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A Review on Chemical Constituents of Acacia Tortilis (Leguminosae)

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ABSTRACT
Acacia tortilis is one of the important species of genus *Acacia* belonging to family Leguminaceae. Though there is no more study performed on this plant but it plays important role in the countries where it found. These countries includes North Africa, Arabian Peninsula and Asian countries. The various part of *Acacia tortilis* plant say leaves, pods, gum exudates and bark was used as antidiabetic, antidiarrhoeal, antiasthmatic and also had several other medicinal benefits. In this review article the main focus is on the chemical composition of *Acacia tortilis.* A number of secondary metabolites have been reported including fatty acids, amino acids, terpenes, hydrolyzable tannins, flavonoids. The most evident and best known are polysaccharides (gums).
KEYWORDS: Acacia tortilis, , flavonoids, terpenoids, tannins, amino and fatty acids.

I. INTRODUCTION

The genus Acacia comprising over 1200 species, found in the warmer drier parts of the World, chiefly in Arabia countries, Australia, Peninsula and Africa.[1] In India, there are about 22 indigenous species, distributed throughout the plains. Some of ***Acacia*** are of considerable value for reafforestation and reclamation of wasteland. They are the good source for tannin, gum and timber[2]. ***Acacia tortilis*** wild. **(Syn: A. Raddiana Savi)** was found to be a very useful source of protein[3]. The acid digest of cell wall constituents fibers and cellulose found in the leaves provide nutrients for the animals as fodders[4]. It is also used for the relaxation of smooth muscle[5]. Traditionally acacia is used as anthelmintic, antidiarrhoeal, anti asthmaticand in pulmonary diseases[6]. The generic name 'Acacia' derived from the Greek word 'akis', meaning a point or a barb. The name 'tortilis' means twisted and refers to the pod structure. It is also known as umbrella thorn (Africa); haaken- steekdoring (South Africa); Israeli babool (India), samor (Egypt and Sudan); acacia de copa plana, espino de parasol (Spanish); acacia faux gommier (French); acacia ad ombrello (Italian); qurac (Somali); Mgunga Mwavuli (Swahili); שיטת הסוכך (Hebrew); سمر [Persian], and in Arabic it is commonly known as sunut, samar, sammar, samra, sayyal, seyal, seyyal[7].

II. BOTANICAL DESCRIPTION

A. tortillis tree that may reach 20 in height with an umbrella-shaped and flat top canopy. Stem and branches are dark brown in the mature and reddish brown with grey lenticels in the young. Leaves are smooth to densely pubescent, 1-7 cm long, with 2-14 pinnae each with 6-22 pairs of leaflets. Flowers white or pale yellowish-white, fragrant, in round heads, solitary or in fascicles. Bark is grey-brown-black, rough and fissured. The spines are in pairs, some short and hooked up to 5 mm long, mixed with long straight slender spines up to 10 cm long. a contorted or spirally twisted pod, yellow brown, 5-15 cm long, with longitudinal veins and slightly constricted between the seeds. There are 5-18 seeds/pod. Semi-dehiscent, i.e. the ripe pods open but remain on the tree without releasing the seed[8].

III. SECONDARY METABOLITES OF ACACIA TORTILIS (FORSSK.) HAYNE SSP. RADDIANA

A. tortillis have been reported to contain proximate and minerals, amino acid, fatty acids from seed oils, gum (polysaccharide from gum), terpenes, hydrolyzable tannins and flavonoids.

IV. PROXIMATE AND MINERALS

Dry mature seeds of *Acacia tortilis* (Forssk.) Hayne ssp. raddiana were collected from different areas of Egypt. Whole seeds from each area were separately ground using an electric mill then used for chemical composition. Proximate composition of the *Acacia* seeds was determined by using the standard Association of Official Analytical Chemists. Moisture content was evaluated by the loss of weight upon drying in an oven at

100 °C to a constant weight. Ash was assessed by incineration at 550 °C of known weights of the samples in a muffle furnace. Crude fat was found out by exhaustively extracting a known weight of sample in petroleum ether (60–80 °C) in a Soxhlet extractor. Protein amount (N × 6.25) was measured by the Kjeldahl method (Method No. 978.04). Crude fiber quantity was ascertained after digesting a known weight of fat-free sample in refluxing 1.25% sulfuric acid and 1.25% sodium hydroxide (Method No. 930.10). Carbohydrates were calculated by difference. The result presented in Table-1 shows the Proximate composition of *A. tortilis* seeds[9].

Sample of Acacia *tortilis* seeds were digested with concentrated nitric acid and perchloric acid (4:1, v/v) and heated to 70–90 °C for 10 min and cooled before injection. Minerals including iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) were estimated in the digested *Acacia* samples, using an Atomic Absorption spectrophotometer. Potassium (K) and sodium (Na) contents of the digests were determined calorimetrically using Flame photometer model. Phosphorus (P) content was measured by using the phosphomolybdovanate method. Calcium (Ca) and magnesium (Mg) were assessed by using the titration method with a 0.02 M EDTA solution. Table-1 shows the minerals composition of *A. tortilis* seeds[9].

Table-1
Proximate and minerals composition of *Acacia tortilis* seeds (dry weight).

