EXTRACTION AND PURIFICATION OF URSOLIC ACID FROM TULSI LEAVES

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ABSTRACT: Ursolic acid is a triterpene molecule present widely in the medicinal plants. The major sources include tulsi leaves (*Ocimum sanctum*) and nilgiri leaves (*Eucalyptous alba*). Ursolic acid is having many biological activities including anti-diabetic, muscle building, antimicrobial activity, anti-obesity etc. Ursolic acid is being used in ayurvedic and nutraceutical formulations. The current project involves the process development for extraction and purification of ursolic acid from tulsi leaves. The extraction process development includes optimization of different process parameters such as solid-solvent ratio, time, agitation speed, solvent used for extraction etc. The optimization would done to make the process more efficient in terms of selectivity, yield and productivity. The crude extract obtained after extraction contains many other compounds other than ursolic acid as extraction is not that selective for natural products. The presence of other compounds may increase or decrease the activity of ursolic acid. Hence the further purification is required to get pure ursolic acid.The purification process involves the use of different synthetic adsorbents and silica column chromatography. The optimization of chromatographic purification will be done with respect to the flow rate, feed volume, concentration of feed, fraction volume, column height, column volume, composition of mobile phase etc.Characterization of the purified product will be done using HPLC, LC-MS, FTIR and NMR.

Keywords: Extraction, optimization, Chromatography, composition.

INTRODUCTION

In recent years, extraction and purification of bioactive compounds from natural sources has become very important for the use of phyto chemicals in the preparation of food supplements or nutraceuticals, functional food ingredients, food additives, pharmaceutical and cosmetic products. This diversified use of bio actives has gained scientific and industrial importance for their production and also led to the identification of new bio-resources. Holy basil (OS Linn.) or 'Tulsi' possesses valuable antioxidant properties for culinary and wide spectrum of medicinal uses viz. anti-carcinogenic, anthelmintic, antirheumatic, antibacterial, antidepressant, antiepileptic, hepatoprotective, radioprotective OS Linn. leaves contain 0.7% volatile oil comprising about 71% eugenol and 20%methyl eugenol. UA, carvacrol, caryophyllene, apigenin, luteolin,apigenin-7-*O*-glucuronide, orientin and molludistin areother additional phyto constituents found in the OS. UA is a triterpene compound found in many plants like apple, basil, bilberries, elder flower, and peppermint.UA have shown diverse pharmacological activities such as anti-inflammatory, antitumor, hypoglycemic, antiulcer, antilipidemic. UA and its derivatives have antiviral potential and also inhibit the development of several viruses including HIV. Usually, the separation of medicinally active components from plant parts (seeds, flowers, roots, leaves) is carried out by solid–liquid extraction process with selective solvents.

There are several novel extraction methods such as supercritical fluid extraction, Ultrasound assisted extraction and microwave assisted extraction have been reported in the literature for the extraction of natural products. These methods have shown several advantages over conventional extraction methods with increased extraction efficiency. The extraction yield of phyto component is mainly affected by operating conditions under which the process is carried out. The kinetics of solute extraction from natural sources involves releasing solute from porous matrices into a solvent phase by means of mass transfer. From an engineering point of view, understanding mass transfer at the solid liquid interface plays significant role in scaling-up of the process. Applications of various conventional techniques such as soxhlet, precipitation, column chromatography and novel technique like counter current chromatography for the extraction and purification of UA from OS leaves have been already reported in the literature. UA has been isolated from several sources, but the authors have not found any literature on the extraction of UA from OS. Hence, the objective of the present study is to develop the optimized batch extraction process for the extraction of UA from OS leaves. The influence of various extraction process parameters such as solvents, extraction time, solute to solvent ratio, speed of agitation, on extraction yield has also been studied

MATERIALS AND METHODS

The raw material used in the project is Tulsi leaves. The tulsi powder size used was 0.50–1.00 mm. The OS powder is greenish brown in color The solvents are methanol, ethanol, tertiary butanol, hexane, ethyl acetate and isopropyl alcohol are used in the experiment. The resins are silica gel, polystyrene polymethacrylate are depending upon its mesh size use it. The Chemicals are acids, alkali, HCL, NaoH, KOH, are used depending upon solvent.

