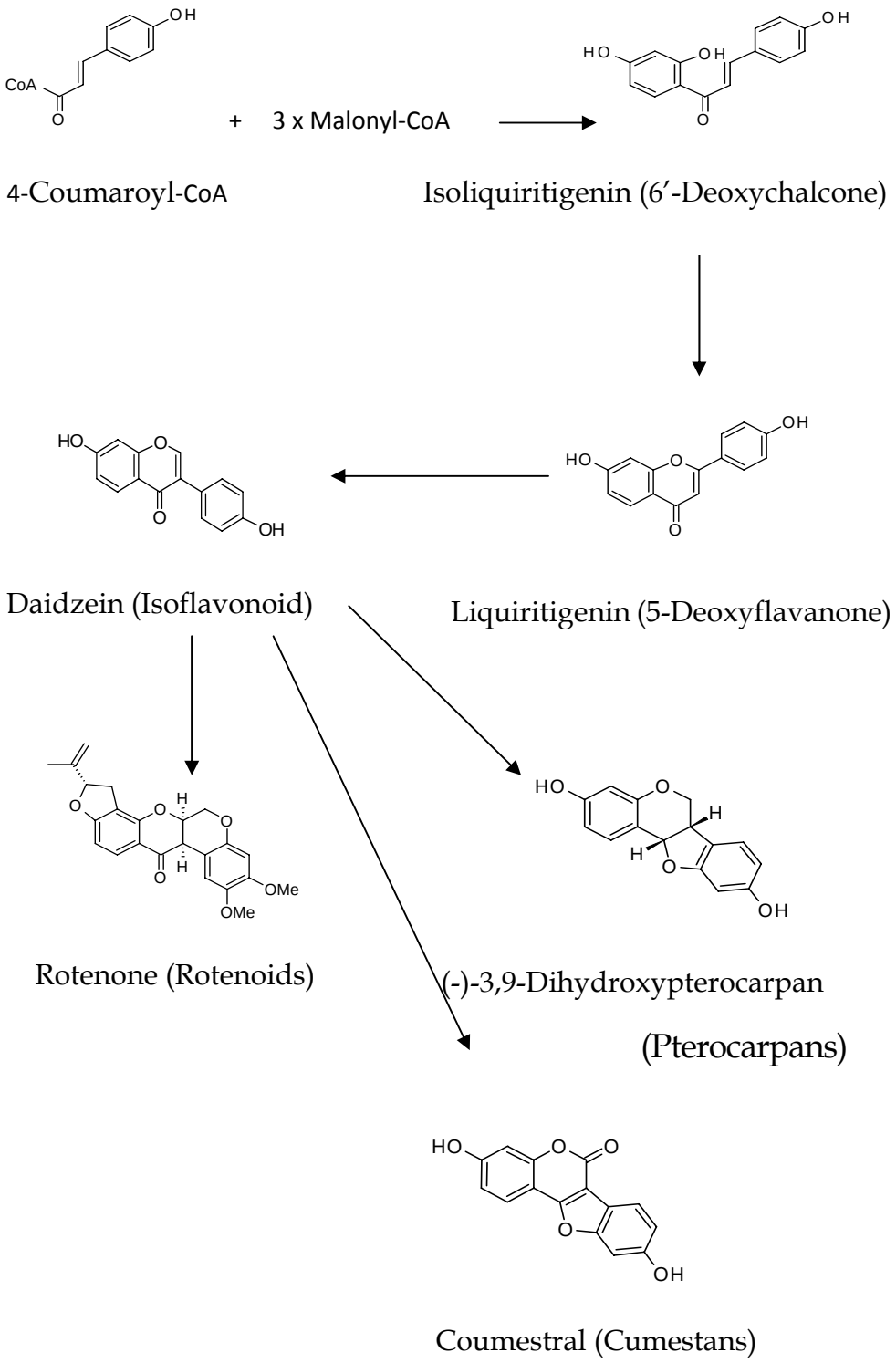


Figure 3. Biosynthetic route of isoflavonoids.

2.1.2. Pharmacological Activity of Flavonoids

It has been reported that flavonoids have many biological activity which include: anti-inflammatory, antibacterial, antiviral, anti-allergic (Cushnie and Lamb, 2005; Murray 1998; Cook and Samman, 1996), cytotoxic, antitumor, treatment of neurodegenerative diseases and vasodilatory action (Williams *et al.*, 2004; Murray 1998; Tsuchiya 2010; Chebil *et al.*, 2006).

Moreover, flavonoids inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities. These effects are exerted as antioxidants, free radical scavengers, chelators of divalent cation (Cook and Samman, 1996; Hebil *et al.*, 2006; Middleton *et al.*, 2000). Accordingly, it inhibits a variety of enzymes which include hydrolases, hyalouronidase, alkaline phosphatise, arylsulphatase, cAMP phosphodiesterase, lipase, α-glucosidase, and Kinase (Narayanna, 2001).

2.1.3. Chemotaxonaomy

Chemosystematics is the application of chemical data to tackle systematic problems. Chemotaxonomy is developed as hybrid between the chemistry of natural products and systematics. It offers a fertile field for investigation and is

gaining considerable importance in the classification at various taxonomic levels (Jones and Luchsinger, 1979).

Application of chemical data to systematic has shown that macromolecular data have practical utility taxonomic problems at the generic level or below. The flavonoids are the most widely used of all secondary metabolites in taxonomic studies. This is partially due to their ubiquitous occurrence in higher plants and their great structural diversity (Harborne and Turner, 1984).

The considerable potential of the prenylated flavonoids as useful taxonomic and phylogenetic markers to resolve a number of systematic problems among some genera of the tribe Tephrosieae such as *Pongamia, Millettia, Derris, Lonhocarpus* and *Mundulea* has been attempted by Khalid (1982).

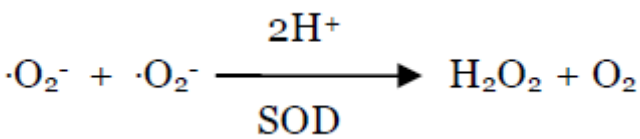
2.1.4. Flavonoids as Antioxidants

Flavonoids are known to be powerful free radical scavengers. This activity is attributed to their ability to donate hydrogen atom. The hydrogen atom is free to be donated from its phenolic groups available on the flavonoid structure. When the hydrogen atom is donated the subsequent radical

produced can be delocalized over the flavonoid stricture (Tripoli *et al.*, 2007). The reaction is as follows:

In vitro, flavonoids inhibit lipid peroxidation by scavenging the superoxide anion and hydroxyl radicals. This happens in two steps. First, the flavaonid donate its hydrogen atom to the peroxy radical, thus terminating the reaction and forming the flavonoid radical. Then, the flavonoid free radical undergoes further reaction with other free radicals to terminate chain propagation (Cook *et al.*, 1996; Ferreira *et al.*, 2010).

In vivo, the organisms have developed defensive systems against toxic radicals such as peroxynitrite and nitrous acid. Most of these systems are depending on enzymes such as the super oxidase (SOD) which concerts 2 superoxide anions to hydrogen peroxide and oxygen (Havsteen, 2002) as follows:



The structure activity relationship studies concluded that the antioxidant activity of flavonoids is linked to 3 groups (Tripoli *et al.*, 2007). First is the *ortho*-dihydroxy (catechol)

structure in the B-ring which confers greater stability to aroxyl radical formed by hydrogen bonding and electron dislocation. Second, the 2,3-Double bond in conjugation with the 4-oxo function (carbonyl group) provides an electron cloud of delocalised electrons around the B-ring and finally, the presence hydroxyl group in C_3 and C_5.

Heim *et al.* (2002) concluded that the 3',4'-catechol structure in the B-ring, strongly enhances lipid peroxide inhibition, which provides the scavenging capacity towards peroxyl, superoxide and peroxynitrite radicals. Moreover, Tripoli *et al.* (2007) pointed that the absence of the hydroxyl group linked to C_3 in flavonones and flavones decrease the antioxidant property.

In India, Pavana *et al.,* (2009) has conducted a research on the streptozotocin induced diabetic rats. The hyperglycaemia associated with an altered hexokinase and glucose-6-phosphatase activities, elevated lipid peroxidation, disturbed enzymatic - Superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidise (GPx). Antioxidant status were observed in strepozotocin induced diabetic rats. Oral administration of *Tephrosia purpurea* aqueous seed extract shows significant improvement as have been demonstrated in the previously mentioned parameters. The research concluded that the aqueous extract of *Tephrosia purpurea* has

potent anti-hyperglycaemic and antioxidant effects in the streptozotocin induced diabetic rats.

2.1.5. Effect of flavonoids on inflammation

Flavonoids have anti-inflammatory effect by the perminant inhibition the cyclo-oxygenase (COX) and lipo-oxygenase (LOX) enzymes (Bitis *et al*., 2003; Pal *et al*., 2009), thus they prevent the prostaglandin synthesis and hence suppress the T-cells which is controlled by the inhibited cytokines.

Moreover, it has been reported that they promotes the interferon (IFN) synthesis (Havsteen, 2002), inhibitition of the inducible nitric oxide synthase – by expressing their anti-oxidant activity (Miller, 1996), inhibition of cytosolic and tyrosine kinase (Middleton *et al*., 2000; Kang *et al*., 2009) and the inhibition of the neutrophiles degranulation (Middleton *et al*., 2000).

2.1.6. Flavonoids in treatment of thrombosis

Utilising the free radical scavenging property, flavonoids have an antithrombotic activity by inhibiting the cyclo-oxygenase and lipo-oxygenase pathways. Thus prevent the formation of thromboxane A_2 (Middleton *et al*., 2000; Tapas *et al*., 2008). Example, Pseudosemiglabrin and semiglabrin isolated from the roots of *Tephrosia semiglabra* exhibited an inhibitory effect against human platelet aggregation when

evaluated in an *in vitro* bioassay system using thromboxane A_2 mimetic U46619 (11α, 9α – epoxymethano PGH_2) as an aggregating agent (IC_{50}=240µg/ml) (Jonathan *et al.,* 1990).

2.1.7. Flavonoids in treatment of memory cognition.

In the past, there was a speculation that flavonoids anti-oxidant scavenging property does not give an account for any bioactivity for the flavonoids in the brain due to very low plasma concentration. However, its effect in the brain is thought to be mediated by an ability to protect neurons, enhancing brain functions and stimulate neuronal regeneration and neurogensis (Rijke *et al*., 2006).

Recently, it has been found that flavonoids could be detrimental to memory process in the brain by its ability to inhibit tyrosine kinase enzyme and activate both synaptic plasticity and new neural growth to enhance memory cognition (Spencer *et al*., 2009).

2.1.8. Flavonoids in treatment of allergy

By inhibiting the cAMP phosphodiesterase and calcium dependent ATPase, flavonoids inhibits the release of histamine from mast cells and basophils (Murray, 1998). Example, Quercetin prevents immune cells (Carlo *et al*., 1999) and inhibits both, the histamine production and release

which makes it useful in treating allergic conditions like asthma and hay fever (Carl *et al.*, 1999).

2.1.9. Flavonoids in treatment of Diabetes Mellitus

As diabetes is a genetic disease, no drug can correct it completely, but flavonoids can ameliorate some of its consequences (Havsteen, 2002).

Researches proved that flavonoids inhibit the aldose reductase enzymes (Patra and Chua, 2010) and hence helps in the regeneration of the pancreatic islets cells. Moreover, flavonoid like quercetin, has been reported to stimulate the Ca^{2+} uptake by the islet cells and thus stimulate the insulin release, which makes it effective for the non insulin dependent diabetes mellitus NIDDM (Tapas *et al.*, 2008).

2.1.10. Flavonoids in treatment of hepatotoxicity

Flavonoids, by binding to DNA-dependant RNA polymerase I, they activate it to increase the protein synthesis, which leads to the regeneration and production of hepatocytes (Murray, 1998), Example of the currently available in the market medication include silymarin.

2.1.11. Spectroscopic methods used in the structure elucidation of flavonoids

Many spectroscopic instruments could be used for the structure elucidation of the flavonoids and isoflavonoids. The spectroscopic techniques currently available include ultraviolet-visible (UV), infra red (IR), nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry.

UV –Vis spectroscopy- There is no single wavelength for all classes of flavonoids since they display absorbance maxima at distinctly different wavelengths. It helps to identify the compound subclass or perhaps even the compound itself. Typically, absorption at λmax 270 and 330 to 365nm for flavones and flavonols, at 290 nm for flavanones, at 236 or 260 nm for isoflavones, at 340 to 360 nm for chalcones, at 280nm for dihydrochalcones, at 502 or 520nm for anthocyanins and at 210 or 280 nm for catechins (Anderson and Markham, 2006).

Today, the major use of the UV-Vis spectroscopy applied to flavonoids is in quantitative analysis, and the value of this method for some structural analysis is diminishing compared to the level of the information gained by the modern spectroscopic techniques like the NMR and MS (Anderson and Markham, 2006).

NMR Spectroscopy- Is an extremely powerful analytical technique for the determination of flavonoids structure but it is limited by poor sensitivity, slow technique and difficulties in analytical mixtures.

After the incorporation of the ^{13}C NMR at the carbonyl groups, NMR studies allowed the measurement of two- and three- bond carbon-carbon coupling constants, ranging from 1.4 to 3.5 Hz, and the measurement two-, three-, and four-bond carbon-hydrogen coupling constants, which ranges from 0.3 tp 3.8 Hz. Complete assignment of the 1H and 13C NMR spectra of several flavones and their proton-proton and carbon-proton coupling constants, including the extreme seven-bond long-range coupling between H-7 and H-3 in 6-hydroxyflavone (0.52Hz) and flavones (0.27Hz) (Anderson and Markham, 2006).

The purpose of the ^{1}H NMR spectrum is to record the chemical shifts, spin-spin coupling and integration data, thus providing information about the number of the hydrogen atoms. Most of the information provided by the ^{1}H NMR is insufficient for a complete structure elucidation and it has to be combined with ^{13}C NMR to imply more accuracy by assigning all of the ^{1}H and ^{13}C NMR signals (Anderson and Markham, 2006).

2.2. *Tephrosia*

Tephrosia is member of the Papilionoideae which represents the largest of the three subfamilies of Fabaceae with 476 genera and 13860 species.

The genus *Tephrosia* encompasses about 300 species found in the Tropical and subtropical regions of the world. The generic name is derived from the Greek word τεφρος (*tephros*), meaning "ash-colored," referring to the greyish tint given to the leaves by their dense trichomes (Quattrocchi and Umber, 2000).

There are more than 22 species of *Tephrosia* reported to occur in Sudan (Andrews, 1952), which are widely distributed in the Northern, Central and Southern regions.

2.2.1. Ethno –pharmacological uses of *Tephrosia*

Tephrosia has many pharmacological uses (Barnes, 1967; Gaskins, 1972). In many African countries, *Tephrosia* species are used for fish poisoning (Matsumura, 1975) as well as the other aquatic organisms (Blommaert, 1950; Bourgois, 1989; Bossard, 1993). Moreover, it has molluscicidal (Cowper, 1948) as well as insecticidal (Shin, 1989) and pesticidal properties (Guadin, 1938). These activities may be associated with the presence of rotenoids. Table 1, illustrates some *Tephrosia*

species with different ethno-pharmacological uses in Africa as well as in Asian countries.

Table 1. Ethno-pharmacological uses of *Tephrosia spp* in African and Asian countries.

Botanical Name	Country	Ethno-medical use	Reference
***T. bracteolate* Guill & Perr.**	Tropical Africa	Grazing of horses and live-stock.	Dalziel, 1937.
	Ivory Cost	Fish poison	Burkill, 1995.
	Tanzania	The roots used for pregnant women with syphilis	Burkill, 1995.
	Northern Nigeria	Leaves and roots used by hunters and warriors for charms against injury.	Dalziel, 1937.
***T. flexuosa* G. Don**	Tropical Africa	Grazing of horses and live-stock.	Dalziel, 1937.
***T. candida* (Roxb) DC**	Bangladesh, Burma, Sierra Leone.	Fish poison.	Chadha, 1976.
T. densiflora	West Africa	Fish poison	Kerharo, 1950.
		Abortifacient properties, anti-parasitic against the parasitic skin infections.	Oliver, 1960.
***T. elegans* Schum**	South Africa	Fish poison	Kerharo, 1950.
		The roots used to prepare arrow poison.	Watt, 1950.

Table 1. Ethno-pharmacological uses of *Tephrosia spp*. in African and Asian countries. (*continued*)

Botanical Name	Country	Ethno-medical use	Reference
***T. linearis* Willd.**	Senegal	Grazing of livestock	Dalziel, 1937.
		Postnatal medicine	Watt, 1962.
***T. lupinifolia* DC**	Senegal	Grazing of livestock	Dalziel, 1937**.**
***T. lupinifolia* DC**	West Zambia	Abortifacient	Watt, 1962.
***T. nana* Kotschy ex Schweinf**	Eastern Cameroon	Grazing the livestock.	Dalziel, 1937.
		Fish poisoning	Kerharo, 1950.
	Congo Brazzaville	Bait in the rodent's snares	
	Nigeria	Granted leaves used for the yaw's sores treatment.	Burkill. 1995.
T. vogelli	Eastern Cameroon	Fish poisoning	Kerharo, 1950.
	India	Root decoction for the treatment of diarrhoea, rheumatism and asthma.	Burkill, 1995.
***T. nubica* (Boiss) Bok**	Kenya	Grazing the livestock.	Dalziel, 1937
		The whole plant infusion is used for women after birth.	Burkill, 1995.
***T. pedicellata* Bak**	Kenya	Fish poisoning	Burkill, 1995.