Component	value
Proximate composition	
Moisture (%)	5.30 ± 0.65
Ash (%)	3.99 ± 0.32
Fat (%)	9.19 ± 0.58
Crude fiber (%)	14.31 ± 0.57
Protein (%)	27.21 ± 1.24
Carbohydrates (%)	45.30 ± 1.74
Minerals	
Ca (mg/100 g)	70.5 ± 1.29
P (mg/100 g)	67.8 ± 5.89
K (mg/100 g)	448 ± 12.5
Mg (mg/100 g)	56.5 ± 3.22
Na (mg/100 g)	48.0 ± 4.47
Mn (mg/100 g)	3.89 ± 0.27
Cu (mg/100 g)	0.74 ± 0.09
Zn (mg/100 g)	3.76 ± 0.47
Fe (mg/100 g)	6.79 ± 0.54

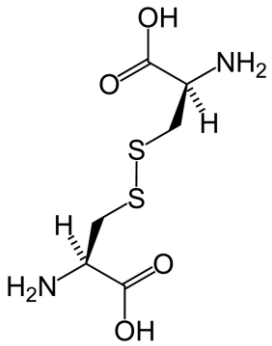
V. AMINO ACIDS

Dry mature seeds of *Acacia tortilis* were collected from Egypt. Amino acid composition was analyzed using High-Performance Amino Acid Analyzer (Biochrom 20, Auto sampler version, Amersham Pharmacia Biotech., Sweden). The sample (100 mg) was hydrolyzed with 5 ml of 6 M HCl in a sealed tube at 110 °C in an oven for 24 h. The hydrolyzed sample was re-dissolved in Na citrate buffer (pH 2.2) and filtered using a 0.2 lm membrane filter then injected into the amino acid analyzer. The contents of the various recovered amino acids were presented as grams per 100 g of protein. The amino acid profile and essential amino acid score *A. tortilis* seeds are listed in Table-2[9].

Table-2
Amino acid composition and essential amino acid score of *Acacia tortilis* seed.

Amino acid	value
Essential	
Cystine **(1)**	1.64 ± 0.26
Valine **(2)**	3.49 ± 0.55
Isoleucine **(3)**	2.25 ± 0.63
Leucine **(4)**	10.4 ± 1.36
Tyrosine **(5)**	2.78 ± 0.33
Phenylalanine **(6)**	3.12 ± 0.39
Histidine **(7)**	3.41 ± 0.14
Lysine **(8)**	3.32 ± 0.56
Threonine **(9)**	2.76 ± 0.16

Nonessential	
Aspartic acid **(10)**	3.01 ± 0.21
Proline **(11)**	1.14 ± 0.17
Serine **(12)**	3.03 ± 0.24
Glutamic acid **(13)**	3.29 ± 0.54
Glycine **(14)**	2.04 ± 0.20
Alanine **(15)**	1.65 ± 0.27
Arginine **(16)**	4.22 ± 0.39



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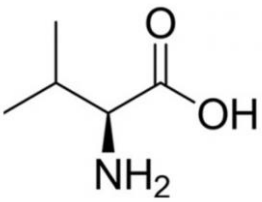

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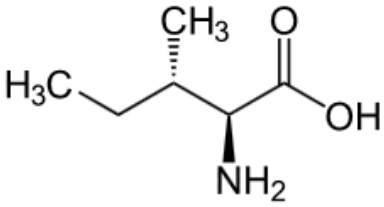

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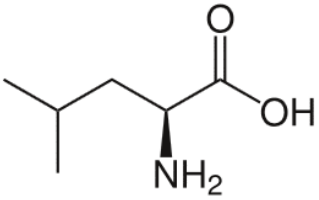

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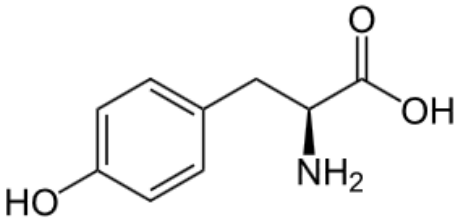

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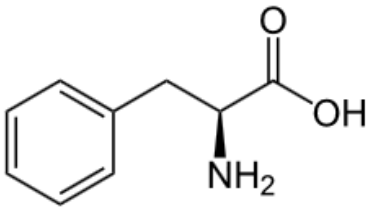

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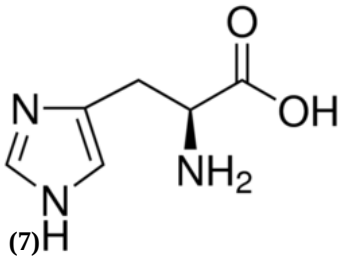

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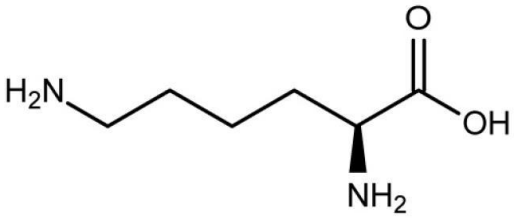

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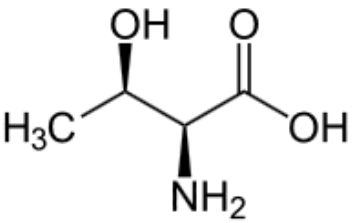

