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Determination of steroid compounds in Hawthorn (Crataegus azarolus) and Raspberry (Rubus idaeus)Fruits in Sulaimani City Kurdistan region –north Iraq by HPLC technique

Science

KEYWORDS : Steroids, raspberry, hawthorn, HPLC analysis, stigmasterol, campesterol, β-sitosterol,avenasterol, clerosterol

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ABSTRACT

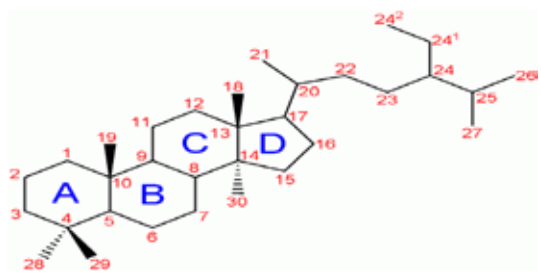
The different steroid compounds in three types of hawthorn fruit (Crataegus azarolus)including Hawraman , Qaradax, house garden , and Raspberry fruit (Rubus idaeus) which grows in Kurdistan Region-North Iraq (season 2011) were determined by RP-HPLC technique including stigmasterol, campesterol, B-sitosterol, avenasterol,and clerosterol. The amount of these steroid compounds in three different types of hawthorn fruit (crataegeus azarolus) Hawraman, Qaradax, house garden were Hawraman type : 0.000 mg/ml, 65.4605mg/ml,62.7443mg/ml,87.9297mg/ml,120.2875mg/ml.Qaradaxtype:78.4247,151.0314mg/ml,0.00mg/ml, 79.7487mg/ml ,and 94.5103mg/ml. House garden type: 75.2227mg/ml ,246.6280 mg/ml,229.2604 mg/ml ,240.9853mg/ml,169.1141mg/ml. While amount these steroids compounds in raspberry fruit (Rubus idaeus) were : 73.2117mg/ml ,0.00mg/ml,83.4229mg/ml ,122.8670mg/ml , and 0.00mg/ml 83.4229mg/ml ,122.8670mg/ml ,and 0.00mg/ml respectively .

Introduction

Hawthorn is a spiking bush or tree, the tree reaches 13feet in height and grows along the edges of woods and forests .hawthron has smooth, gray dark and sharp thorns wich grow along the branches. The medicinal parts are the flowers and the fruits (1).The Latin names is crataegus azarolus it belong to the genus (crataegus), the genus (crataegus) is a member of the rose family (rosaceae), it is cultivated and grows wild in Kurdistan Region –north Iraq , a local name of hawthorn fruit in Kurdish is (goezh)Fig [1] Raspberry is a plant which stand about 6 feet tall , It is often found growing wild in hedges . It may be vining or shrubby but tends to grown in thickets, it belongs to the genus to (rubus) , the genus rubus is a member of rose family (rosaceae). It is cultivated and grows wild in Sulaimani City – Kurdistan Region north Iraq .A local name of raspberry fruit in Kurdish is totirk (1) Fig[2].Steroids are lipids that contain four fused carbon rings that form the steroid nucleus, cyclopentan perhydrophenanthrenp Fig[3] Phytosterol, wich encompass plant sterol and stanols are steroid compounds similar to cholesterol which occur in plants and vary only in carbon side chains and or absence of double bond.Stanols are saturated sterols, having no double bonds in the sterol ring structure. More than 200 sterols and related compounds have been identified (2).Free phytosterols extracted from oils are insoluble in water, relatively insoluble in oil and soluble in alcohols. Phytosterol enriched foods and dietary supplements have been marketed for dec

Fig(1)hawthorn fruit

Fig(2)raspberry fruit



Fig(3) Steroid skeleton

The richest occurring source of phytosterol are vegetable oils and products made from them(3).The intake of naturally occurring phytosterol ranges between 150-450mg/day depending on eating habit(4) specially designed vegetarian experimental diets having been produced yielding upwards = 700mg / day(5). The most commonly accruing phytesterols in the human diet are β- sitosterol , and stigmasterol which account for approximately 65%, 30% and 3% of diet contents, respectively (6) . The most common plant stanols in human diet are Sitostanol and campestanol which combined make up about 5% of dietary phytosterol (7). The ability of phytosterol to reduce cholesterol levels was first demonstrated in humans in 1953(8).They were subsequently marketed as a pharma ceutical under the name Cytellin as treatment for elevated cholesterol from 1954 .It has been postulated that campestrol is most efficient in membrane permeability and fluidity regulation and three are evidence that stigma sterol play an important role in cell proliferation but has reduced ordering effected (10) campesterol and avenasterol are phytosterol. Phytosterol are relevant in pharmaceuticals (production of therapeutic steroids), nutrition (anti –cholesterol additives in functional foods, anti cancer properties),and cosmetics (cream lip stick) β-sitosterol is one of several physterols (plantsterols) β-sitosterol reduces blood levels of cholesterol and is sometimes used in treating hypercholesterolemia, inhibits cholesterol absorption in the intestine (11) when the sterol is absorbed in the intestine, it is transported by lipoproteins and incorporated in to the cellular membrane(12) phytosterols and phytostanuls both inhibit the uptake of dietary and biliary cholesterol , decreasing the level of LDL and serum total cholesterol (13)