Table 1. Ethno-pharmacological uses of *Tephrosia spp*. in African and Asian countries. (*continued*)

Botanical Name	Country	Ethno-medical use	Reference
***T. pedicellata* Bak**	Sudan	Chewing the roots for the throats and lung complains	Burkill, 1995.
***T. platycarpa* Guill and Perr**	Sudan-Kordofan	Production of cooking oil	Burkill, 1995.
T. hamiltonii	India	Tonic, laxative, diuretic, deobstrunit. Roots and seeds have insecticidal and pesticidal effect. Decoction of pods is vermifuge and stop vomiting.	Burkill, 1995.
***T. purpurea* (Linn.)**	India	Tonic, laxative, diuretic, treatment of boils, pimples and bleeding piles, cough & kidney disorders. Seed oil used for treating scabies, itching, eczema and skin eruptions	Chadha, 1976, Rastogi, 1990.
***T. purpurea* (Linn.)**	Tanzania	Roots are chewed for pain relief. Leaves and roots have purgative and emetic effect.	Burkill 1995

Table 1. Ethno-pharmacological uses of *Tephrosia spp*. in African and Asian countries. (*continued*)

Botanical Name	Country	Ethno-medical use	Reference
	Senegal	diarrhoea, whooping cough, vaginal douches, spasmodic coughing, fever, sterility, rickets and syphilis.	Kerharo 1964
***T. purpurea* (Linn.)**	Nigeria	Diuretic, blood purifier, gargle and internally for coughs and colds.	Ainslie 1937.
***T. uniflora* Pers**	Ethiopia	Stems are used as toothbrushes and roots used to kill the fresh water snail *Bulinus globules,* vector schistosomosis.	Adewunmi 1980.

2.2.2. Previous Phytochemical Investigations.

An in-depth literature search based on the Dictionary of Natural Products (2011) revealed that 186 flavonoids have been reported from the 49 *Tephrosia* species previously investigated. All the reported compounds are arranged in Tables (2 – 13) along with their botanical origin and references. The literature search strictly followed the

biosynthetic pathway as chalcone (Table 2), flavan (Table 3), flavonone (Table 4), flavone (Table 5), flavanol (Table 6), flavene (Table 7), isoflavan (Table 8), isoflavone (Table 9), carpans (Table 10), coumstan (Table 11), rotenoids (Table 12) beside some other miscellaneous compounds (Table 13). It is interesting to note that the bulk of the reported flavonoids from this genus are prenylated at A-ring and devoid of oxygenation at B-ring as previously predicted by Waterman and Khalid (1985).

2.2.3. Chalcones.

To date the number of chalcones reported from *Tephrosia species* is 22 compounds. They are arranged in Table 2 on the basis of their complexity and substitution patterns. The chalcones isolated from this genus are characterised by the absence of the hydroxyl group from C_4 in the B-ring and $C_{2'}$ in the A-ring with frequent prenylation involving mainly A-ring at $C_{3'}$ and/or $C_{5'}$, while absence of hydroxylation at $C_{6'}$ seems to be correlated by the synthesis of certain types of isoflavonoids (Andersen and Markham, 2006).

Table 2. Chalcones isolated from *Tephrosia spp*.

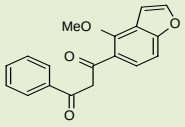
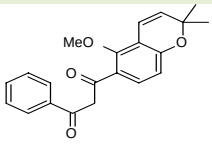
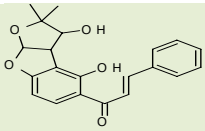
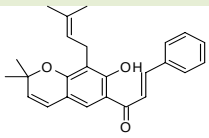
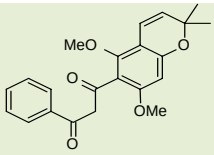
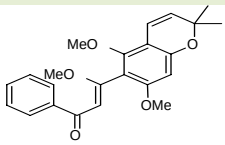
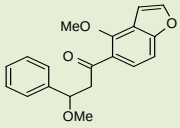
Compound	Botanical Origin	Reference
 **Pongamol**	*T. lanceolata* *T. purpurea.*	Lee and Morehead, 1995. Pelter *et al*., 1981; Sinha *et al*., 1982; Paramar *et al*., 1989.
 **Purpurenone**	*T. purpurea*	Rao and Raju, 1984.
 **Tephrosone**	*T. purpurea*	Chang *et al*., 2000.
 **Spinochalcone C**	*T. spinosa*	Rao and Prasad, 1992.
 **Praecansone B**	*T. praecans* *T. procumbens*	Camele *et al*., 1980. Venkataratnam *et al*., 1987.
 **Praecansone A**	*T. praecans* *T. procumbens*	Camele *et al*., 1980 Venkataratnam *et al*., 1987
 ***O*-Methylpongamol**	*T. purpurea*	Pelter *et al*., 1981; Sinha *et al*., 1982; Paramar *et al*., 1989.

Table 2**.** Chalcones isolated from *Tephrosia spp*. *(continued)*

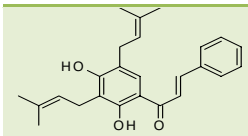
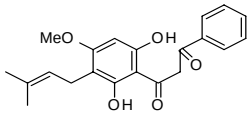
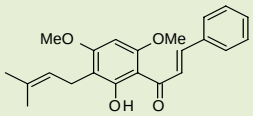
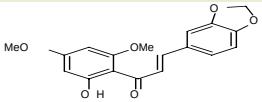
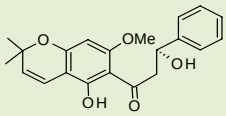
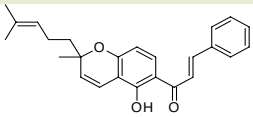
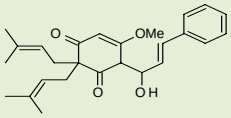
Compound	Botanical Origin	Reference
 **2′,4′-Dihydroxy-3′,5′-diprenylchalcone**	*T. spinosa*	Rao and Prasad, 1992 Sharma and Rao, 1992.
 **β,2′,6′-Trihydroxy-4′-methoxy-3′-prenyl-chalcone**	*T. major*.	Gomez-Garibay *et al.*, 2002.
 **Ovalichalcone**	*T. candida*.	Roy *et al.*, 1986.
 **Tephrone**	*T. candida,*	Chibber and Dutt, 1982.
 **Elatadihydochalcone**	*T. elata*	Muiva *et al.*, 2009.
 **Spinochalcone B**	*T. spinosa*	Rao and Prasad, 1992.
 **Tunicatachalcone**	*T. tunicate.*	Andrei *et al.*, 2000.

Table 2. Chalcones isolated from *Tephrosia spp*. *(continued)*

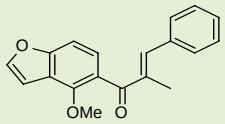
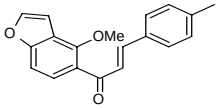
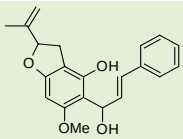
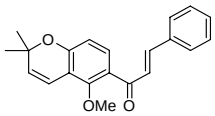
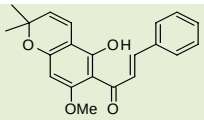
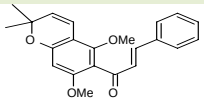
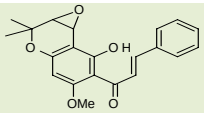
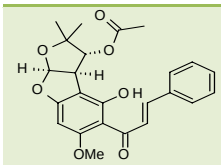
Compound	Botanical Origin	Reference
 **Purpuritenin B**	*T. purpurea*	Sinha *et al*., 1982.
 **Purpuritenin A**	*T. purpurea*.	Sinha *et al*., 1982; Lee and Morehead, 1995.
 **Crassichalcone**	*T. crassifolia*	Gomez–Garibay *et al*., 1999.
 **Spinochalcone D**	*T. spinosa.*	Prasad *et al*., 2005.
 **Obovatachalcone**	*T. carrollii,* *T. obovata*	Gomez–Garibay *et al*., 2001. Chen *et al*., 1978.
 **5-*O*-Methylobovata-chalcone**	*T. pulcherrima*	Ganapaty *et al*., 2008.
 **Epoxyobovatachalcone**	*T. carrollii*	Gomez-Garibay *et al*., 2001.

Table 2. Chalcones isolated from *Tephrosia spp*. *(continued)*

Compound	Botanical Origin	Reference
 Tephropurpurin	*T. purpurea*.	Change *et al*., 1997; Chang *et al*., 2000.

2.2.4. Flavan

To date the number of flavans reported from *Tephrosia* species is 11 compounds. They are arranged on the basis of their complexity and substitution patterns in Table 3, where their names, structure, botanical origin and references are presented. All flavans reported from this taxon seem to be devoid of oxygenation at B-ring with prenylation at C_8.

Table 3. Flavans isolated from *Tephrosia spp*.

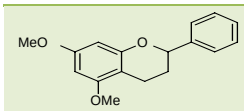
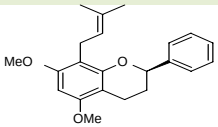
Compound	Botanical Origin	Reference
 Tephrowatsin E	*T. watsoniana*	Gomez-Garibay *et al*., 1985.
 5,7-Dimethoxy-8-prenylflavan	*T. watsoniana*	Gomez-Garibay *et al*. 1985.

Table 3. Flavans isolated from *Tephrosia spp*. (*continued)*

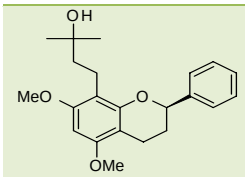
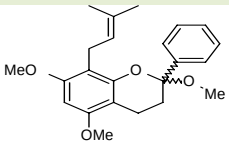
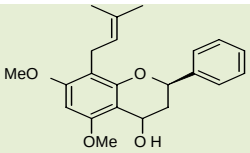
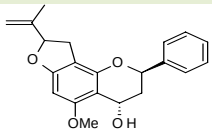
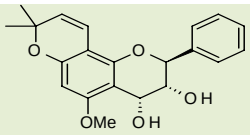
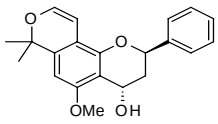
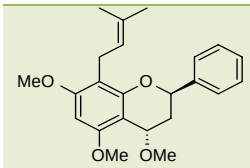
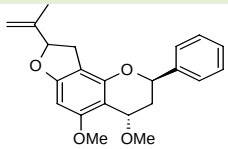
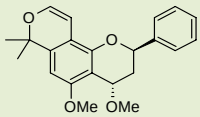
Compound	Botanical Origin	Reference
 **Nitenin**	*T. nitens*	Gomez-Garibay *et al*., 1984.
 **Tephrowatsin D**	*T. watsoniana*	Gomez-Garibay *et al*., 1985.
 **Tephrowatsin A**	*T. watsoniana*	Gomez-Garibay *et al*., 1985.
 **Hildgardtol A**	*T. hildebrandtii* *T. crassifolia.*	Monache *et al*., 1986. Gomez – Garibay *et al*., 1999.
 **Quercetol A**	*T. quercetorum*	Gomez-Garbay *et al*., 1988.
 **Hildgardtol B**	*T. hilderbrandtii, T. abbottiae,* *T. quercetorum.*	Monache *et al*., 1986. Gomaz–Garibay *et al*., 1986; Gomez – Garibay *et al*. 1988.

Table 3. Flavans isolated from *Tephrosia spp*. (*continued)*

Compound	Botanical Origin	Reference
 **Quercetol B**	*T. quercetorum*	Gomez-Garbay *et al*., 1988.
 **Methylhildgardtol A**	*T. hildebrandtii* *T. crassifolia.*	Monache *et al*., 1986. Gomez – Garibay *et al*., 1999.
 **Methylhildgardtol B**	*T. hilderbrandtii*	Monache *et al*., 1986

2.2.5. Flavanone

To date the number of flavanones reported from *Tephrosia* species is 34 compounds. They are arranged in Table 4 on the basis of their complexity and substitution patterns. The pattern of lacking oxygenation at B-ring seems to be consistent with very few exceptions.

Table 4. Flavanones isolated from *Tephrosia spp.*

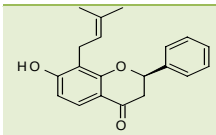
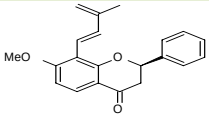
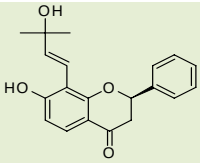
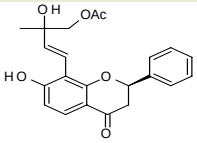
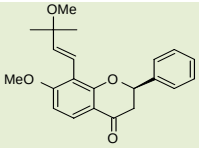
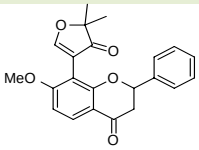
Compound	Botanical Origin	Reference
 7-Hydroxy-8-prenyl-flavanone (S-form)	*T. falciformis*	Khan *et al*., 1986
 Dehydroisoderricin	*T. purpurea*	Rao and Raju, 1984.
 Tephroapollin A	*T. apollinea*	Abd El-Razek *et al*., 2007.
 Tephroapollin B.	*T. apollinea*	Abd El-Razek *et al*., 2007.
 7-Methoxy-8-(3-methoxy-3-methyl-1-butenyl)flavanone	*T. purpurea*	Kiuchi *et al*., 1989
 Tephroglabrin	*T. purpurea*.	Pelter *et al*., 1981.

Table 4. Flavanones isolated from *Tephrosia spp*. *(Continued)*

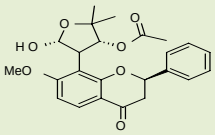
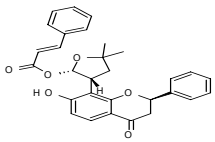
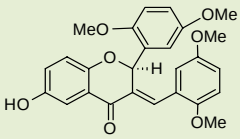
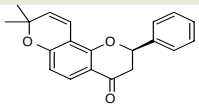
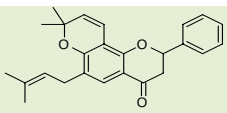
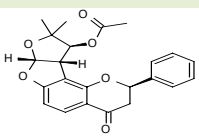
Compound	Botanical Origin	Reference
 Tephrorin A	*T. purpurea*.	Chang *et al*., 2000.
 Tephrorin B	*T. purpurea*.	Chang *et al*., 2000.
 3-(2,5-Dimethoxy-benzylidene)-6-hydroxy-2′,5′-dimethoxyflavanone. (R,E)-form	*T. calophylla*	Reddy *et al*., 2009.
 Isolonchocarpin	*T. purpurea*	Rao and Raju, 1979
 Maximaflavanone A	*T. maxima*	Rao *et al*., 1994
 Purpurin	*T. purpurea*.	Rao and Raju, 1984 Pirrung *et al*., 1998.

Table 4. Flavanones isolated from *Tephrosia spp*. *(Continued)*

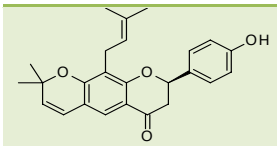
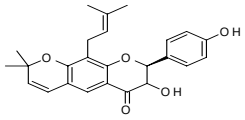
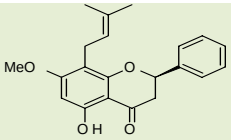
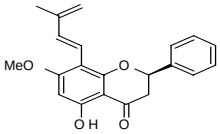
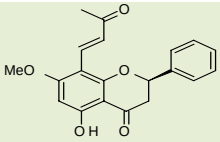
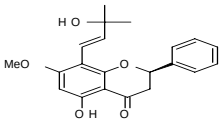
Compound	Botanical Origin	Reference
 Lupinifolin	*T. lupinifolia*	Smalberger *et al.*, 1974
 Lupinifolinol	*T. lupinifolia*	Smallberger *et al.*, 1974
 Tephrinone	*T. villosa* *T. calophylla*	Hossain *et al.*, 1999. Kishore *et al.*, 2003.
 Tephroleocarpin B	*T. leiocarpa.*	Gomez-Garibay *et al.*, 1991.
 5-Hydroxy-7-methoxy-8-(3-oxo-1-butenyl)-flavanone	*T. toxicaria*	Jang *et al.*, 2003.
 Tephroleocarpin A	*T. leiocarpa*	Gomez-Garibay *et al.*, 1991.

Table 4. Flavanones isolated from *Tephrosia spp*. *(continued)*

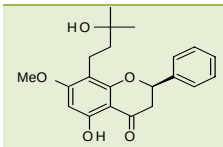
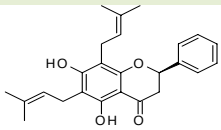
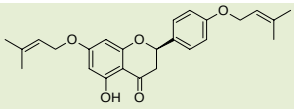
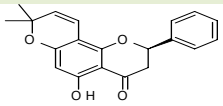
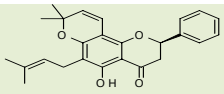
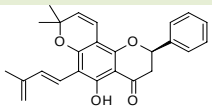
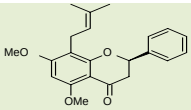
Compound	Botanical Origin	Reference
 Tephrowatsin C	*T. watsoniana*	Gomez-Garibay *et al*., 1985.
 5,7-Dihydroxy-6,8-diprenylflavanone	*T. spinosa*	Rao and Prasad, 1992.
 5-Hydroxy-4′,7-di-prenyloxyflavanone	*T. calophylla*	Reddy *et al*., 2009.
 Obovatin	*T. obovata*	Chen *et al*., 1978
 Fulvinervin A	*T. Fulvinervis*	Rao *et al*., 1985.
 Spinoflavanone A	*T. spinosa*	Rao and Raju, 1992.
 Candidone	*T. candida,* *T. elata,*	Roy *et al*., 1986. Lwande *et al*., 1985.