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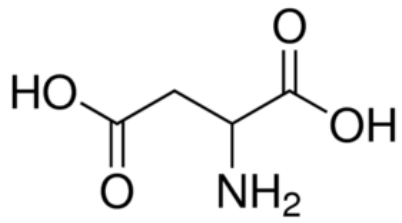

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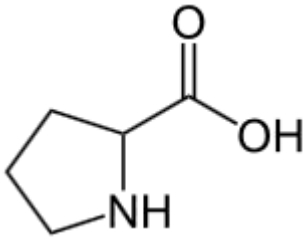

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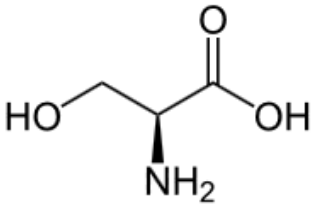

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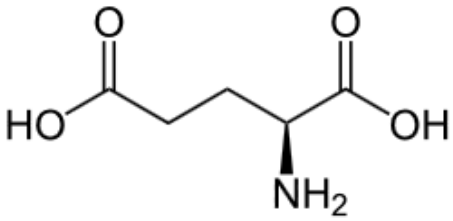

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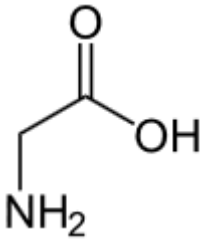

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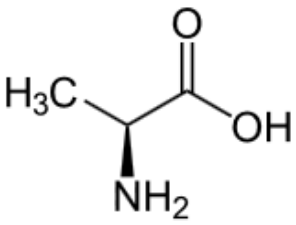

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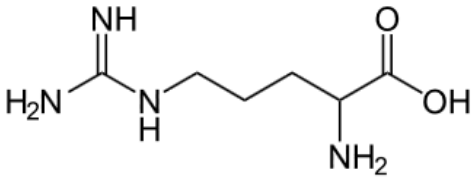

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VI. FATTY ACIDS FROM SEED OILS

Dry mature seeds of *Acacia tortilis* (Forssk.) Hayne ssp. raddiana were collected from Egypt. Fatty acid analysis was performed using a Hewlett Packard Gas Chromatograph (HP 6890 series), equipped with a flame ionization detector and a capillary column, HP5, (30 m; i.d. 0.32 mm; 0.5 lm film thickness). The column temperature was programmed from 150 ^{o}C for 1 min then elevated to 235 ^{o}C at a rate of 17 ^{o}C /min and then raised to 245 ^{o}C at a rate of 1 ^{o}C /min and hold at 245 ^{o}C for 5 min. The injector and detector temperatures were 260 and 275 ^{o}C, respectively. Nitrogen was the carrier

gas at a flow rate of 1.5 ml/min. Identification of the peaks was achieved by retention times and by comparing them with authenticated standards analyzed under the same conditions[9]. Table-3 shows that seed oil consists mainly of essential saturated and unsaturated fatty acid. These results were similar to profiles obtained from Indian[10] and Iranian[11] *Acacia tortilis* seeds.

Table-3
Fatty acid composition of Acacia tortilis seed oil (% of total fatty acids).

S..No.	Fatty Acid	Values (%)		
		Egypt	India	Iran
1	Palmitic Acid (C16:0)	12.31 ± 0.83	6.4174	20.6
2	Stearic Acid (C18:0)	11.88 ± 0.42	0.6304	-
3	Oleic Acid (C18:1)	7.740 ± 0.30	3.9630	-
4	Linoleic Acid (C18:2)	66.57 ± 0.36	36.7484	70.0
5	Arachidic (C20:0)	1.500 ± 0.12	-	-
6	Epoxy(C18:1)	-	1.8013	-

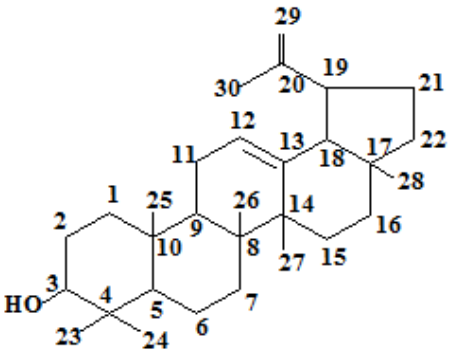
7	Linolenic Acid(C18:2)	-	50.4390	-
8	Vaccenic acid(C18:1)	-	-	2.1

VII. GUM (POLYSACCHARIDE FROM GUM)

Acacia tortilis was collected from India. Gum exudate was crushed into fine particles using laboratory grinder. Fine powder of gum exudates (100 gm) was stirred vigorously in distilled water (200 mL) for 6 hours at room temperature and centrifuged to remove water-insoluble part. The supernatant solution was decanted off. The concentrated aqueous solution was poured into 3 times its volume of ethanol with constant stirring. The polysaccharide was precipitated out in the form of a fluffy precipitate. The precipitate was again dissolved in water and added to ethanol. Precipitate was treated successively with dry solvent ether and acetone. It was filtered under vacuum and dried in vacuum desiccators at room temperature. The pure polysaccharide was subjected to hydrolysis with sulfuric acid (2N) for 18 hr on steam bath.The hydrolyzate was cooled, neutralized with saturated solution of barium carbonate by drop wise addition till the pH of the solution reached at 7, filtered, and the residue washed with water. The combined filtrate was concentrated at or below 40 ^{0}C in rotary evaporator under reduced pressure. This hydrolyzed mass was used for paper chromatography. Complete hydrolysis of the polysaccharide followed by paper chromatography revealed the presence of four spots, corresponding to D-galactose, D-glucose, L-rhamnose and D-glucuronic acids, respectively[12-13]. Although initial studies indicated that gums of species of *Acacia tortilis* were characterized by low Galactose content 23, low Mannose 3, low Uronic acid 8 and high arabinose 66 ratio[14].