BATCH EXTRACTION

Batch extraction was carried out in a glass reactor of 150 ml capacity equipped with a six bladed (pitched blade) glass turbine for agitation. The measured quantity of the tulsi leaves powder was taken in a glass reactor and required amount of solvent was added to it. The mixture was then agitated for 45 min. Samples were withdrawn and filtered. Different parameters affecting the extraction such as extraction time, solute to solvent ratio, speed of agitation and extraction temperature were optimized and the final extraction experiment at the optimized conditions was carried out to get maximum recovery.

PURIFICATION

For the purification of ursolic acid we use column chromatography. the column is 250ml and has 1.8 cm diameter. we use all samples and purify in the column and samples are analysis using HPLC analysis.

RESULT AND DISCUSSION

Effect of solvent

The choice of an extracting solvent is the important step towards parameter optimization which has significant effect on the extraction yield. It is difficult to predict the interaction between natural product and extracting solvent due to diverse chemical structure of natural product. Different solvents will yield different amount and composition of extract In the present study solvents of varying polarity, such as methanol, Hexane and Isopropyl alcohol were used as the extracting solvent. Other experimental parameters were kept constant (solute to solvent ratio 1:80; speed of agitation of 400 rpm and extracting time, 45 min). The polarity of the solvent plays an important role in the extraction process. Ursolic Acid is polar in nature, and the solubility of Ursolic Acid increases with an increase in polarity of the solvents (methanol > isopropyl alcohol > hexane)

SOLVENT	WEIGHT OF POWDER (gm)	SOLVENT (ml)	EXTRACTION YIELD (mg)
HEXANE	20	160	1.2
METHANOL	20	160	1.8
ISOPROPYL ALCOHOL	20	160	1.6

Effect of solid solvent ratio, agitation speed and time

In this parameter we take solid solvent ratio as 1:5 and 1:8; agitation speed as 400, 800 and time 45 in different combination. At high speed of agitation more turbulence is generated which results in high yield of solute in the batch reactor. It was observed that the amount of ursolic acid extracted per gram of tulsi powder increases with increase in larger solvent ratio and agitation speed upto 1:8, 800 and time 45min. But beyond that no significant change was observed in yield of ursolic acid.

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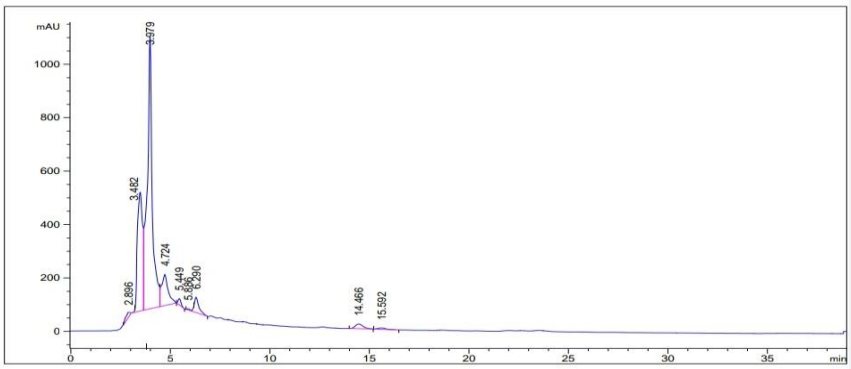
Solid solvent ratio	Agitation speed (rpm)	Time (Min)	Extraction yield (mg)
1:5	400	45	2.3
1:8	800	45	3.4
1:5	800	45	2.9
1:8	400	45	2.7