Table 4. Flavanones isolated from *Tephrosia spp*. *(Continued)*

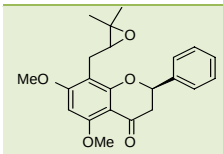
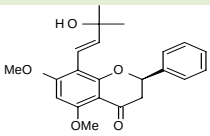
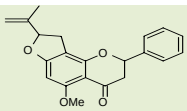
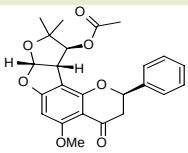
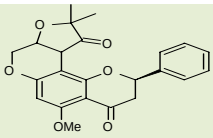
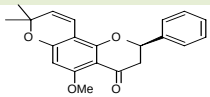
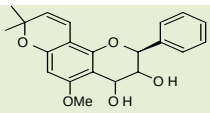
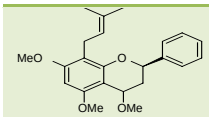
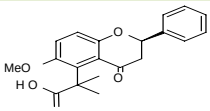
Compound	Botanical Origin	Reference
 Epoxycandidone	*T. hamiltonii*	Hussaini and Shoeb, 1987.
 Quercetol C	*T. quercetorum*	Gomez-Garbay *et al*., 1988.
 Emoroidenone	*T. emoroides*	Machocho *et al*., 1995.
 Apollineanin	*T. apollinea*	Hisham *et al*., 2006.
 Stachyoidin	*T. polystachyoides*	Antus *et al*., 1977.
 Pongachin	*T. woodii*, *T. obovata*, *T. candida*,	Dominguez *et al*., 1983. Chen *et al*., 1978 Chibber and Dutt, 1982.
 Quercetol A	*T. quercetorum*	Gomez-Garibay *et al*., 1988.

Table 4. Flavanones isolated from *Tephrosia spp*. *(Continued)*

Compound	Botanical Origin	Reference
 Quercetol B	*T. quercetorum*	Gomez-Garibay *et al*., 1988.
 Falciformin	*T. falciformis*	Khan *et al*., 1986 Parmar *et al*., 1990.

2.2.6. Flavone

To date the number of flavones reported from *Tephrosia* species - is 30 compounds. They are presented on Table 5 on the basis of their complexity and substitution patterns. All flavones reported so far are showing the same pattern of lacking oxygenation at B-ring and most of them bear prenylation at C_8 with few of them are prenylated at C_6 and C_8.

Table 5. Flavone isolated from *Tephrosia spp.*

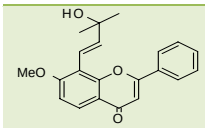
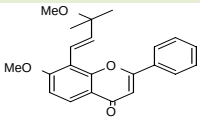
Compound	Botanical Origin	Reference
 Lanceolatin A	*T. lanceolata,* *T. apollinea*	Ayengar *et al*., 1973 Waterman and Khalid, 1980 Abou-Douh *et al.* 2005 Khalafallah *et al*., 2009
 Tephroapollin D	*T. apollinea*	Abd El-Razek *et al*., 2007.

Table 5. Flavones isolated from *Tephrosia spp*. (*Continued*)

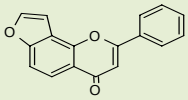
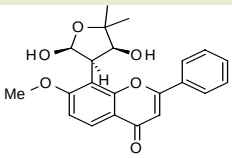
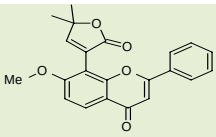
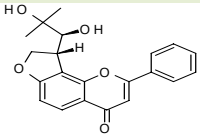
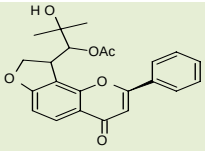
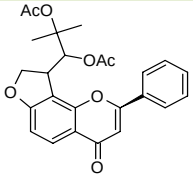
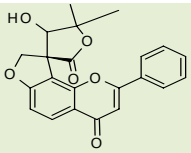
Compound	Botanical Origin	Reference
 Lanceolatin B	*T. lanceolata.*	Lee and Morhead, 1995.
 Tepurindiol	*T. purpurea.*	Pelter *et al.* 1981.
 Apollinine	*T. apollinea,*	Waterman and Khalid, 1980.
 Tephroapollin E	*T. apollinea,*	Adb ElRazek *et al*., 2007 Khalafallah *et al.*, 2009.
 Tephroapollin F	*T. apollinea*	Abd El-Razek *et al*., 2007.
 Tephroapollin G	*T. apollinea*	Abd El-Razek *et al.*, 2007.
 Glabratephrinol	*T. apollonea*	Waterman and Khalid, 1980; Khalafallah *et al.*, 2009.

Table 5. Flavons isolated from *Tephrosia spp.* (*Continued*)

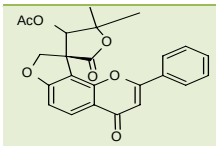
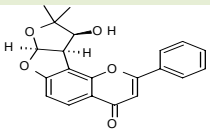
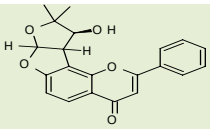
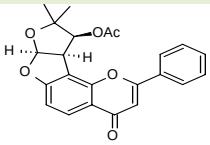
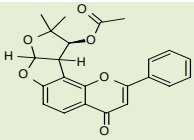
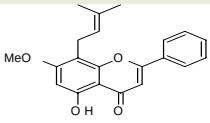
Compound	Botanical Origin	Reference
 Glabratephrin	*T. apollinea*	Waterman and Khalid, 1980; Khalafallah *et al.*, 2009.
 Semiglabrinol	*T. semiglabra*	Smalberger *et al.*, 1973 Ahmed, 1986.
 Pseudosemiglabrinol	*T. apollinea*	Ahmed, 1986
 Semiglabrin	*T. apollinea* *T. semiglabra*	Smalberger *et al.*, 1973; Waterman and Khalid, 1980; Ahmed, 1986; Abou-Douh *et al.*, 2005. Jonathan *et al.*, 1990; Pirrung *et al.*, 1995;
 Pseudosemiglabrin	*T. apollinea*	Waterman and Khalid, 1980; Jonathan *et al.*, 1990; Pirrung *et al.*, 1995.
 5-Hydroxy-7-methoxy-8-prenylflavone	*T. abbottiae*	Jain and Sharma, 1974.

Table 5. Flavons isolated from *Tephrosia spp.* (*Continued*)

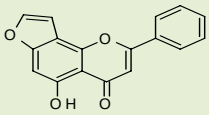
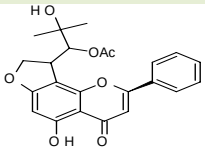
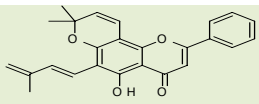
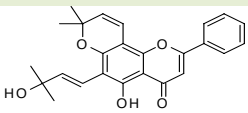
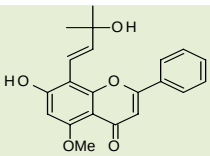
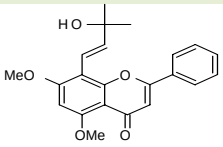
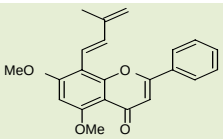
Compound	Botanical Origin	Reference
 Pongaglabol	*T. purpurea.*	Ahmed *et al.*, 1999.
 5-Hydroxy-tephroapollin F	*T. apollinea*	Khalafallah *et al.*, 2009.
 Fulvinervin B	*T. Fulvinervis*	Rao *et al*. 1985.
 Fulvinervin C	*T. Fulvinervis*	Venkataratnam *et al.*, 1986.
 Emoroidone	*T. emoroides*	Machocho *et al.*, 1995.
 Tephrostachin	*T. bracteolata*	Watermann and Khalid, 1981.
 Anhydrotephro-stachin	*T. bracteolata*	Watermann and Khalid, 1981.

Table 5. Flavons isolated from *Tephrosia spp.* (*Continued*)

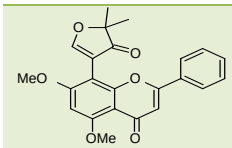
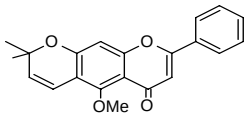
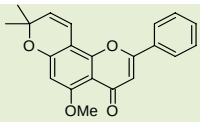
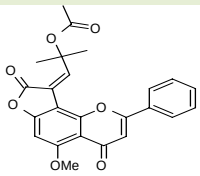
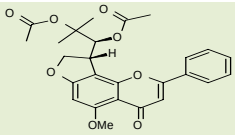
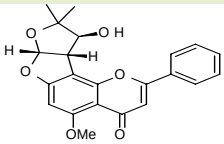
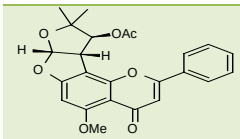
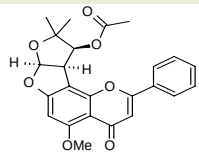
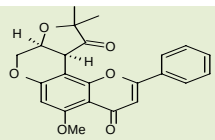
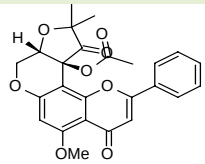
Compound	Botanical Origin	Reference
 Tachrosin	*T. polystachyoides.*	Antus *et al.*, 1977
 5-Methoxy-6,6-dimethyl-pyrano-[2,3:7,6]flavone.	*T. Praecans*	Camele *et al.*, 1980
 Isopongaflavone	*T. braceolata* *T. elata* *T. Praecans*	Watermann and Khalid, 1981 Lwande *et al.*, 1985 Camele *et al.*, 1980
 Tephrorianin	*T. hookeriana*	Vanangamudi *et al.*, 1997.
 Polystachin	*T. polystachyoides.*	Vleggaar *et al.*, 1978.
 Multijuginol	*T. multijuga*	Velggaar *et al.*, 1975

Table 5. Flavons isolated from *Tephrosia spp.* (*Continued*)

Compound	Botanical Origin	Reference
 Multijugin	*T. multijuga*	Vleggaar *et al*,. 1975.
 Enantiomultijugin	*T. viciodes*	Gomez-Garibay *et al.*, 1992.
 Stachyoidin	*T. polystachyoides*	Antus *et al.*, 1977
 Tephrodin	*T. polystachyoides*	Antus *et al.*, 1977

2.2.7. Flavonol

To date the number of flavonols reported from *Tephrosia* species is 30 compounds. They are presented in Table 6 on the basis of their complexity and substitution patterns. Unlike all other classes of flavonoids, the flavonols are characterized by B-ring and C_3 hydroxylation concomitantly with O-glycosylation at C_3 and C_7.

Table 6. Flavanols isolated from *Tephrosia spp*.

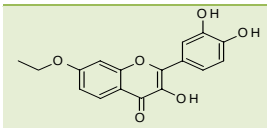
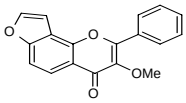
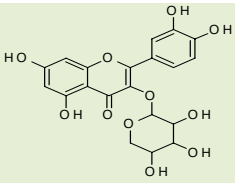
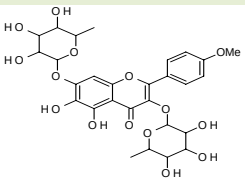
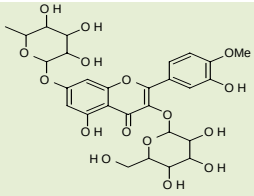
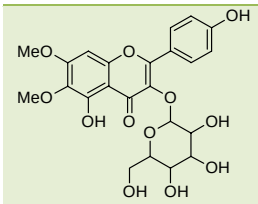
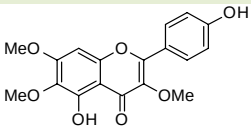
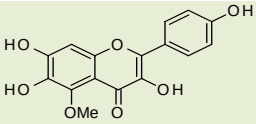
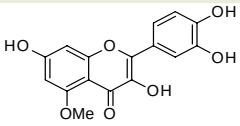
Compound	Botanical Origin	Reference
 7-Ethoxy-3,3′,4′-trihydroxyflavone	*T. procumbens*	Venkataratnam *et al*., 1987
 Karanjin	*T. purpurea*.	Aneja *et al*., 1958.
 3-O-Arabino-pyronoside Queractin	*T. vogelii*	Marston *et al*., 1984.
 4′-Methoxy-3,7-di-O-α-L-rhamno-5,6-dihydroxyflavone	*T. candida*	Sarin *et al*., 1976.
 Tamarixetin 3-glucoside 7-rhamnoside	*T. purpurea*	Kassem *et al*., 2006.

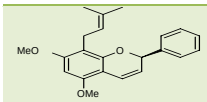
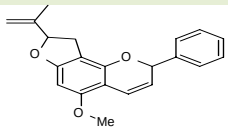
Table 7. Flavanols isolated from *Tephrosia spp*.

Compound	Botanical Origin	Reference
 Tephrospinosinol	*T. spinosa.*	Khajuria *et al*., 1982.
 Penduletin	*T. candida,*	Flores *et al*., 1958.
 Vogeletin	*T. vogelii*	Rangaswami *et al*., 1959; Sambamurthy *et al*., 1962
 Azaleatin	*T. vogelf*	Jurd and Horowitz, 1957 Harborne 1969.

2.2.8. Flavene

Only 2 flavenes are reported from *Tephrosia* species as shown in Table 7. The two compounds are lacking oxygenation at B-ring and bearing prenylation at C_8 with methoxylation at C_5.

Table 8. Flavene isolated from *Tephrosia spp*.

Compound	Botanical Origin	Reference
 Tephrowatsin B	*T. watsoniana*	Gomaz-Garibay *et al*., 1985a.
 Abbottin	*T. abbottiae*, *T. crassifolia*, *T. emoroides*, *T. hildebrandti*	Gomez-Garibay *et al*., 1986. Gomez – Garibay *et al*. 1988. Machocho, 1995. Monache *et al*., 1986;

2.3. The Isoflavonoids

The isoflavonoids are almost entirely restricted to the plant family Fabaceae. Some of the many variant are shown in Table 8-11.

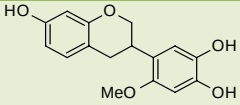
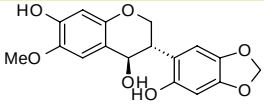
From comparative studies (Khalid, 1982) it appears likely that all different sub-classes of isoflavonoids are derived from a common primary isoflavone prototype. Once this step is acquired the elaboration of all other isoflavonoids sub-classes represents minor modifications along the biosynthetic pathway proposed for isoflavone synthesis. The most striking fact regarding the isoflavonoids as a group is their frequent co-occurrence with the much abundant isoflavone derivatives. The present literature search enumerates the co-occurrence of different sub-

classes of isoflavonoids within the genus *Tephrosia* (Tables 8-11). Each of the typical isoflavonoids sub-classes reported from *Tephrosia* including isoflavans, isoflavone, carpans, and rotenoids is presented separately here under.

2.3.1. Isoflavan

To date there are only 2 isoflavan been reported from *Tephrosia species*. Their names, structures, botanical origin and reference are presented on Table 8.

Table 9. Isoflavan isolated from *Tephrosia spp*.

Compound	Botanical Origin	Reference
 Astraciceran	*T. strigosa.*	Rao and Sridhar, 1999.
 Pumilanol	*T. pumila*	Ganapaty *et al.*, 2008.

2.3.2. Isoflavone

To date there are 25 isoflavone been reported from *Tephrosia species*. Their names, structures, botanical origin and reference are presented on Table 9. They are characterised by the lack of C_5 and $C_{4'}$ oxygenation with either C- or O-prenylation.

Table 10. Isoflavones isolated from *Tephrosia spp.*

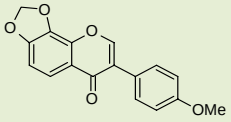
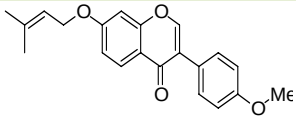
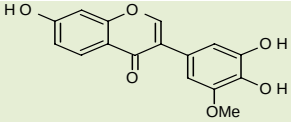
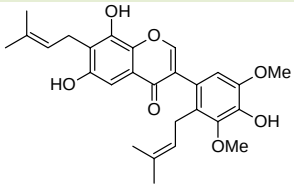
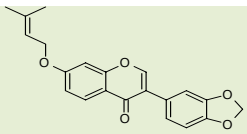
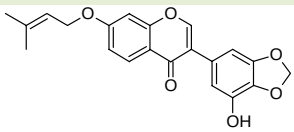
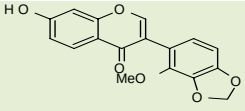
Compound	Botanical Origin	Reference
 Maximaisoflavone H	*T. maxima*	Rao and Murthy, 1985
 Maximaisoflavone J	*T. maxima*	Rao and Murthy *et al.*, 1985.
 4′,7-Dihydroxy-3′,5′-dimethoxyisoflavone	*T. purpurea*	Chang *et al.*, 1997
 Pumilaisoflavone C	*T. pumila*	Yenesew *et al.*, 1989.
 Maximaisoflavone B	*T. maxima*	Rao and Murthy, 1985.
 Maximaisoflavone K.	*T.maxima*	Rao *et al.*, 1994.
 Maximaisoflavone G	*T. maxima*	Rao *et al.*, 1984

Table 9. Isoflavones isolated from *Tephrosia spp*. *(Continued)*

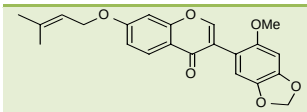
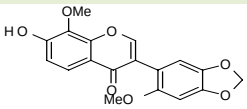
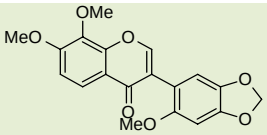
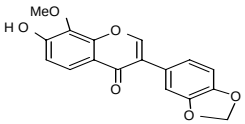
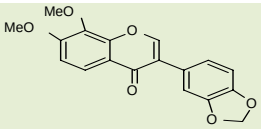
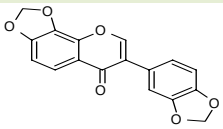
Compound	Botanical Origin	Reference
 **Maximaisoflavone C**	*T. maxima*.	Rangaswami *et al*., 1959.
 **Maximaisoflavone F**	*T. maxima*	Rao *et al*., 1984
 **2′,7,8-Trimethoxy-4′,5′-methylene-dioxy-isoflavone**	*T. maxima,*	Galina *et al*., 1974.
 **Maximaisoflavone E**	*T. maxima*	Rao *et al*., 1984
 **Purpuranin A**	*T. purpurea,*	Rao *et al*., 1984.
 **Maximaisoflavone A**	*T. maxima*	Kulka and Seshadri, 1962.