VIII. TERPENES FROM LEAVES

The dried and powdered leaves of *Acacia tortilis* procured from Yaman, were exhaustively extracted with light petroleum ether (60-80 ^{o}C), benzene and finally with methanol. On TLC examination, these concentrates showed number of spots in different solvent systems (petrol-benzene and petrol-ether) with the same R_f values. The above two concentrates were therefore mixed together. The combined concentrate was chromatographed over silica-gel column, using successively petrol (A), Petrol-benzene (9:1-B_1, 8:2-B_2, 7:3-B_3, 6:4-B_4, 1:1-B_5) and benzene (C) as eluting solvents. The fractions B_2 and B_3 on TLC examination (silica-gel, petrol-benzene 1:1) showed two major spots with the same R_f values. The above two fractions were therefore mixed together and subjected to column chromatography over silica-gel followed by fractional crystallization, afforded two crystalline TLC homogenous compounds, marked as Lupan-3-ol,12,20-diene **(17)** and Lup-12,20-dien 3-one **(18).** The fractions B_4, B_5 and C were found to be having the same composition with varying concentrations of the compounds. The three fractions were combined together. Repeated column chromatography over silica gel column using petrol-benzene mixture in different ratios gave three compounds containing some minor impurities. Repeated crystallization by an appropriate solvent, gave pure comp-ounds labeled as friedelin **(19),** β-Amyrin **(20)** and β-sitosterol **(21)**[15].



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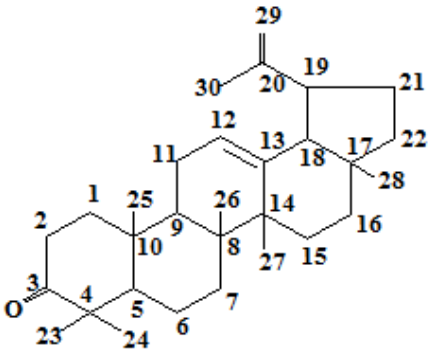

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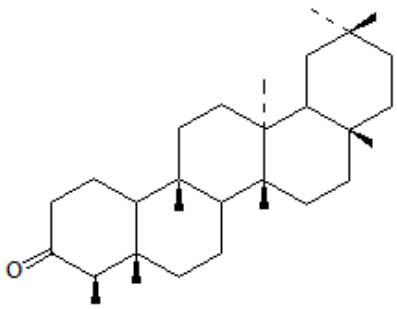

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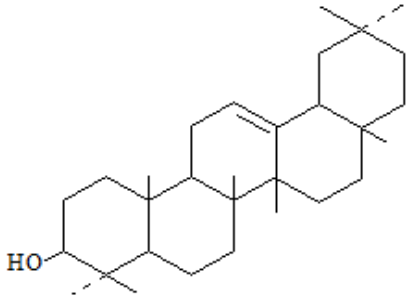

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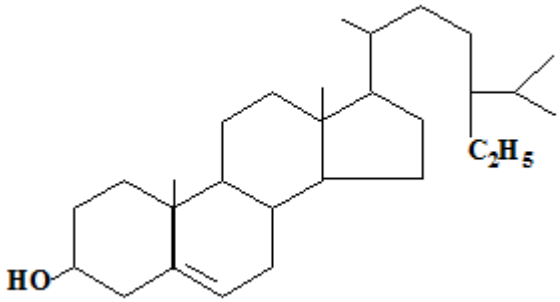

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The leaves of *A. tortilis* were collected from Nigeria. The essential oils of leave ***Acacia tortilis*** subjected to GC analysis was performed on a Shimadzu GC-18A gas chromatograph chromatography system, fitted with two capillary columns, coated with DB-5 and DB-FFAP (fused silica, 30m x 0.32 mm, 0.25 µm film thickness, J&W Scientific, Folsom, CA) and flame ionization detector (FID). The oil was dissolved in 1:10 diethyl ether. The volume injected was 0.2 µL and split ratio was 1: 10. Oven temperature was programmed from 60–240°C at 3°C/min and held at 240°C for 10min, using hydrogen as carrier gas at a flow rate of 1.0mL/min. Injection and detector temperatures were maintained at 240°C. A total of seventy-five compounds representing 89.8% of the total oil fraction could be identified in the essential oil. The identified constituents consisted of diverse classes of compounds such as terpenoids, aliphatic and aromatic compounds. These included 25 monoterpenes (20.4%), 28 sesquiterpenoids (52.1%),18 aliphatic (15.5%) and 3 aromatic (1.7%) compounds. Table-4 shows composition of the leaf oil of *Acacia tortilis*[16].