Material and methods

Three types of hawthorn, (crataegus azarolus) fruits including Hawraman ,qaradax,and house garden with Raspberry fruit (Rubus idaeus)were harvested by hand in its optimum state for two consecutive seasons in 2011 in sulaimani city –Kurdistan Region –Iraq. After, a morphological and chemical characterization, the samples were prepared for determind steroid compounds.

Extraction procedure for steroid compounds (14)
Active ingredient sterols:
The extract were separated on FLC (fast liquid chromatography) column , C- 18,13 µm particle size (50x4.6mm ID),mobile phase 0.1% acetic acid in deionized water (solvent A) and acetonitrile (solvent B)using linear gradient from 0 -100% B in 10 minutes , detection UV set at 275 nm, flow rate 1.2 ml/min, temperature 30^{0}C . The separation occurred on liquid –chromatography shimadzu 2010 LC equipped with binary delivery pump model 2010 shimadzu ,the eluted peaks were monitored by UV-Vis 2010 SPD spectrophotometer.

Extraction procedure
The sample powder 1.0 has been dissolved in 5ml hot water for 2 hour, then using ultrasonic bath for 20 minutes to get all extract dissolved in hot water 60^{0}C, then the extracts were filtered on filter paper no.1,0.5 mm to separate the fiber , 20 µl from the samples injected on HPLC system. The sequence of the eluted material of the standard were as follow, each standard was 25µg/ml.

Calculation: Concentration of sample µg/ml = [area of sample/area of standard] x conc. of standard x dilution factor

no	subjects	Rt/ minute	area/ standard	df	area/ sample	conce.µg/ ml	conce.of sample
1	stigmasterol	0.99	21221	3		25	0.0000
2	campesterol	2.15	21597	3	18850	25	65.4605
3	β-sitosterol	3	22973	3	19219	25	62.7443
4	avenasterol	3.92	24496	3	28719	25	87.9297
5	clerosterol	4.99	19878	3	31881	25	120.2875

Table(1) Phytosterol compounds content in Hawraman Hawthorn fruit

no	subjects	Rt/minute	area/ standard	df	area/ sample	conce.µg/ ml	conce.of sample
1	stigmasterol	0.99	21221	3	22190	25	78.4247
2	campesterol	2.15	21597	3	43491	25	151.0314
3	β-sitosterol	3	22973	3		25	0.0000
4	avenasterol	3.92	24496	3	26047	25	79.7487
5	clerosterol	4.99	19878	3	25049	25	94.5103

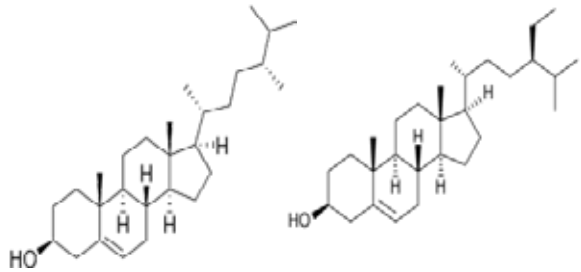
Table(2) Phytosterol compounds content in Qaradax Hawthorn fruit

Table(3) Phytosterol compounds content in House garden Hawthorn fruit

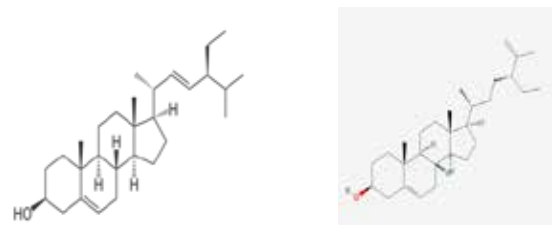
no	subjects	Rt/ minute	area/ standard	df	area/ sample	conce. µg/ml	conce.of sample
1	stigmasterol	0.99	21221	3	20715	25	73.2117
2	campesterol	2.15	21597	3		25	0.0000
3	β-sitosterol	3	22973	3	25553	25	83.4229
4	avenasterol	3.92	24496	3	40130	25	122.8670
5	clerosterol	4.99	19878	3		25	0.0000

no	subjects	Rt/minute	area/ standard	df	area/ sample	conce.µg/ ml	conce.of sample
1	stigmasterol	0.99	21221	3	21284	25	75.2227
2	campesterol	2.15	21597	3	71019	25	246.6280
3	β-sitosterol	3	22973	3	70224	25	229.2604
4	avenasterol	3.92	24496	3	78709	25	240.9853
5	clerosterol	4.99	19878	3	44822	25	169.1141