Table 9. Isoflavones isolated from *Tephrosia spp*. *(Continued)*

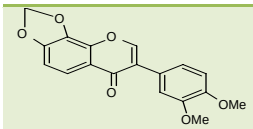
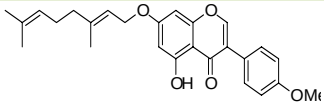
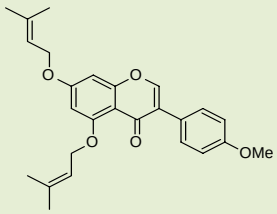
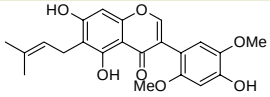
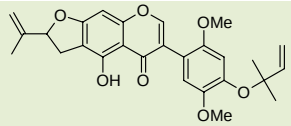
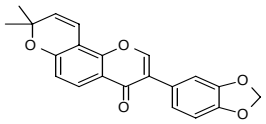
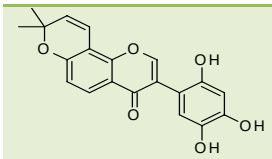
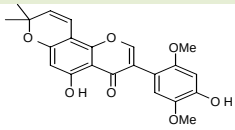
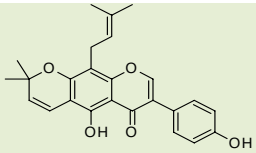
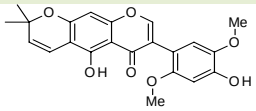
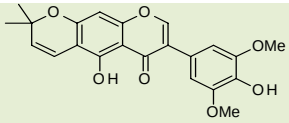
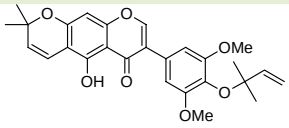
Compound	Botanical Origin	Reference
 Maximaisoflavone D	*T. maxima*	Rao *et al.*, 1984.
 7-O-Geranylbio-chaninA	*T. tinctoria*	Reddy *et al.*, 2007.
 5,7-Di-O-prenyl-biochaninA	*T. tinctoria.*	Khaivulla *et al.*, 2008.
 Viridiflorin	*T. viridiflora*	Gomez-Garibay *et al.,* 1985b.
 Pumilaisoflavone B	*T. pumila.*	Dagne *et al.*, 1988.
 Calopogonium-isoflavone B	*T. maxima.*	Murthy and Rao *et al.*, 1985.

Table 9. Isoflavones isolated from *Tephrosia spp*. *(Continued)*

Compound	Botanical Origin	Reference
 Barbigerone	*T. barbigera,*	Vilian, 1980
 4′-*O*-Demethyl-toxicarolisoflavone	*T. polyphylla.*	Dagne *et al.*, 1992.
 Scandenone	*T. elata*	Jain *et al.*, 1974.
 Elongatin	*T. elongate* *T. viridiflora.*	Smalberger *et al.*, 1975 Gomez-Garibay *et al.*, 1985.
 Pumilaisoflavone D	*T. pumila*	Dagne *et al.*, 1988; Yenesew *et al.*, 1989.
 Pumilaisoflavone A	*T. pumila*	Dagne *et al.*, 1988; Yenesew *et al.*, 1989.

2.3.3. Carpans

To date, 9 carpans have been reported from *Tephrosia species*. Their names, structure, botanical origin and references are presented on Table 10. They are characterised by C5 deoxygenation and B-ring *ortho* hydroxylation or methylene dioxy formation.

Table 11. Carpans Isolated from *Tephrosia spp*.

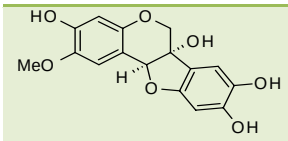
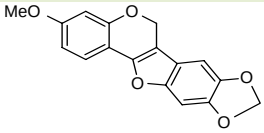
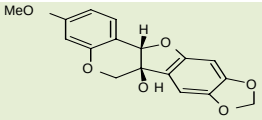
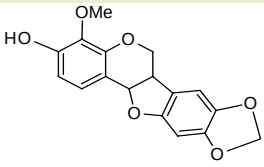
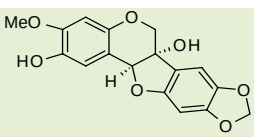
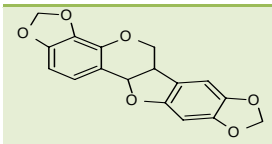
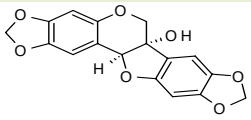
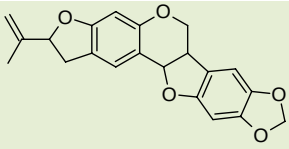
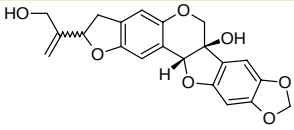
Compound	Botanical Origin	Reference
 Hildecarpin	*T. hildebrandtii*	Lwande *et al*., 1985, Lwande *et al*., 1987.
 Flemichapparin B	*T. candida*	Adityachaudhury *et al*., 1987.
 Pisatin	*T. bidwilli*	Ingham and Markham, 1980.
 4-Methoxymaackiain	*T. bidwilli*	Chaudhuri *et al*., 1995.
 Tephrocarpin	*T. bidwilli*	Ingham and Markham, 1980.

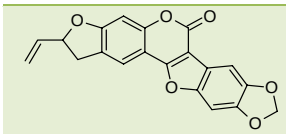
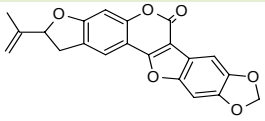
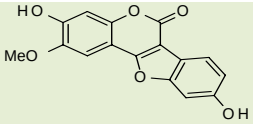
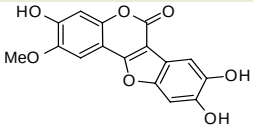
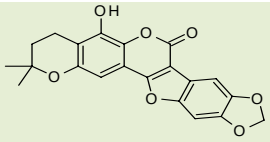
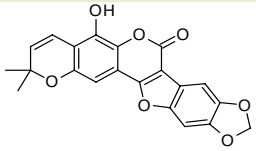
Table 10. Carpans isolated from *Tephrosia spp*. (*continued*)

Compound	Botanical Origin	Reference
 3,4:8,9-Bis-(methylenedioxy)pterocarpan	*T. aequilata*	Tarus *et al*., 2002.
 Acanthocarpan	*T. bidwilli*	Ingham and Markham, 1980.
 Emoroidocarpan	*T. emoroides*	Machocho *et al*., 1995.
 Hildecarpidin	*T. hildebrandtii*	Lwande *et al*., 1987.

2.3.4. Coumestan

To date, only 6 Coumestans have been reported from *Tephrosia species.* Their names, structures, botanical origin and references are presented on Table 11.

Table 12. Coumestans isolated from *Tephrosia spp*.

Compound	Botanical Origin	Reference
 Tephcalostan B	*T. calophylla*	Ganapaty *et al*., 2009.
 Tephcalostan	*T. calophylla*.	Kishore *et al*., 2003.
 2-*O*-Methyllucernol	*T. hamiltonii*	Rajani and Sarma, 1988
 Tephrosol	*T. villosa*.	Rao and Srimannarayana, 1980.
 Tephcalostan C	*T. calophylla*	Ganapaty *et al*., 2009.
 Tephcalostan D	*T. calophylla*	Ganapaty *et al*., 2009.

2.3.5. Rotenoids

To date, there are 23 rotenoids have been reported from *Tephrosia species*. Their names, structures, botanical origin and references are presented on Table 12. Almost all of the reported rotenoids have oxygenated at C_7 and prenylated at C_8 with the bulk of them containing hydroxylation at C_5.

Table 13. Rotenoids isolated from *Tephrosia spp*.

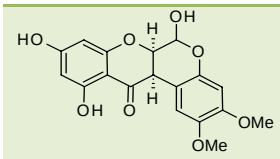
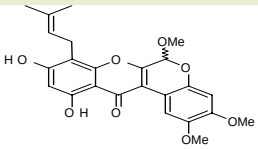
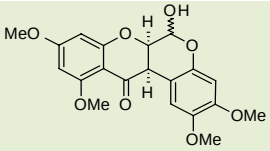
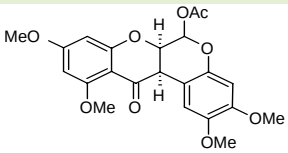
Compound	Botanical Origin	Reference
 9-Demethyldihydro-stemonal	*T. pentaphylla*	Dagne *et al.*, 1989.
 9-O-Demethyl-6-Omethyl-8-prenyl-stemonal	*T. villosa*.	Prashant and Krupadanum, 1993.
 Dihydrostemonal	*T. pentaphylla*	Dagne *et al.*, 1989.
 6-O-Acetyl-dihydrostemonal	*T. pentaphylla*	Dagne *et al.*, 1989.

Table 12. Rotenoids isolated from *Tephrosia spp*. *(Continued)*

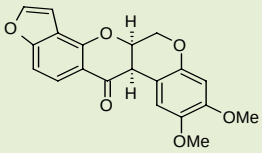
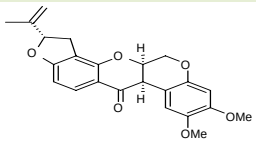
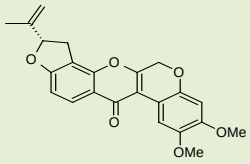
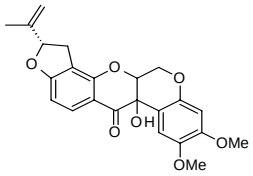
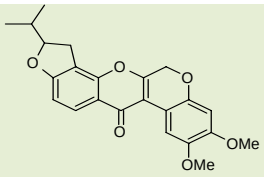
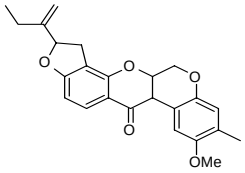
Compound	Botanical Origin	Reference
 Elliptone	*T. strigosa,* *T. vogelii,*	Kamal and Jain, 1980. Carlson *et al*., 1973.
 Rotenone	*T. vogelii* *T.fulvineris* *T. pentaphylla*	Bhandari *et al*., 1992 Crombie and Whiting, 1998.
 Dehydrorotenone	*T. falciformis* *T. virginiana*	Bose *et al*., 1976.
 *Cis*-Rotenolone	*T.vogelii*	Carlson *et al*., 1970
 1′,2′-Dihydro-dehydro-rotenone	*T. candida.*	Roy *et al*., 1987.
 Wallichin	*T. wallichi*	Bose *et al*., 1981.

Table 12. Rotenoids isolated from *Tephrosia spp*. *(Continued)*

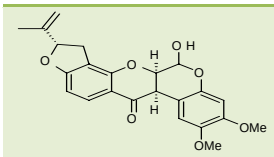
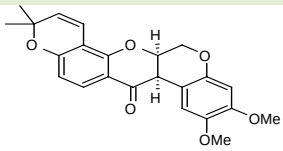
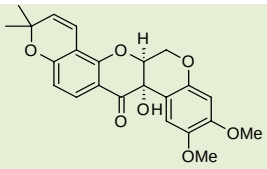
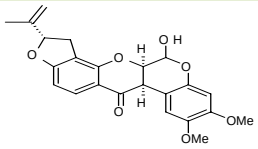
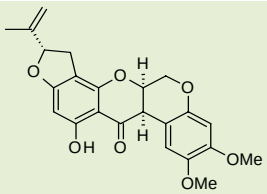
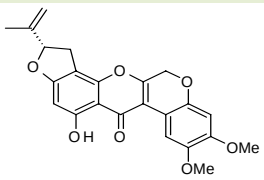
Compound	Botanical Origin	Reference
 6-Hydroxyrotenone	*T. pentaphylla*	Dagne *et al*., 1989.
 Deguelin (-) form	*T. candida* *T. vogelii*	Marston *et al*., 1984. Carlson *et al*., 1970.
 Tephrosin	*T. vogelii*	Marston *et al*., 1984.
 6-Hydroxyrotenone	*T. pentaphylla*	Dagne *et al*., 1989.
 Sumatrol	*T. toxicaria*	Crombie and Peace, 1961. Harper *et al*., 1940.
 Villosol	*T. villosa*	Sarma *et al*., 1976

Table 12. Rotenoids isolated from *Tephrosia spp*. *(Continued)*

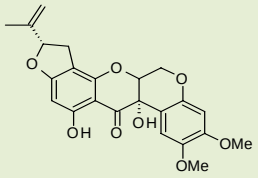
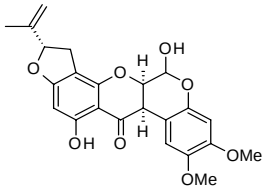
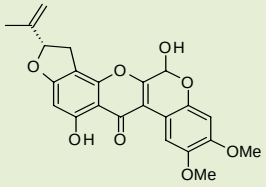
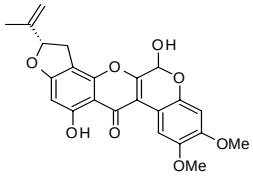
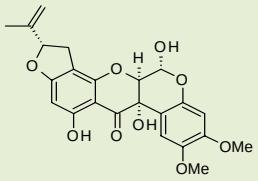
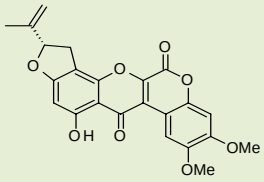
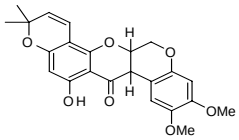
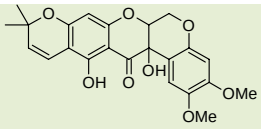
Compound	Botanical Origin	Reference
 Villosinol	*T. villosa*.	Sarma *et al*., 1976; Krupadanam *et al*., 1977.
 6-Hydroxysumatrol	*T. toxicaria*	Crombie and Peace, 1961. Harper *et al*., 1940.
 Didehydrovillosin	*T. villosa*	Prashant *et al*., 1993.
 Villinol	*T. villosa*	Krupadanam *et al*., 1977.
 Villol	*T. villosa*.	Sarma *et al*., 1976; Krupadanam *et al*., 1977.

Table 12. Rotenoids isolated from *Tephrosia spp*. *(Continued)*

Compound	Botanical Origin	Reference
 Villosone	*T. villosa*	Krupadanam *et al.*, 1977.
 α-Toxicarol	*T. toxicaria,* *T. candida*	Harper, 1939 Andrei *et al*., 1997.
 12*a*-Hydroxy-β-toxicarol	*T. candida*.	Andrei *et al*., 1997.

2.4. Miscellaneous

To date, 10 more compounds, mainly dimmers have been reported from *Tephrosia species*. Their structures, names, botanical origin and references are presented on Table 13.

Table 14. Miscellaneous of compounds from *Tephrosia spp*. *(continued)*.

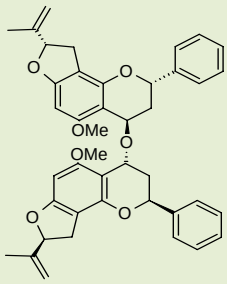
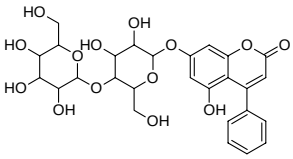
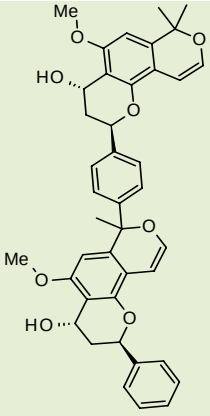
Compound	Botanical Origin	Reference
 Crassifolin	*T. crassifolia*	Gomez–Garibay *et al*., 1999.
 7-O-[β-D-Glucopyranosyl-(1›4)-β-D-glactopyrano-side]-5,7-Di-hydroxy-4-phenyl-2H-1-benzopyran-2-one	*T. purpurea*.	Saxena and Choubey, 1997.
 Hildgardtol B	*T. abbottiae* *T. hilderbrandtii, T. quercetorum*.	Gomaz–Garibay *et al*., 1986; Monache *et al*., 1986; Gomez–Garibay *et al*. 1988.