Table-4
Composition of the leaf oil of *Acacia tortilis*

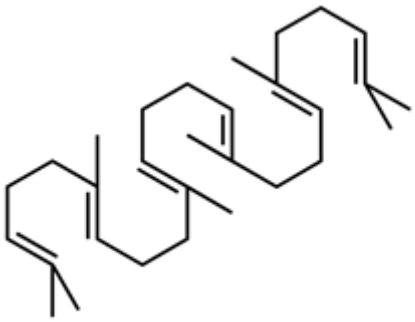
Compound [a]	RI [b]	Percentage (%)	Compound [a]	RI [b]	Percentage (%)
Monoterpenes		**20.4**			
α-pinene	939	Tr	nerolidol *	1540	9.9
α-phellandrene	1008	4.5	γ-cadinene	1543	7.4
p-cymene	1027	4.2	α-calacorene	1548	Tr
limonene	1033	1.0	caryophyllene alcohol c	1557	2.2
γ-terpinene	1060	2.0	germacrene B	1563	0.9
terpinolene	1087	Tr	caryophyllene oxide	1576	1.8
α-terpineol	1187	0.9	viridiflorol	1590	Tr
trans-carveol	1217	3.1	isoelemicin *	1598	1.9
β-cyclocitral	1223	Tr	10-epi-γ-eudesmol	1609	Tr
nerol	1230	Tr	τ-muurolol	1635	0.7
citronellol	1236	0.4	humulene oxide *	1642	0.8
piperitone	1242	Tr	bulnesol	1651	Tr
neral	1247	Tr	α-cadinol	1672	10.6
carvone	1256	Tr	α-eudesmol	1682	Tr
linalyl acetate	1260	Tr	(*E*, *E*)-farnesol	1722	Tr

geraniol	1278	0.6	**Aliphatic compounds**		**15.5**
(*E*)-cinnamaldehyde	1282	Tr	heptanal	902	0.1
methyl cinnamate	1318	Tr	(*E*)-2-heptenal	951	4.7
citronellyl acetate	1357	0.1	2-octanone 965 0.3		
eugenol	1364	Tr	(*E*)-2-octenal	1055	6.0
methyl eugenol	1406	2.0	2-nonanone	1092	Tr
geranyl acetone	1448	0.1	nonanal	1104	0.6
cinnamic acid	1467	0.9	(*E, E)*- 2, 4-nonadienal	1201	0.2
p-menth-9-en-1-ol	1486	0.6	decanal	1209	0.9
(E)-β-ionone	1494	Tr	decanol	1263	Tr
Sesquiterpenes		**52.1**	nonanoic acid	1275	2.3
δ-elemene	1341	Tr	tridecane	1300	Tr
α-cubebene [c]	1347	1.2	decanoic acid	1373	0.1
α-copaene	1372	0.4	ethyl decanoate	1398	Tr
β-elemene [c]	1397	Tr	ethyl 2, 4-decadienoate *	1479	Tr
α-gurjunene	1413	0.6	tridecanol	1592	0.3
β-caryophyllene	1418	0.3	hexadecane	1600	Tr
γ-elemene [c]	1425	0.2	pentadecanoic acid	1800	Tr
trans-α-bergamotene	1430	0.9	hexadecanol	1880	Tr
α-humulene	1454	12.0	**Aromatic compounds**		**1.7**
aromadendrene	1475	Tr	acetophenone	1041	1.7
β-guaiene *	1483	0.3	benzyl benzoate	1732	Tr
germacrene D	1487	Tr	2-phenylethyl benzoate	1836	Tr
calamenene *	1526	Tr			

[a] Compound eluted from DB-5 column; [b] RI on DB-5 column; [c] Identified by MS;
*** correct isomer not identified; Tr- Trace amount < 0.1%**

8.1. Terpenes from seed oil

A. tortilis seeds were collected from Iran. Oil extraction was performed with a Soxhlet apparatus using *n*-hexane as the solvent. 100 g of powdered seeds was extracted for 6 h and then the solvent was evaporated by using a rotary evaporator at40 ÚC. The pure oil was transferred into a small glass vial, flushed with nitrogen and maintained at -18°C until analyzed composition. Oil analyses were performed on a Hp-6890 gas chromatograph (GC) equipped with a FID and a DB-5 capillary column, 30 m × 0.25 mm, 0.25 ¼m film thickness, temperature programmed as follows: 60°”240 °C at 4°C/min. The carrier gas was N_2 at a flow of 2.0 ml/min; injector port and detector temperature were 250 °C and 300 °C, respectively. Sample was injected by splitting and the split ratio was 1:10.GC/MS analysis was performed on a Hewlett-packard 6890 /5972 system with a DB-5 capillary column. The triterpene known Squalene **(22)** were identified by their retention time, retention indices, relative to C9-C40 *n*-alkanes. Squalene identified with a considerable amount of 2.8% in the oil[11].