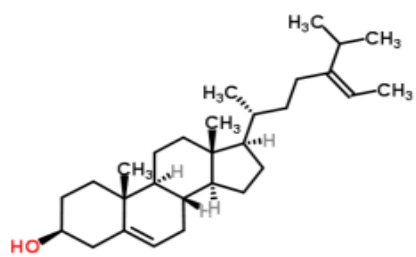
Table(4) Phytosterol compounds content in raspberry fruit



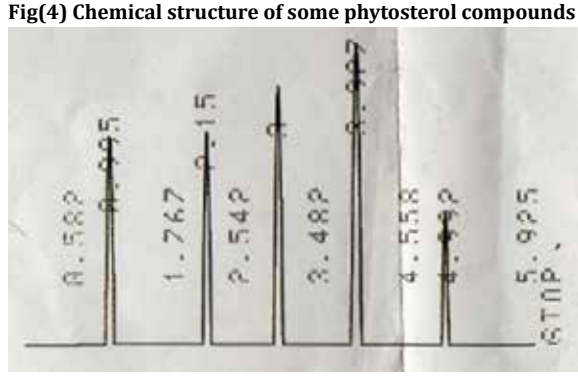
Campesterol **β-Sitosterol**



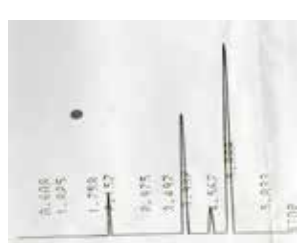
Stigmasterol **Clerostero**



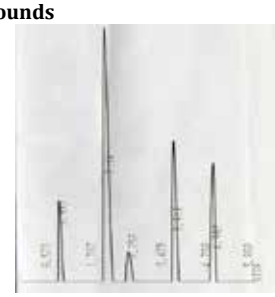
Avenasterol
Fig(4) Chemical structure of some phytosterol compounds



Fig(5) HPLC chromatograms of a mixture of authentic standers of phytosterol compounds



Fig(6)



Fig(7)

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Fig(8) **Fig(9)**

Fig(6,7,8,9) HPLC chromatograms of phytosterol compounds in Hawraman, Qaradax, house garden and raspberry fruits respectively

Result and discussion

The HPLC analysis of phytosterol compounds in the three types of hawthorn fruits with raspberry fruits are presented in table [1-4] from the result House garden hawtorn fruit fig[8]table[3] contain five types of phytosterols like stigmasterol , campesterol, β-sitosterol, avensatesterol, and clerosterol Fig[4]. Qaradax hawthorn fruit fig[7]table[2] contain four type of phytosterols like stagmasterol,campesterol ,avenasterol and clerosterol. Hawraman hawthorn fruit fig[6]table[1] contain four types of phytosterols like campesterol, β-sitosterol and avensatesterol, and clerosterol, raspberry fruit contain three types of phytosterol like stigmasterol , β-sitosterol and avenasterol .All fruits contain avenasterol while Hawraman hawthorn fruit not contain stigmasterol.Qaradax hawthorn fruit not contain β-sitosterol, and raspberry fruit not contain campesterol.From the result appear that the house garden hawthorn fruit contain high percent of compesterol,β-sitosterol , avensatesterol, and clerosterol,.246-6280mg/ml,229.2604mg/ml, 240.9853mg/ml,169.1141mg/ml than other fruits

Conclusion

Phytosterols, also called plant sterol occur in relatively high concentration in the tree types of hawthorn fruits (Hawraman, Qaradax,and house garden)with raspberry fruit were grows in Sulaimani City, Kurdistan Region –Iraq like stigmasterol ,campsterol, , β-sitosterol and cholesterol .Physterol occur as organic compounds and essential constituents of cell membranes in all plant oils. Recently increased interest in phytosterol lies in their potential to reduce plasma. Low-density lipoprotein cholesterol level decreasing coronary mortality and there for acting as naturally preventive dietary product, High expectation have already been put forward regarding phytosterol analysis and traditional plant –breeding applications in developing improved cultivars with desirable phytosterol composition and increased content

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