Table 13. Miscellanoius of compounds from *Tephrosia spp*. *(Continued)*

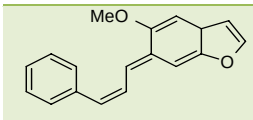
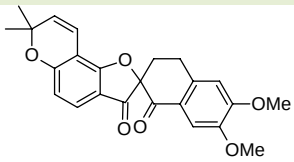
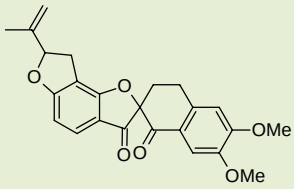
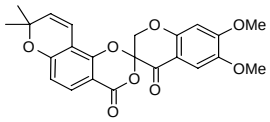
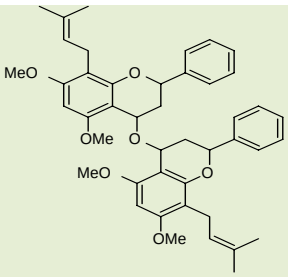
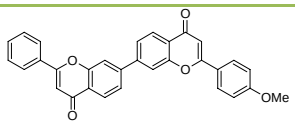
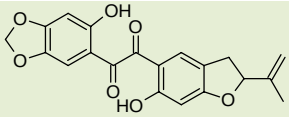
Compound	Botanical Origin	Reference
 Purpureamethide	*T. purpurea*.	Sinha *et al.*, 1982.
 Tephrospiroketone I	*T. candida*.	Andrei *et al.*, 2002.
 Tephrospiroketone II	*T. candida*.	Andrei *et al.*, 2002.
 Tephrospirolactone	*T. candida*.	Andrei *et al.*, 2002.
 Tepicanol A	*T. tepicana*.	Gomez-Garibay *et al.*, 1997.

Table 13. Miscellaneous of compounds from *Tephrosia spp*. *(Continued)*

Compound	Botanical Origin	Reference
 4'-Methoxy-7,7'-biflavone	*T. purpurea*	Vasudeva *et al.*, 2008.
 Calophione A	*T. calophylla*	Ganapaty *et al.*, 2009.

2.5. *Tephrosia apollinea* (Del.) DC.

T. apollinea (Del.) DC. which is locally known as Amayoga, has been reported to be toxic to goats (Suliman *et al.*, 1982). Interestingly, there is no record for any use of *T. apollinea* in the Sudanese traditional medicine, which makes it a vital point to justify the study of its secondary plants constituents and evaluate its bioactivity.

2.5.1. Geographical Distribution

In addition to Sudan, *T. apollinea* also found to be growing wildly in Pakistan, Iran, Saudi Arabia, Egypt, Eritrea, Somalia Republic, Socotra and Ethiopia.

T. apollinea grows wildly in the northern, central and southern parts of Sudan on the Nile bank areas.

2.5.2. Pharmacognostical aspects of *Tephrosia apollinea* (Del.) DC.

Morphologically, *T. apollinea* (Del.), DC. is a bushy plant12-18 inches high, diffusely branched from the base. Leaflets ¾ -1 ½ inches long, 5-7 (rarely 9) in number, oblanceolate to obovate, more or less pubescent above and beneath. Flowers red, in lax 6-16 flowered terminal or axillary racemes. Pod linear, 1 ½ - 1 ¾ inches long, glabrous or pubescent 6-9 seeded (Andrews, 1952). (Figure 4).

The fruit of *T. apollinea* is the distinctive character which differentiates it from the other species in being straight and open by rupturing with dispersing the seeds for spreading and the survival of kind (Al-Ghamdi and Al Zahrani, 2010).

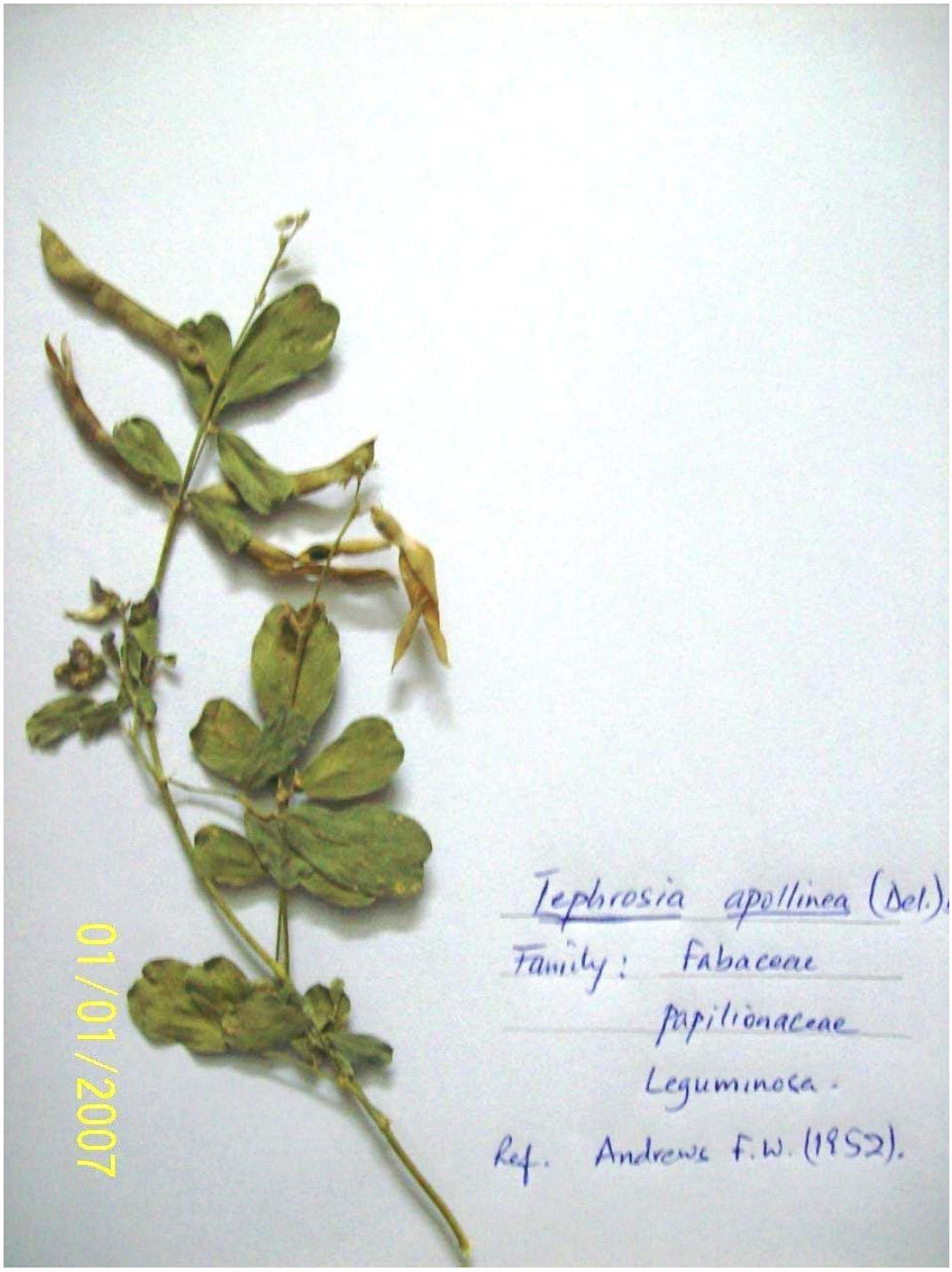


Figure 4. *Tephrosia apollinea* (Del.) DC herbrium

2.5.3. Flavonoids of *Tephrosia apollinea*

To date, the number of flavonoids reported from Tephrosia apollinea is 15 compunds. 3 of them are flavanones (Table 4) which are Tephroapollin A (p.34), Tephroapollin B (p.34) and Apollineanin (p.38) while the rest are flavones (Table 5) which are lanceolatin A (p.39), tephroapollin D (p.39), Apollinine (p.40), Tephroapollin E (p.40), tephroapollin F (p.40), tephroapollin G (p.40), glabratephrinol (p.40), glabratephrine (p.41), pseudosemiglabrinol (p.41), semiglabrin (p.41), pseudosemiglabrin (p.41) and 5-hydroxy tephroapollin F (p.42). Interstingly, there is no isoflavonoids reported yet from *T. apollinea.*

3. Methodology

3.1. Preamble

All solvents used were of reagent grade quality.

Melting points (uncorrected) were deterimined on Kofler Hot stage, Ultraviolet (UV) spectra were recorderd on Unicam SP 800A spectrometer in ethanol (EtOH). Infra-red (IR) spectra were recorded, as KCL discs on Perkin-Elmer 341 spectrometer using a Na/Hal lamp (589 nm) at Optical rotation $[\alpha]_D$ were measured on Perkin-Elmer polarmeter, Model 241.

Nuclear magnetic resonance spectra were measured on Bruker DRX 500 spectrometer, measuring frequency 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR). Samples were dissolved in deuterated chloroform and spectra were generally referenced to the solvent signal.

Mass (MS) spectra and accurate mass measurements were determined on an AEI MS 902 spectrophotometer at 70 eV using probe insertion and elevated temperature.

Petroleum ether refers to the b.p. 60-80° fraction except where otherwise stated, MeOH is methanol, EtOAc is

ethyl acetate, $CHCl_3$ is chloroform and DMSO is dimethyl sulphoxide.

3.2. Plant Material

3.2.1. Documentation of Plant Material

All the different morphological parts of *T. apollinea* were collected from Abo Hamad, in the Nahr ElNile state. Voucher specimen is deposited at Medicinal and Aromatic Plants Research Institute Herbarium.

The whole plant was allowed to dry on shade and dry air current.

3.2.2. Preparation of the powdered plant materials

The dried roots, leaves and seeds were cut, dried and separately moulded on a manual mould into smaller pieces. The collected broken pieces were grounded mechanically to a fine powder to provide a larger surface area for providing efficient extraction.

3.3. Extraction Procedure

The plant material was macerated on the next day in 200 ml of chloroform for 24 hours. The extract was decanted and the residue was allowed to dry.

The residue was macerated on the next day in 150 ml of 80% methanol for 24 hours. The extract was decanted and concentrated to remove the methanol.

The remaining aqueous residue was partitioned with ethyl acetate. The fractionation usually made 3 consecutive times.

The separated fraction of ethyl acetate was passed through anhydrous sodium sulphate and then used for TLC applications.

3.3.1. Thin Layer Chromatography (TLC)

Silica gel 60 F_{254} precoated plates (Merck, Darmstadt, Germany) were used for analytical TLC. Different solvent systems were used depending on the polarity. The following solvent systems were used as mobile phases:

- Toluene: ethyl acetate: formic acid (5:4:1).
- Petroleum ether: Chloroform (7:3).
- Ethyl acetate: chloroform (60:40).

- Petroleum ether : Ethyl acetate (6:4)

3.4. Analysis of *Tephrosia apollinea* Roots

3.4.1. Extraction and Isolation of Compounds

Ground roots (50 g) were defatted with petroleum ether and then extracted with $CHCl_3$. Examination of the chloroform extract by TLC on silica gel G using Petroleum ether: ethyl acetate (7: 3) revealed the presence of a number of blue, blue green and yellow fluorescent compounds. Concentration of the $CHCl_3$ extract yielded brown oil (3.2 g). An aliquot of this oil (2.5 g) was subjected to column chromatography over silica gel. Elution with petroleum ether: EtOAc (3:2) gave TA-1 (2.7 mg, R_f 0.43 in Petroleum ether: EtOAc (6:4) followed by a precipitate which on repeated recrystallization gave TA-2 (3.1 mg, R_f 0.60 on Silica G) using Petroleum ether: EtOAc (6:4).

3.4.2. Sample purity

The compounds isolated were developed on 3 different mobile phases and a single spot has been observed.

The TLC plate was then sprayed with vanillin sulphuric acid and placed inside the oven. One single spot has been observed on each sample.

3.4.3. Characterisation of TA-1 as (-)-Semiglabrin (1)

Needles from $CHCl_3$: MeOH, mp 253-256°, Smalberger *et al*., 1973). Found: M^+ 392.1258; $C_{23}H_{20}O_6$ requires 392.1260. UVγ_{max} nm: 249, 257, 310. IR ν_{max} cm^{-1}: 1740, 1645 (2 x C=O). ^1H NMR ($CDCl_3$): δ 1.11, 1.33 (2 x 3H, 2 x *s*, 4″-Me_2), 2.22 (3H, *s*, 5″-OAc), 4.32 (1H, *d*, *J* = 7Hz, 6″-H), 5.68 (1H, *s*, 5″-H), 6.67 (1H, *d*, *J* = 7Hz, 2″-H), 6.80 (1H, *s*, 3-H), 6.96 (1H, *d*, *J* = 8 Hz, 6-H), 7.50 – 7.63 (3H, *m*, 2′,4′,6′-H), 7.88 – 8.0 (2H, *m*, 3′,5′-H), 8.18 (1H,*d*, *J = 8Hz, 5-H).* ^{13}C NMR:.EIMS *m/z* (rel. int.): 392 $[M]^+$ (22), 332 (100), 317 (32), 303 (13), 290 (22), 289 (22), 263 (18), 251 (6), 160 (7), 149 (5), 105 (3), 102 (4), 95(3), 89 (68). The physical and spectroscopic data are in full agreement with (-)-semiglabrin isolated from the seeds of T. apollineas by Waterman and Khalid (1981).

3.4.4. Characterisation of TA-2 as (-)-Pseudo-semiglabrin (2)

Plates from EtOH, mp 181-183°, $[\alpha]^{24}$ -282 (*C*. 0.49; $CHCl_3$). Found: M^+ 392.1258; $C_{23}H_{20}O_6$ requires 392.1260. UVγ_{max} nm: 215, 248sh, 257, 309. IR ν_{max} cm^{-1}: 1760, 1630 (2 x C=O), 1600, 1450. ^1H NMR ($CDCl_3$): δ 1.16, 1.40 (2 x 3H, 2 x *s*, 4″-Me_2), 1.51 (3H, *s*, 5″-OAc), 4.63 (1H, *dd*, J_1 = 8Hz, J_2 = 7Hz, 6″-H), 5.60 (1H, *d*, J_1 = 8Hz, 5″-H), 6.53 (1H, *d*, J_2 = 7Hz, 2″-H), 6.78 (1H, *s*, 3-H), 6.96 (1H, *d*, *J* = 8 Hz, 6-H), 7.52 – 7.63 (3H, *m*,

2′,4′,6′-H), 7.80 – 7.95 (2H, *m*, 3′,5′-H), 8.19 (1H,*d*, *J = 8Hz, 5-H)*. ^{13}C NMR: ($CDCl_3$, 150 MHz) δ 177.7 (C4), 169.8 (CO-C5′′), 164.6 (C7), 162.7 (C2), 153.8 (C9), 131.8 (C4′), 131.3 (C1′), 129.1 (C3′, C5′), 128.7 (C2′, C6′), 118.3 (C10), 111.7 (C2′′), 109.0 (C6), 107.5 (C3), 84.6 (C4′′), 77.0 (C5′′), 47.9 (C6′′), 27.6, 23.2 (Me x2, C4′′), 20.3 (COCH3-C5′′). EIMS *m/z* (rel. int.): 392 $[M]^+$ (21), 333 (4), 317 (13), 303 (5), 289 (4), 263 (9), 251 (6), 133 (4), 102 (29). The physical and spectroscopic data are in full agreement with (-)-pseduosemiglabrin isolated from the seeds of T. apollineas by Waterman and Khalid (1981).

3.5. DPPH radical scavenging assay

The DPPH radical scavenging was determined according to the method of Farazana *et al.,* (2005) with some modification. In 96-wells plate, the test samples were allowed to react with 2,2-Di (4-tetraoctylphenyl)-picryl hyrdazyl stable free radical (DPPH) for half an hour at 37^{o}C. The concentration of DPPH was kept as (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiple reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group.

3.6. Iron chelating activity assay

The iron chelating ability was determined according to the modified method of Kexue *et al.,* (2006). The Fe 2+ were monitored by measuring the formation of ferrous ion-ferrozine complex. The experiment was carried out in 96 microtiter ELISA plate. The plant extract was mixed with FeSO4. The reaction was initiated by adding 5mM ferrozine. The mixture was shaken and left at room temperature for 10 minutes. The absorbance was measured at 562nm. EDTA was used as standard and DMSO as control. All tests and analysis were run in triplicate.

4. Results and Discussion

4.1. Isolation of compounds from the chloroform extract of *T. apollinea* roots.

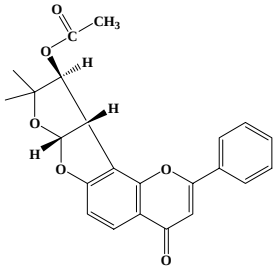
Concentration of the chloroform extract yielded brown oil. An aliquot of this oil was subjected to column chromatography over silica gel. Elution with petroleum spirit (b. p. 40- 60°C): ethyl acetate) (3: 2) gave crude TA-1 followed by TA-2. Subsequent purification by Chromatotron centrifugal reparative chromatography yielded pure TA-1 and TA-2 consecutively.

4.2. Characterization of TA-1 as (-)-Semiglabrin

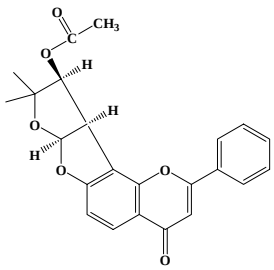
TA- 1, which was optically active, was obtained as needles from chloroform: methanol, melting at 253-256°C. Exact mass measurement established the molecular formula as $C_{23}H_{20}O_6$.

The UV spectrum showed maxima at 249, 392 and 310 nm, typical of a flavone (Mabry *et al*., 1970) and was unchanged on addition of base. The IR spectrum showed features characteristic of a flavones system with a band at 1645 cm^{-1} attributable to the γ-pyrone moiety (Wanger,

1964). The band at 1740 cm^{-1} suggested the presence of an acetyl group.