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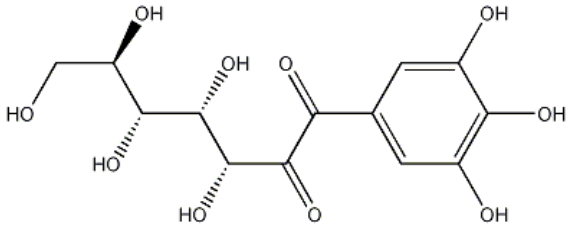
IX. HYDROLYZABLE TANNINS

Samples of *Acacia raddiana* leaves were collected from Sinai peninsula, Egypt. Fresh leaves were extracted with EtOH-H_2O (3:1) eventually gave the known phenols: 1-galloyl glucose **(23)**, 1,6-digalloyl glucose **(24)**, 1,3,6-trigalloylglucose **(25)** and 1,3-di-O-galloyl-4,6-(-)-hexahydroxydiphenoyl-β-glucopyranose

(26). The coned extract was applied to a polyamide column and eluted with H_2O-EtOH. The successive elutes were individually dried in vacuo and subjected to 2DPC, whereby four out of eight different phenolic frs (I-VIII) were obtained. Compound 26 was pptd from an acetone soln of fr. VI by Et_2O and purified through repeated pptn. Cellulose column of fr. II afforded crude 23 which was purified through Me_2CO-Et_2O pptn, while compounds 24, were eluted from a polyamide column of fr. III. Compound 24 was purified through Me_2CO-Et_2O pptn. Compound 25 was isolated from fr. IV through Me_2CO-Et_2O pptn[17].



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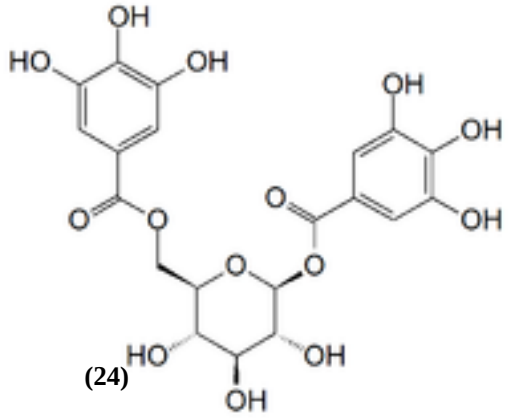

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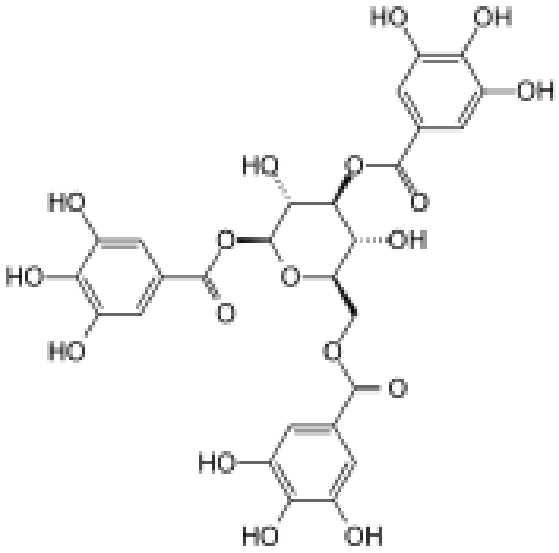

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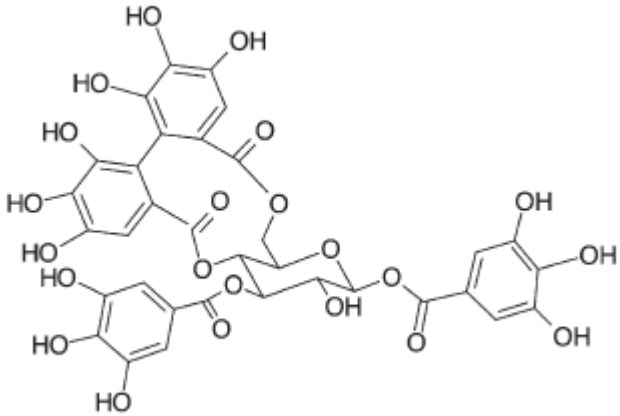
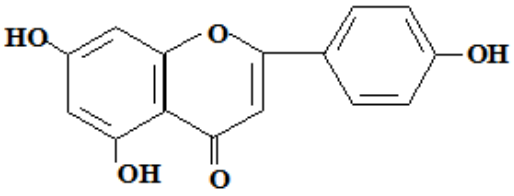


(26)

X. FLAVONOIDS

The dried and powdered leaves of *Acacia tortilis* procured from Yaman, were exhaustively extracted with light petroleum ether (60-80 °C), benzene and finally with methanol. The methanol extract was concentrated by heating over a boiling water bath under reduced pressure, a brown gummy mass was obtained. It gave positive colour test for flavonoids. The examination in TEF and BPF systems showed it to be mixture of several compounds. The brown gummy mass was purified by refluxing it with petroleum ether, benzene and chloroform. The semi-solid mass left behind was chromatographed over silica gel column. Fractional elution with benzene-ethylacetate (1:1) and ethylacetate yielded four compounds. They were purified by repeated crystallization and labeled as 5,7,4'-trihydroxy flavone (Apigenin) **(27),** 5,7,3',4' tetrahydroxy flavone (Luteolin) **(28),** 3, 5,7, 3',4'-pentahydroxy flavone (quercetin) **(29)** and 5,7-dihydroxy-4'-p-methyl benzyl isoflavone **(30)** [18].