TA-1



TA-2

The ^1H NMR spectrum (Figure 6) was typical of 7-oxygentaed 8-substituted flavone showing coupled resonances (J = 8 Hz) at δ_H 6.96 and 8.18 for C_6 and C_5 protons; the paramagnetic shift of the C_5 proton being due to the deshielding effect of the *peri* situated carbonyl. Two multiplets centred at δ_H 7.50 (3H) and 7.90 (2H) were attributed to an unsubstituted B-ring. The C_3 proton appeared as a singlet at δ_H 6.80. The two high filed singlets at δ_H 1.11 and 1.33 were assigned to *gem* dimethyls adjacent to an oxygen function and the protons of the OAc group appeared as a singlet at δ_H 2.22. The doublet centred at δ_H 4.32 was assigned to the benzylic proton at $C_{6''}$ and the other doublet centred at δ_H 6.67 to $C_{2''}$ proton [deshielded by being attached to a carbon atom bearing two oxygen atoms (Pachler *et al.,* 1976)]. A *cis* configuration between H-2'' and H-6'' was assigned on the basis of the coupling constant (J = 7Hz). The

slightly broadened singlet at δ_H 5.86 is due to the $C_{5''}$ methin proton. The lack of any discernible coupling between $C_{5''}$ and $C_{6''}$ protons suggests, through application of the karplus equation (karplus, 1959), a dihedral angle of 90° between these protons thus requiring H-5'' to be equatorial also. These data are in close agreement with that previously published for semiglabrin (Smalberger *et al.*, 1973), although TA- 1 had higher melting point and lower optical rotatory value.

A ^{13}C NMR of TA- 1 was obtained [see Figure 7 (p. 82), Table 15 (p.79)] and compared with resonance data published previously for 7-hydroxyflavone (Wenkert and Gottlieb, 1977) and 7-methoxyflavone (Pelter et al., 1976). The ^{13}C resonance of TA- 2 [Figure 8 (p. 83), Table 15 (p. 79)] was obtained as plates melting at 181-183°C. TLC revealed the presence of a blue fluorescent compound with the same R_f value as TA- 1 and analysed for the same molecular formula, $C_{23}H_{20}O_6$, as TA-1. The UV, IR and EIMS spectra were in most respects identical to those of TA-1, although the OR (-384°C) was somewhat elevated (see Experimental, p.71). However significant differences were observed in the ^1H NMR resonances for the protons of the bisfurano and acetate moieties (see Table 14, p. 78 and Figure 6 and 8, p.81 and 83 respectively). The appearance of additional coupling between

$C_{5''}$ and $C_{6''}$ protons was in close agreement with the observation made by Smalberger et al. (1973).

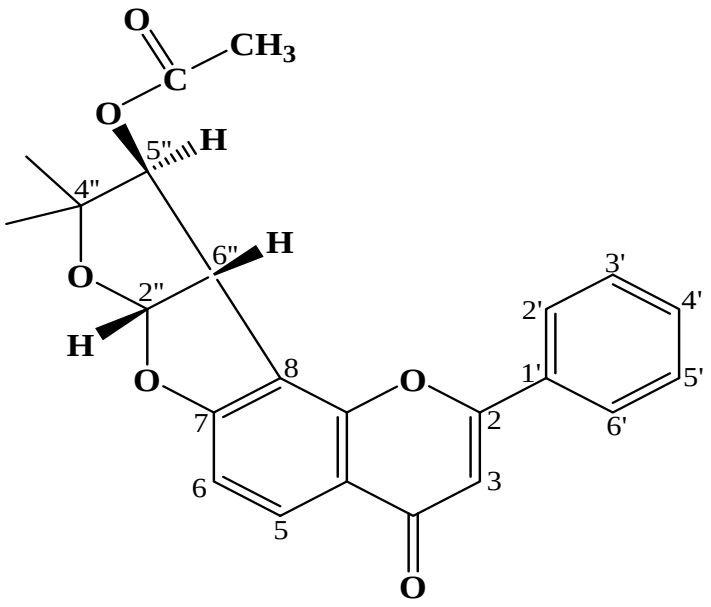


Figure 5. Numbering system of TA-1.

Table 15. Comparison of chemical shift values (δ) of the protons of TA- 1 (1) and TA- 2

	2′, 4′, 6′ – H	**3′, 5′ – H**	**3 – H**	**5 – H**	**6 – H**	**2″ – H**	**6″ – H**	**5″ – H**	**5″ – Ac**	**4″ – Me_2**
TA– 1	**7.50 - 7.63**	**7.88 - 8.00**	**6.80**	**8.18**	**6.96**	**6.67**	**4.32**	**5.68**	**2.22**	**1.11, 1.33**
J (Hz)	*m*	*m*	*s*	*d* 8	*d* 8	*d* 7	*d* 7	*s*	*s*	*s* *s*
TA– 2	**7.52 - 7.61**	**7.80 - 7.95**	**6.78**	**8.19**	**6.96**	**6.53**	**4.63**	**5.60**	**1.51**	**1.16, 1.40**
J (Hz)	*m*	*m*	*s*	*d* 8	*d* 8	*d* 7	*dd* 7/ 8	*d* 8	*s*	**s** **s**

Table 16. Comparison of chemical shift values (δ) of the Carbons of TA- 1 (1) and TA- 2

	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	$C_{1'}$ $C_{4'}$	$C_{2'}$ $C_{6'}$	C_3 $C_{5''}$	$C_{2''}$	$C_{3''}$	$C_{4''}$	$C_{5''}$	$C_{4''}$-Me_2	OAc
TA-1	162.98	107.88	177.57	129.00	109.16	163.83	112.50	153.37	118.86	131.67 131.78	126.48 126.48	129.17 129.17	112.50	52.95	87.96	76.84-80.29	27.60 23.26	20.95
TA-2	162.79	107.77	177.69	128.85	109.07	164.68	111.58	153.95	118.55	131.50 131.89	126.48 126.33	129.17 129.28	111.86	48.08	84.76	76.84-77.48	27.74 23.35	20.46

TA-2 must therefore be the corresponding diastereoisomer of TA- 1 (1, p.75) and can be assigned the structure (2) and the trivial name of the pseudosemiglabrin. The highly shielded resonance position of the acetate substituent of TA-2 (δ, 1.51) is comparable to that observed in TA- 2 (Figure 8, p.83) and contrasts markedly with the more typical value of δ 2.22 observed in semiglabrin (Figure 6, p.81). This shielding is considered to be caused by spatial interaction of the acetate with the heterocyclic ring system, as in TA- 2 (p. 83).

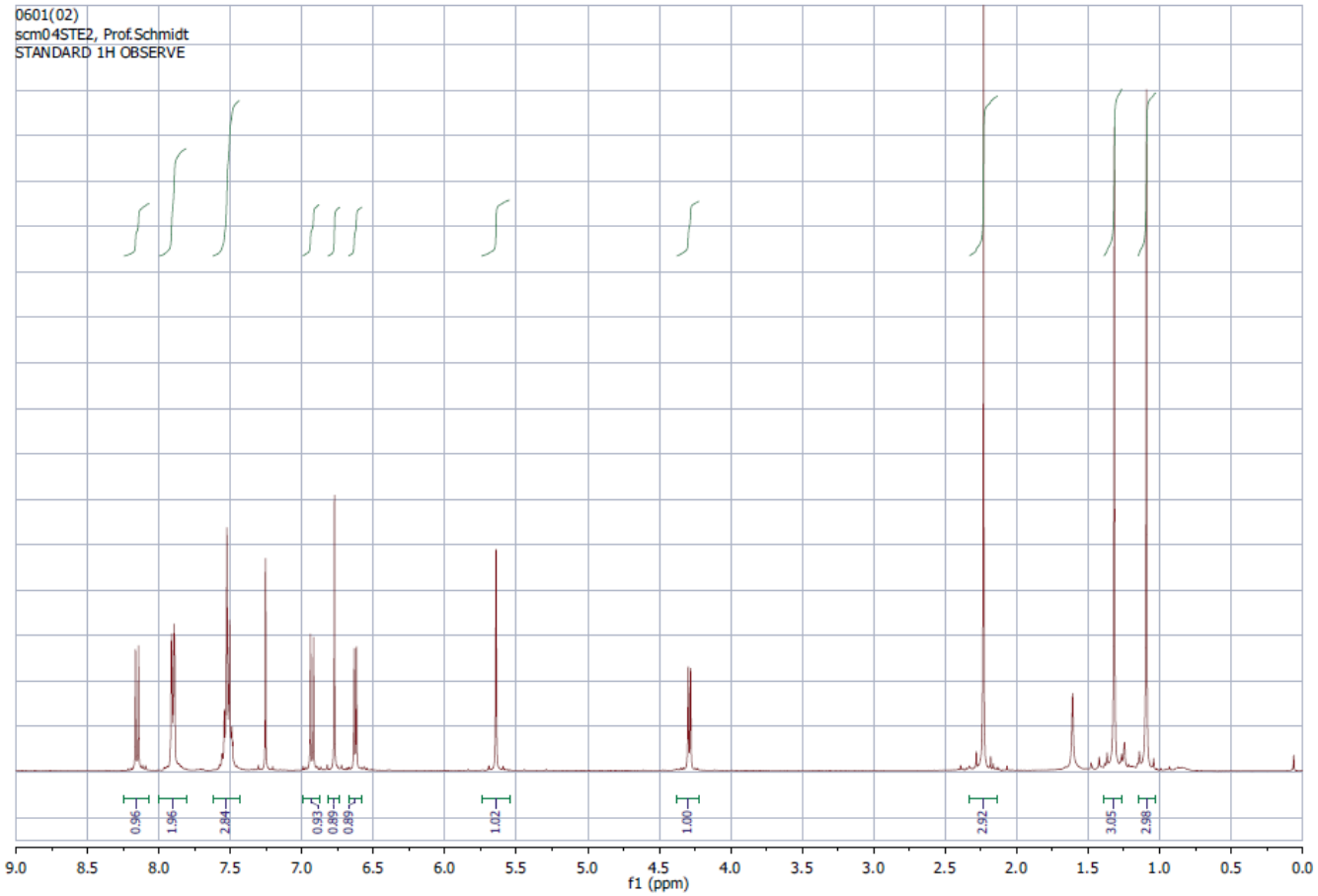


Figure 6. ^{1}H NMR Spectrum of TA-1

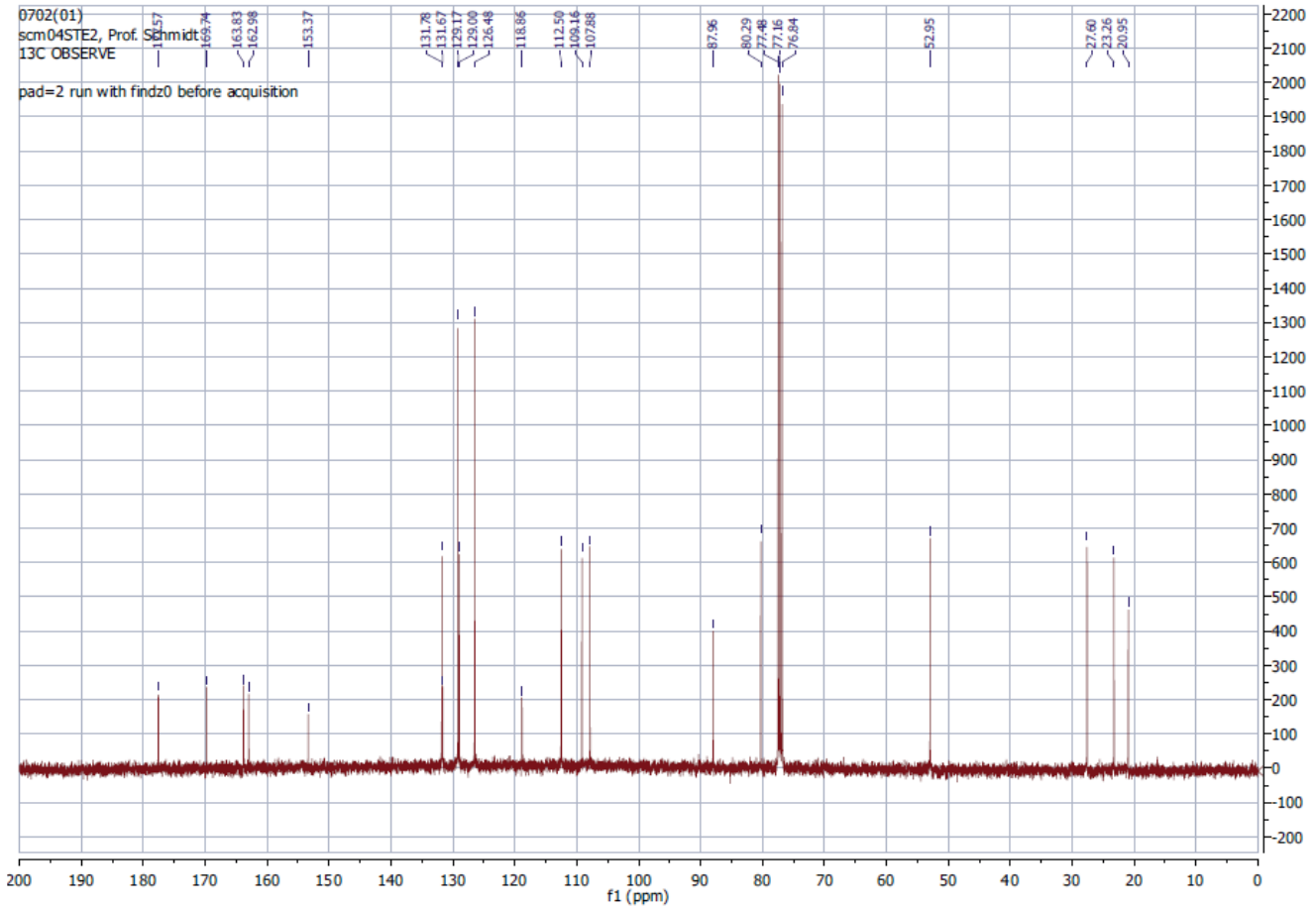


Figure 7. ^{13}C NMR spectrum of TA-1

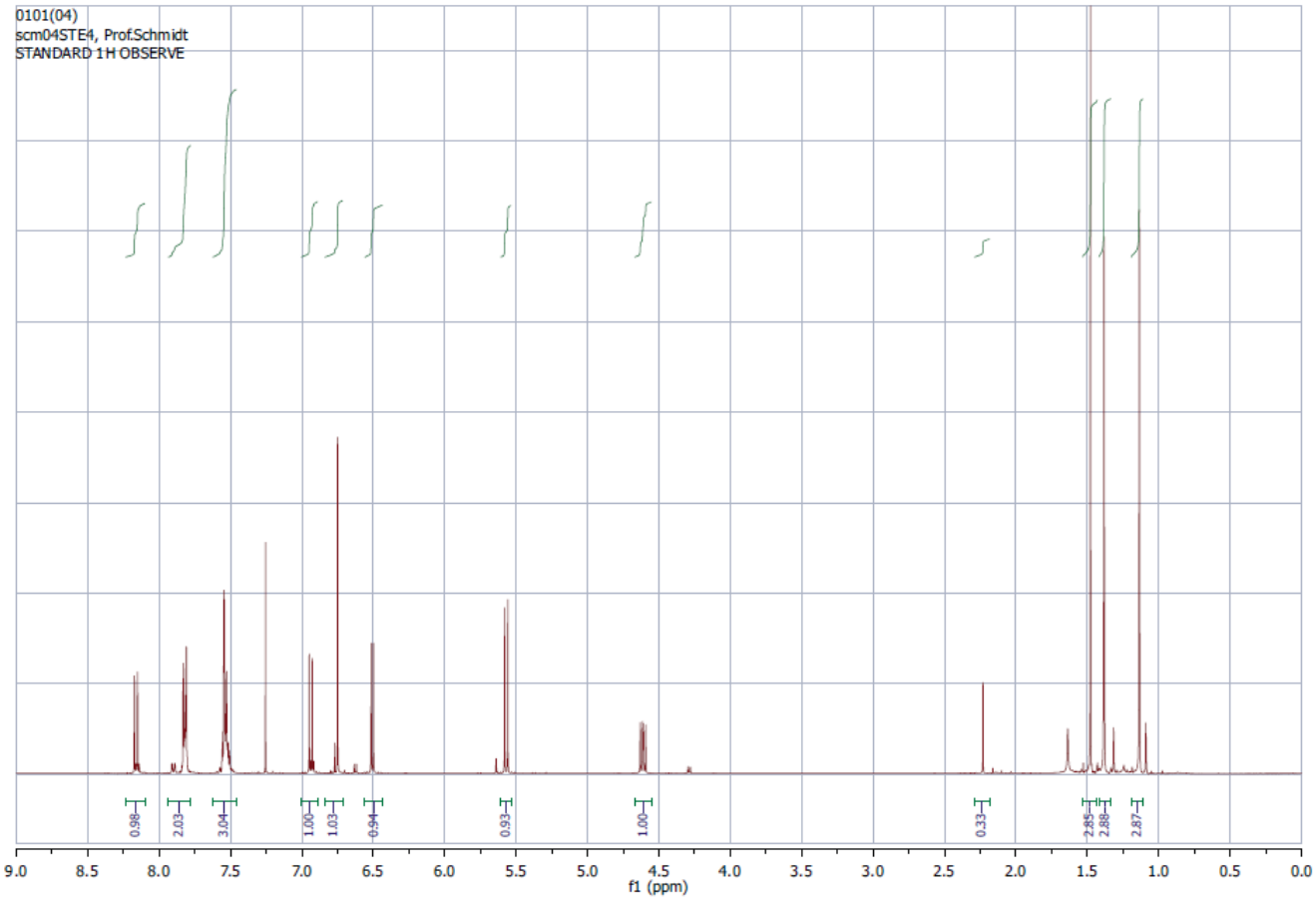


Figure 8. ^{1}H NMR spectrum of TA-2

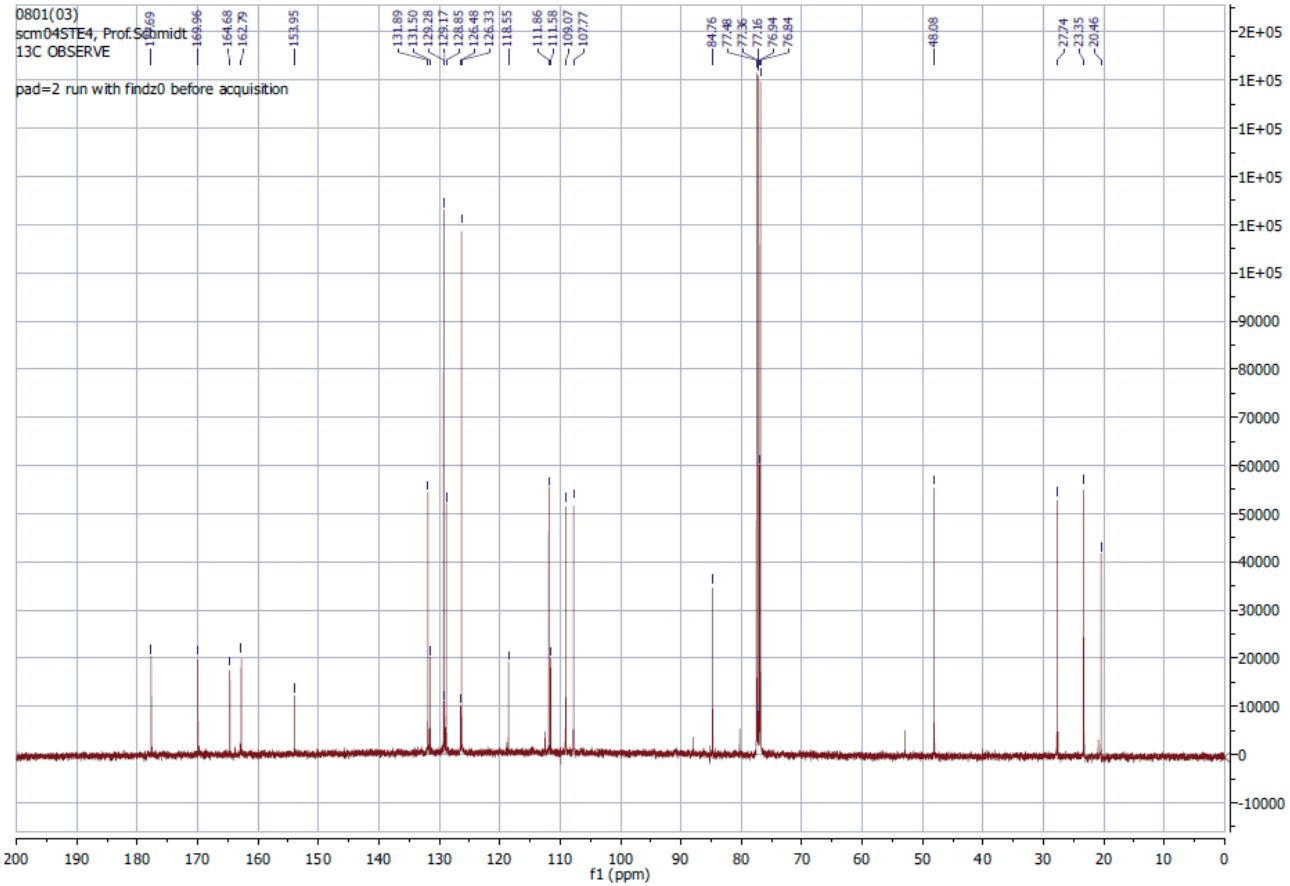


Figure 9. ^{13}C NMR spectrum of TA-2

On overlapping both ^{1}H NMR over each other, as seen on Figure 10 (p.86), the pattern of the signals in both spectra is similar. In which, both spectra shows H-5 at δ_H 8.2, the B-ring protons, H-2′ and H-6′ are at δ_H 7.8 with integral intensity of 2 protons and the H-3′, H-4′ and H-5′ are appeared on δ_H 7.5 with an integral intensity of 3 protons.

The *ortho*-coupling of H-5 and H-6 are also similar in both spectra, as well as the presence of the singlet peak at δ_H 6.8 which stands for the H-3, is the same.

Moreover, protons of the 2 methyl groups attached to $C_{4''}$ showed similar signals at δ_H 1.2 and 1.4 respectively. Each of them has an integral intensity of 3 protons.

The difference is mainly on the H-6′′, H-5′′, H-2′′ and on the signal of the protons of the acetyl group.

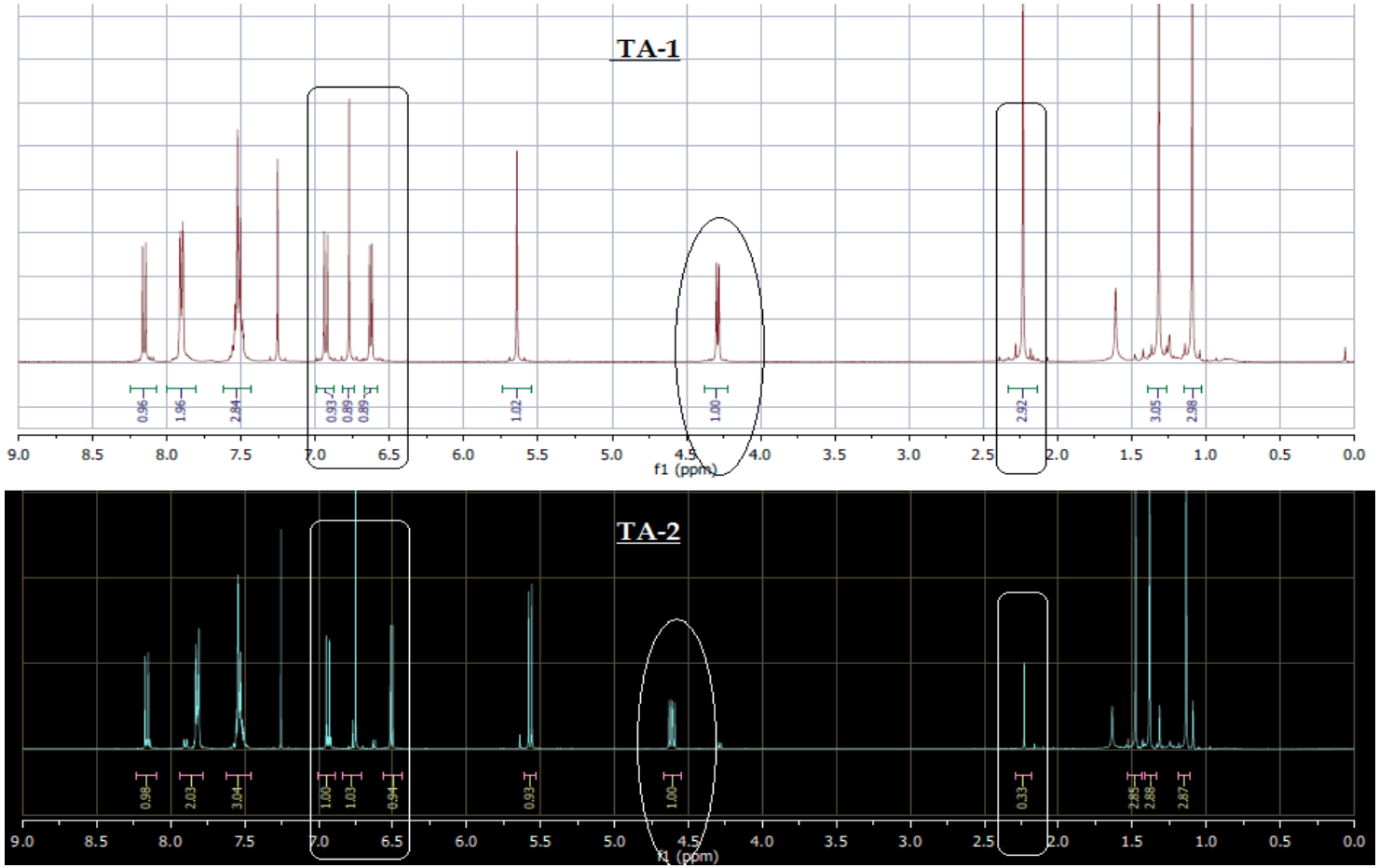


Figure 10. Comparison between the TA-1 and TA-2 ^{1}H NMR spectra

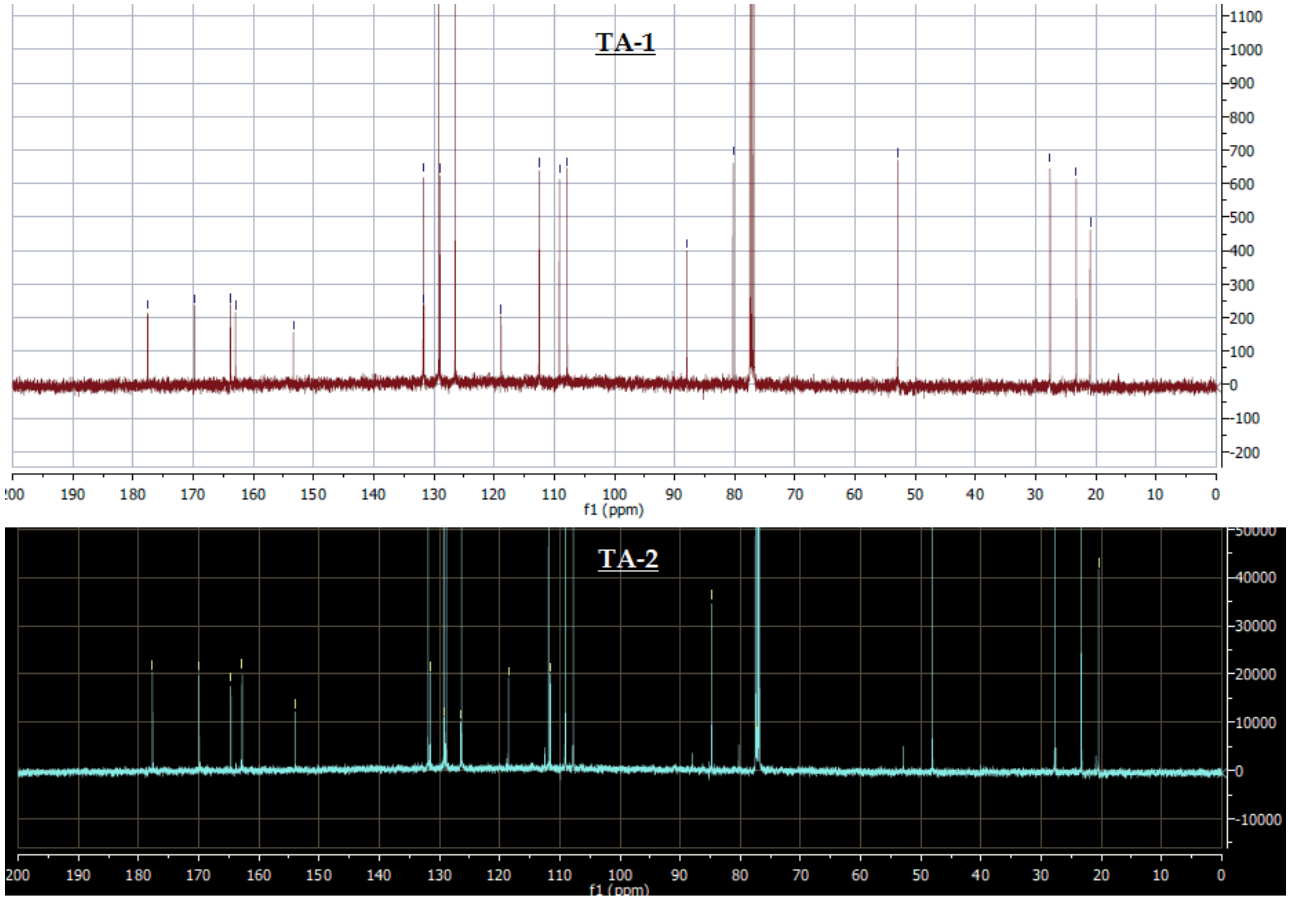
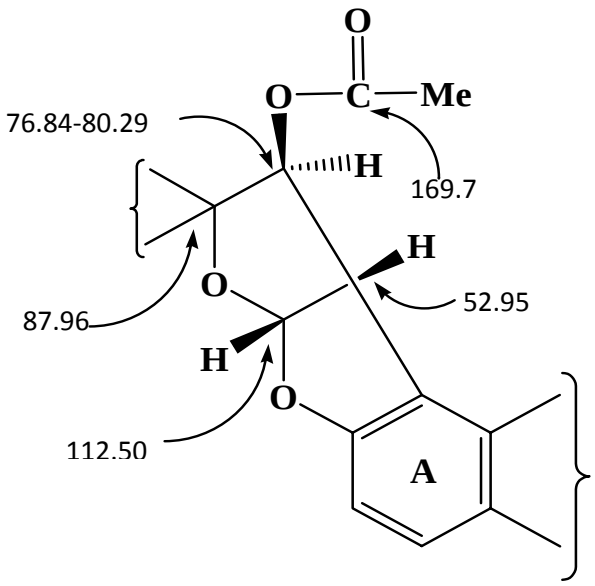
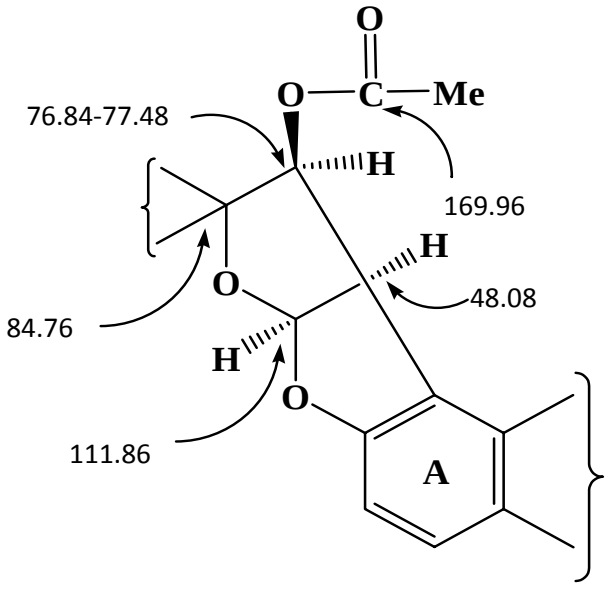