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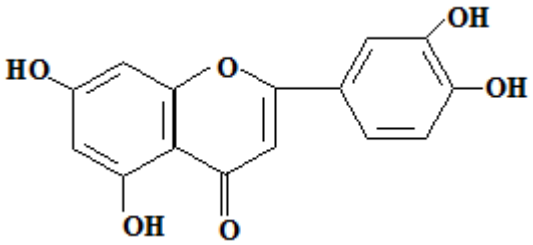

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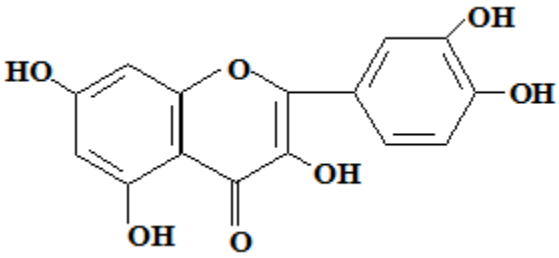

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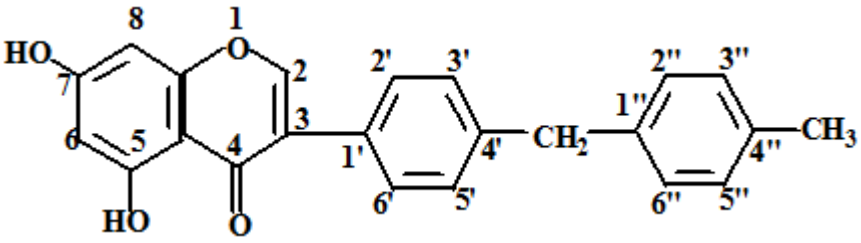
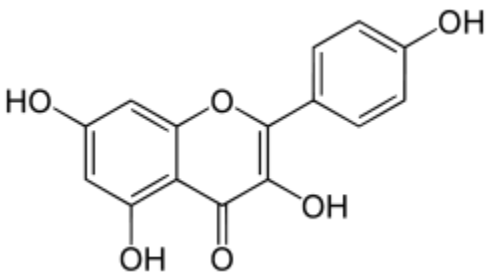


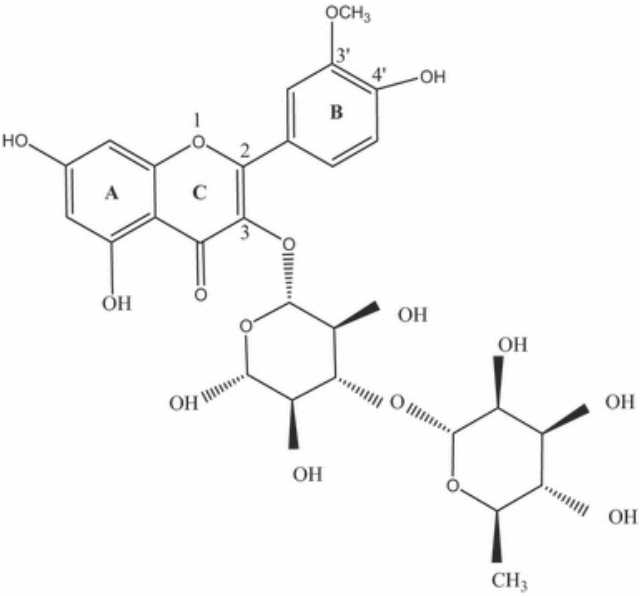
(30)

Dried and powdered leaves of *Acacia tortilis* were collected from Rajasthan - India and separately soxhlet extracted with 80% hot ethanol. On a water bath for 24 hrs. The extracts were concentrated and concentrate re-extracted with petroleum ether (Fraction-I), ether (Fraction-II) and ethyl acetate (Fraction-III) in succession. Fraction-III was dried in vacuo and the resultant was hydrolysed with 7% H_2SO_4 for 2 hrs. The mixture was filtered and the filtrate extracted with ethyl acetate. Concentrated ether and ethyl acetate fraction were applied on TLC. Plates along with standard reference compounds and the plates developed with the solvent system n-butanol, acetic acid and water (4:1:5) when kaempferol **(31)** were detected. The compound was isolated by preparative TLC and crystallized[19].



(31)

Samples of *Acacia raddiana* leaves were collected from Sinai peninsula, Egypt. Extraction of *A. raddiana* leaves with aqueous ethanol (3:1) eventually gave the known phenols: isorhamnetin 3-O-rutinoside **(32),** quercetin 3-O-rutinoside **(33),** quercetin 3-O-gentiobioside **(34),** quercetin 3-O-glucosyl-(l~6)-galactoside, quercetin-3-O-glucoside **(35)** and quercetin-3-O-galactoside **(36)**. The coned extract was applied to a polyamide column and eluted with H_2O-EtOH. The successive elutes were individually dried in vacuo and subjected to 2DPC, whereby three out of eight different phenolic frs (I-VIII) were obtained[17]. compounds 32 and 33 were eluted from a polyamide column of fr. III. Compounds 32 and 33 were purified through crystallization. A combination of a polyamide CC of fr. V followed by prep. PC afforded pure samples of 34 and quercetin 3-O-glucosyl-(l~6)-galactoside. Prep. PC of fr. VII gave pure samples of 35 and 36[17].