Figure 11. Comparison between the TA-1 and TA-2 13 C NMR spectrum

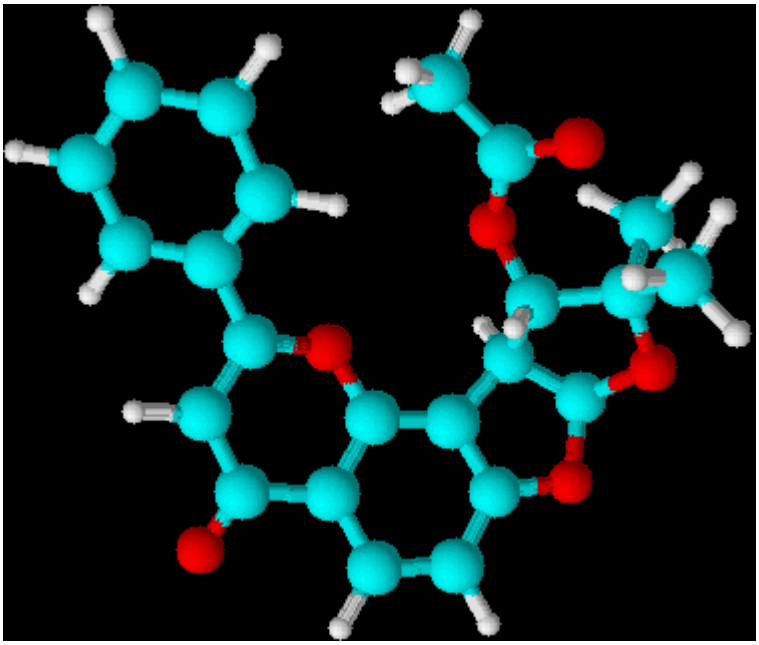


TA-1



TA-2

Figure 12. Comparison of 13C NMR spectra of TA-1 and TA-2



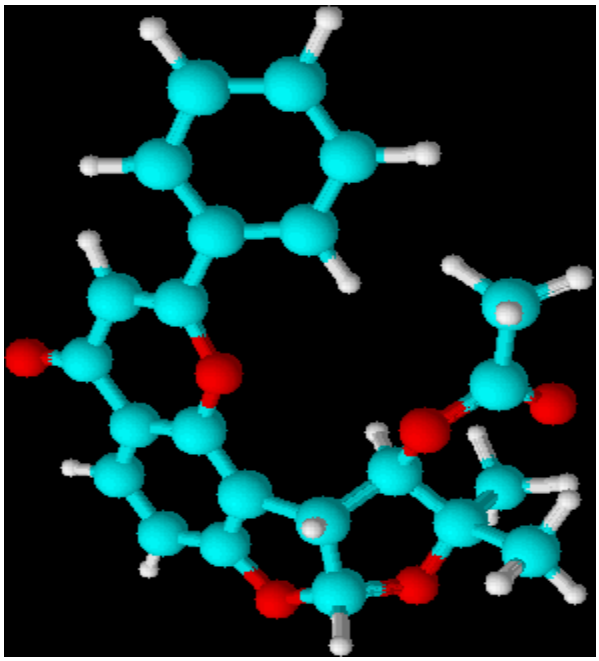


Figure 13. 3D-structure of TA-1

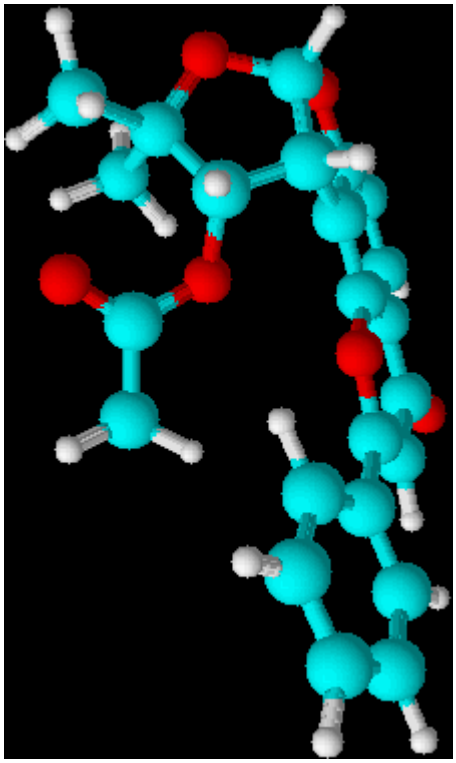


Figure 14. 3D-structure of TA-2

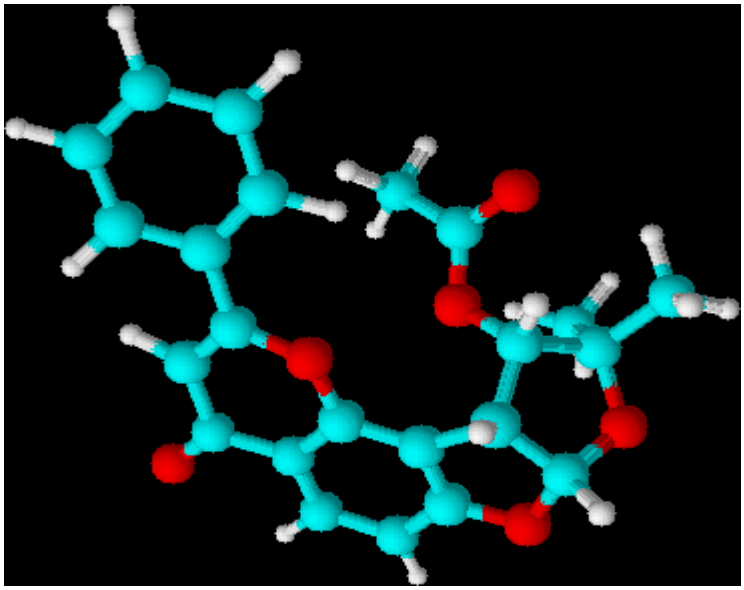


Figure 15. 3D-structure of TA-2 with emphasis on the configuration of the acetoxy group

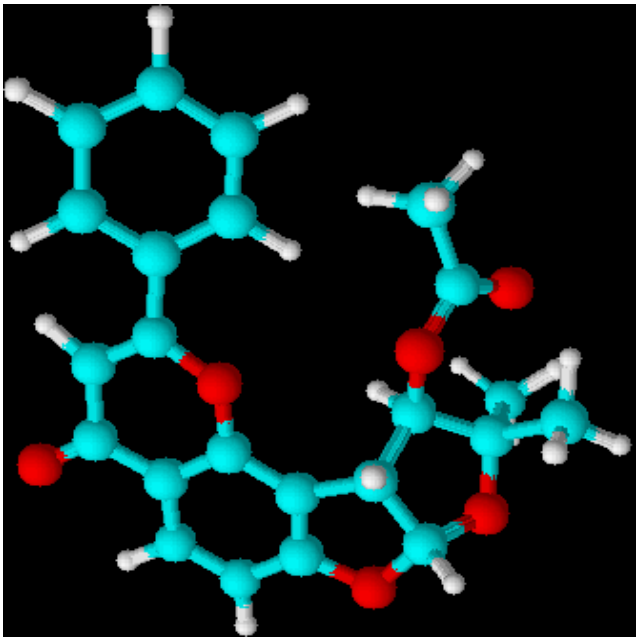


Figure 16. 3D- structure of TA-1 with emphasis on the configuration of the acetoxy group.

4.3. Results of Antioxidant activity:

4.3.1. DPPH results.

Chloroform extract of *T. apollinea* seeds has shown DPPH free radical scavenging activity (RSA) with 32% RSA, while the chloroform extract of the leaves and roots showed 19 and 6% RSA (weak antioxidant activity) respectively. These results (Table 17) were calculated as (RSA%±SD standard deviation of the mean) and carried out in triplicate.

Table 17. The relative scavenging activity for the chloroform extracts of *T. apollinea* seeds, Leaves and roots.

Sample Code	**RSA% ± SD (Conc. 500µg/ml)**
Seeds	32 ± 0.04
Leaves	6 ± 0.07
Roots	19 ± 0.05

4.3.2. Iron chelating assay

All of the chloroform extracts of the seeds, roots and leaves of *T. apollinea*, exhibit no chelating activity towards ferrous iron ferrozine complex (Table 18). The results were calculated as radical scavenging activity (RSA±SD standard deviation of the mean), and carried out in the triplicate.

Table 18.The relative scavenging activity for Iron chelation anti-oxidant assay for chloroform extract of *T. apollinea* seeds, leaves and roots.

Sample Code	RSA% ± SD (Conc. 500µg/ml)
Seeds	(14 ± 0.04)
Leaves	(29 ± 0.05)
Roots	(51 ± 0.06)

The very low antioxidant activity observed of the three extracts corresponds very well with chemical structure of the flavonoids reported from either the seeds or the roots of *T. apollineae*. Hence all the isolated compounds totally lack the presence of hydroxyl groups which are usually associated with the antioxidant activity of the flavonoids.

Due to unavailability of sufficient material of either TA-1 or TA-2 the antioxidant activity of these compounds was not conducted. Based on their chemical structure and the above aregument, however, they are not anticipated to display antioxidant activity.

It is controversial, however, that the platelet aggregation inhibitory of semiglabrin and pseudosemiglabrin reported by Jonathan *et al.,* (1990) have been later attributed by Tapas *et al*., (2008) to their antithrombogenic activity which is due to the fact that the activated platelets adhering to vascular endothelium generate lipid peroxides and oxygen free radicals which inhibit the endothelial formation of prostacyclin and nitrous oxide.

4.4. The Chemotaxonomic Significance of *Tephrosia Flavonoids*

The flavonoids generally have a considerable potential as taxonomic and phylogenetic markers in plant classification (Harborne, 1975; Swain, 1975). The systematic significance of flavonoids and isoflavonoids in the Fabaceae has been previously discussed by Gomes *et al*. (1981). In this section the significance of flavonoids and isoflavonoids within the genus *Tephros*ia is discussed.

To data flavonoids have been studied in only a few taxa of *Tephrosia* (49 out of about 300 species). However, it is already apparent that C–prenylation seems to have a high taxonomic predictability. The species of *Tephrosia* producing flavonoids can be classified broadly into three groups:

A. Those producing flavonoids only (53%).
B. Those producing both flavonoids and isoflavonoids (22%).
C. Those producing only isoflavonoids (25%).

The distribution of these skeletal types among the species of *Tephrosia* is presented in Tables 2-13 (pp. 27-62).

Furthermore it appears possible to recognise three series of *C*–prenylation among *Tephrosia* species, the first two each being confined to a single taxon:

The flavonoids of *Tephrosia* are characterised by an unsubstituted B–ring and are oxygenated at C_5 and C_7 or at C_7 only. So far the two oxygenation patterns appear mutually exclusive for species within the genus. From Tables (2-7) and compounds isolated from *T. apollinea* by other workers (Smalberger *et al.,* 1973; Waterman and Khalid, 1980; Hisham *et al.,* 2006; Abdel El-razek *et al.*, 2007; Khalafallah *et al.*, 2009) and during this study it is obvious that in many cases the C–8–prenyl substituent has undergone a series of oxidative transformation which appears to be typical of *Tephrosia*. This taxon seems to possess a unique ability to oxidise 7–methoxyl substituents to a $-O=CH_2$ group in the same manner that *Tephrosia* and other closely related genera oxidise the 2′–OMe group of isoflavones to give rise to rotenoids.

It is clear from the suggested outline (Figure 17) that the modifications occurring in C–8–prenyl flavonoids where the C–7 methoxyl is present is a parallel to rotenoid formation. Based on the biogenetic pathway proposed for the development of this type of flavonoids in *Tephrosia* (Figure 16, p. 91) the development of this group can be envisaged as involving the progressive transformation of a C–8–isoprenyl side chain from the simple form, like lanceolatin–A (Table 5, p. 39) of *T. lanceolata*, *via* a more complex system, such as that

encountered in *T. polystachyoides* e.g. polystachin (Table 5, p.43), to the highly modified flavonoids of *T. semiglabra*, *T. multijuga* (Table 5, p. 44-45) , *T. apollinea* (p.41) and *T. purpurea* (Pelter *et al*., 1981).

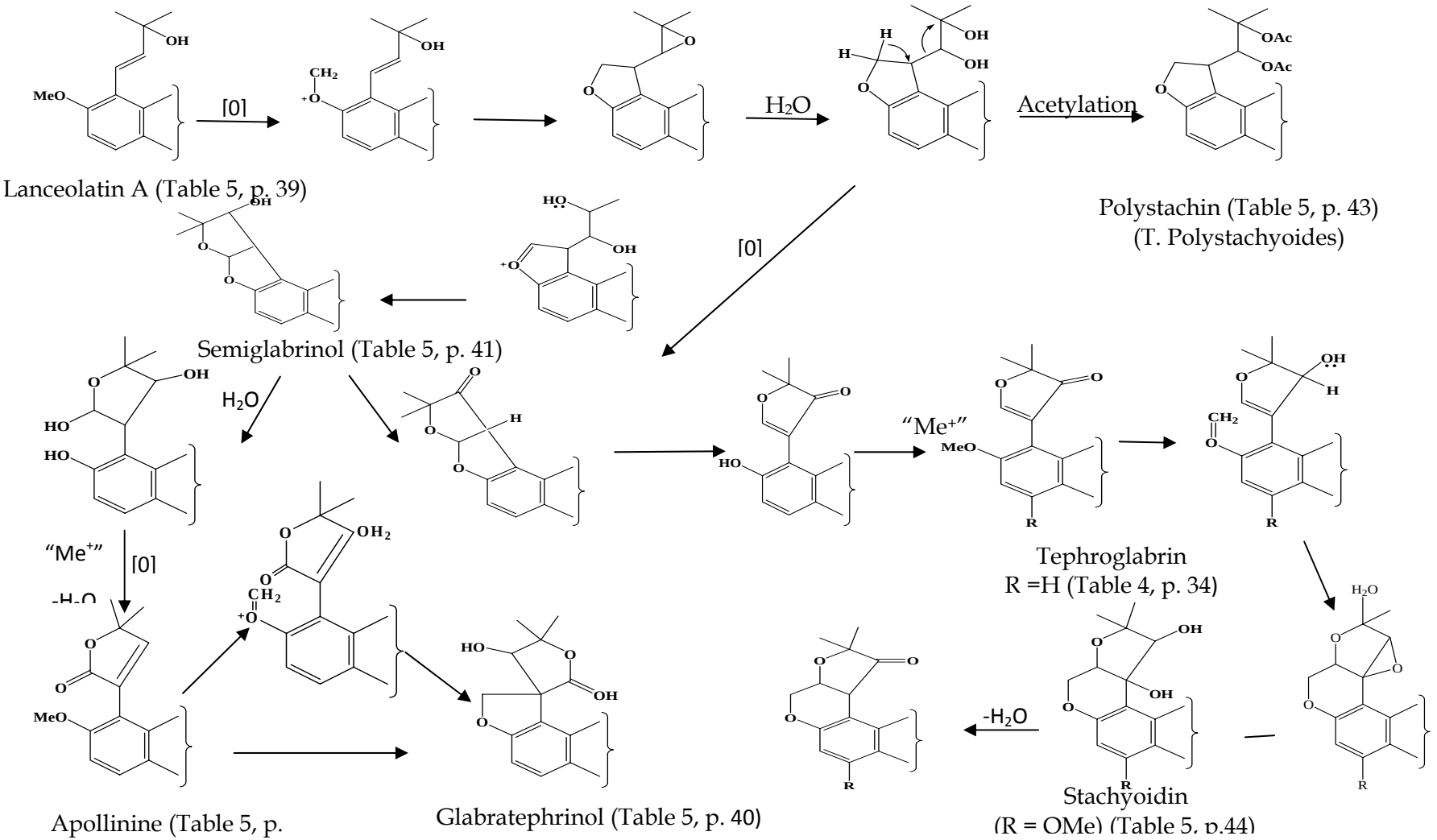


Figure 17. Postulated mechanism for *Tephrosia* flavonoids formation

It is interesting to speculate upon the presence of an enzyme system regulating both processes but to date only the Indian populations of *T. purpurea* have been reported to produce both rotenoids. However, it should be mentioned that there is some doubt regarding the homogeneity of the white and purple flowered forms of *T. purpurea* in India; some authorities (Gupta and Paul, 1978) considering it likely that there is confusion with *T. villosa.* If this is the case then it remains plausible that, to date, no overlap has been noted between the C-7/8 complex modification and rotenoid formation. This suggests that the kinetics of the enzyme in question may be regulated by the presence or absence of isoflavonoids with C-2′-OMe substituents and will only act at C-7 in the absence of isoflavonoids. This further suggests that either the formation of rotenoid or *Tephrosia* 'complex' flavonoids could have arisen secondarily without any further genetic development.

Some other *Tephrosia* species produce simple angular furano and/or 2, 2-dimethylpyrano flavonoids. Examples of such isoprenoidal flavonoids are very few in the plant kingdom and they seem to be largely restricted to the Fabaceae and more particularly to the tribe Tephrosieae. However, their prenylated counterparts among xanthones and coumarins are very numerous.

Although Gomes *et al.* (1981) have not accepted any evolutionary connection between the flavonoids and isoflavonoids Table 2-13 (p.28-63) shows quite clearly that expression of the latter line gave rise to suppression of the former. Furthermore *Tephrosia* provides indirect evidence for their evolutionary connection as when *Tephrosia* species are incapable of producing isoflavonoids (due to the lack of C-4'-oxygenation of the B-ring) they appear to utilise enzymic resources already developed to modify substituents at C_7 and C_8 in the A-ring (C-7-OMe and C-8-prenyl). Thus, *Tephrosia* species, by making use of the genetically controlled abilities inherited from rotenoid forming ancestors can redirect the interaction between enzyme and substrate to bring about observed modifications in the A-ring.

If the phylogenetic scenario given above was followed by *Tephrosia* one can conclude that "complex" *Tephrosia* flavonoids have developed secondarily after evolution of *Tephrosia* unsubstituted B-rings flavonoids had blocked isoflavonoid formation. This implies that *T. apollinea*, *T. purpurea*, *T. polystachyoides*, *T. multijuga* and *T. semiglabra*, represent an advanced group within the genus.

From the above it is possible to construct a tentative phylogeny for the development of *Tephrosia* which is governed by the following :-

(i) *Tephrosia* originated in Africa [most of the species occur in Africa (Geesink, 1981)] and has radiated from there across to Asia.

(ii) *Tephrosia* arises from an evolutionary line where the elaboration of rotenoids had already developed, possibly as feeding deterrents to reduce insect and perhaps fish predation on seeds and roots.

Today we are faced with a contradictory state of affairs in which African species of *Tephrosia* seem largely devoid of rotenoids whereas these occur widely among Asiatic species. Consideration of the above facts in conjunction with Table 12 (p.56) suggests that rotenoid formation in *Tephrosia* originated in Africa and rotenoid containing *Tephrosia* species radiated into the Indian sub-continent, possibly by way of Madagascar (cf *Mundulea*). In Africa however rotenoid formation seems to have been widely supplanted by the 'complex' flavonoids of *T. appollinea* etc. possibly due to a build up of rotenoid resistant insect predators after long exposure to these compounds. It is unfortunate in this respect that, as yet, we know nothing regarding the anti-feedant properties of these 'complex' flavonoids.

5. Conclusion

Two **diastereoisomeric** prenylated flavonoids were isolated from the roots of *Tephrosia apollinea* (Delile), and their structure have been fully elucidated by ^{1}H and ^{13}C NMR besides other spectroscopic techniques as (-)-Semiglabrin and (-)-Pseudosemiglabrin

The chemotaxonomical significance of the isolated compounds is considered in relation to what have been already published and a postulated mechanism for the formation of *Tephrosia* flavonoids is suggested.

The antioxidant activity of the crude extract of the different morphological parts was assessed and the low antioxidant activity was correlated with the chemical structure of the flavonoids occurring in this taxon.

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