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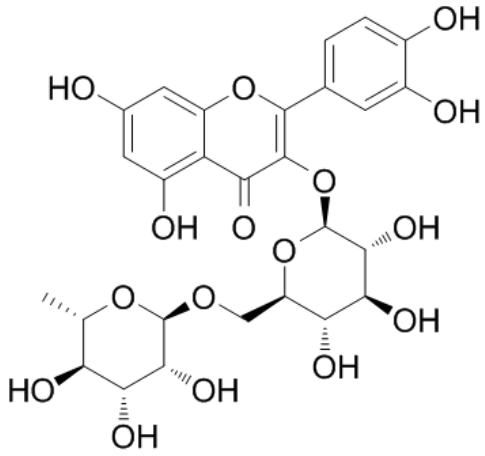

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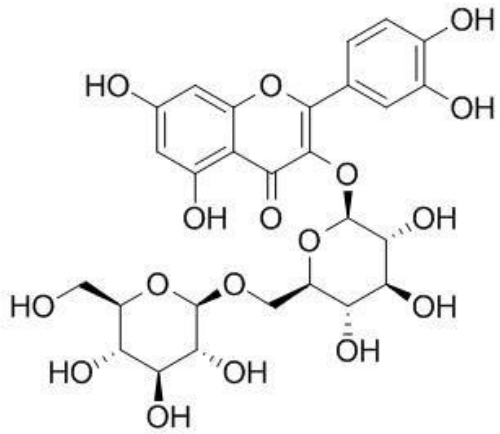

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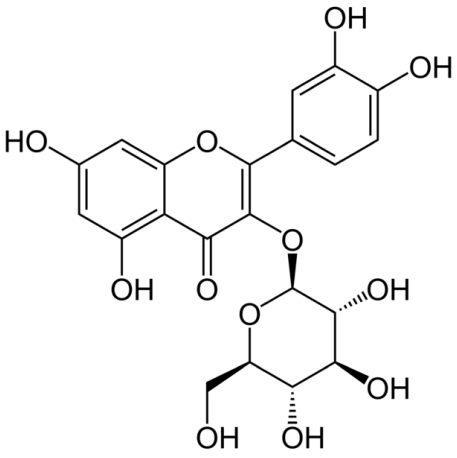

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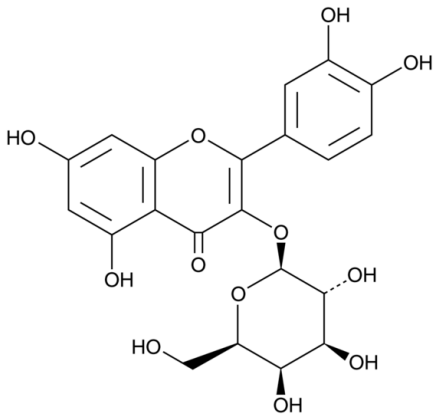
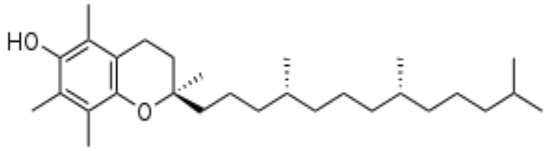


(36)

Apigenin-6,8-bis-D- glycoside and rutin (quercetin 3-*O*-rutinoside) were found in the leaves of Acacia tortilis[20].

10.1 flavonoids From Seed Oils

Samples of seeds of *A. raddiana* were collected from southern Algeria. The seeds of were sorted, crushed and placed in cellulosic Soxlhet cartridges for chemical extraction by hexane. Tocopherols; α-Tocopherol **(37),** β-Tocopherol (39), γ-Tocopherol **(39)** and δ-Tocopherol **(40)** were analyzed by RT-HPLC with detector 10 A.L Shimadza fluorescence and a column of silica[21].



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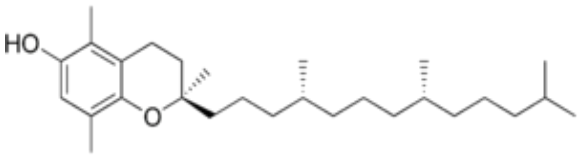

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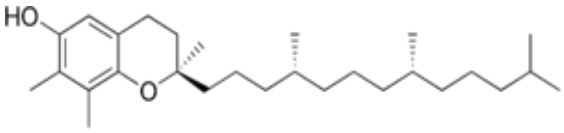

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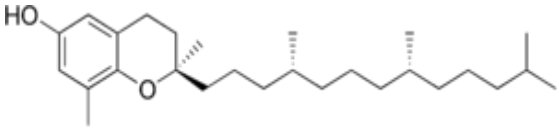

(40)

XI. CONCLUSION

The literature survey revealed that Acacia tortilis, is an important source of many therapeutically and pharmacologically actions. The plant has been widely studied for its pharmacologically activities and finds its position as a versatile plant having a wide spectrum of medicinal activities.

Thus, the growing evidence base for Acacia tortilis suggest that it is very important plant of genus acacia species and it may prove as a boon for arid and sub arid zone, it consist of numerous medicinal as well as commercial value. The best part of this plant is, it has no toxic effect, so it may prove important alternative therapy for treatment of various diseases.

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