Result

Now the samples are analyzed in HPLC analysis

For 1st Run

The following graph is absorbance v/s time, we get



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.896	BB	0.2230	299.61768	18.29955	0.9681
2	3.482	BV	0.2535	7993.60645	445.24326	25.8281
3	3.979	VV	0.2398	1.81528e4	1019.10645	58.6535
4	4.724	VB	0.3066	2678.60205	116.32374	8.6548
5	5.449	BB	0.1547	235.73590	23.55937	0.7617
6	5.886	BV	0.1795	52.02210	4.28998	0.1681
7	6.290	VB	0.2355	918.89105	57.18445	2.9690
8	14.466	BB	0.4204	489.42947	17.93461	1.5814
9	15.592	BB	0.4106	128.56903	4.53693	0.4154

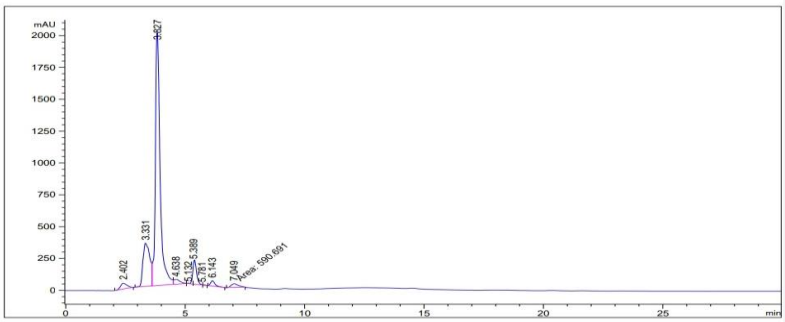
Totals : 3.09493e4 1706.47832

*** End of Report ***

From above graph the highest peak value is 3 which shows that 58.65 % purity

For 2nd Run

The following graph is absorbance v/s time, we get



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.402	BB	0.2893	825.65625	43.93322	2.1173
2	3.331	BV	0.2646	6838.22998	340.02286	17.5355
3	3.827	VV	0.2055	2.74681e4	1984.46899	70.4373
4	4.638	VB	0.2648	691.03680	36.84387	1.7720
5	5.132	BV	0.0970	17.58193	2.81093	0.0451
6	5.389	VV	0.1614	2026.45923	191.53088	5.1965
7	5.781	VB	0.1088	14.57452	2.05287	0.0374
8	6.143	BB	0.1880	524.19647	42.47659	1.3442
9	7.049	MM	0.3490	590.69122	28.20895	1.5147

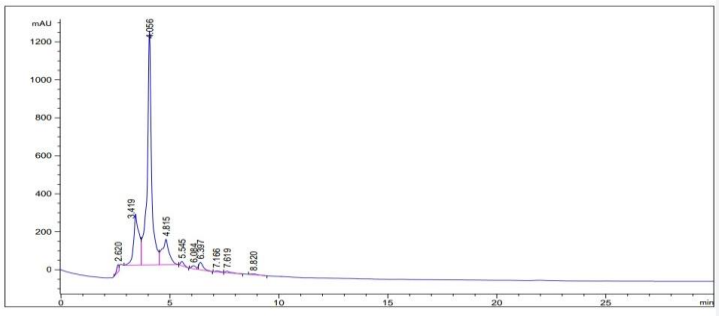
Totals : 3.89965e4 2672.34916

*** End of Report ***

From above graph the highest peak value is 3 which shows that 70.43 % purity

For 3rd Run

The following graph is absorbance v/s time, we get



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.620	BV	0.1066	263.52795	38.13564	0.9894
2	3.419	BV	0.2165	4563.92383	268.29388	17.1353
3	4.056	VV	0.1970	1.74029e4	1230.16541	65.3393
4	4.815	VB	0.2959	3039.49121	134.36461	11.4118
5	5.545	BB	0.1625	240.50912	22.54641	0.9030
6	6.084	BV	0.2149	213.22365	15.85644	0.8006
7	6.397	VB	0.2185	591.47894	39.99599	2.2207
8	7.166	BV	0.2685	98.19545	5.60304	0.3687
9	7.619	VB	0.2420	144.59079	8.35252	0.5429
10	8.820	BB	0.3033	76.79998	3.87321	0.2883

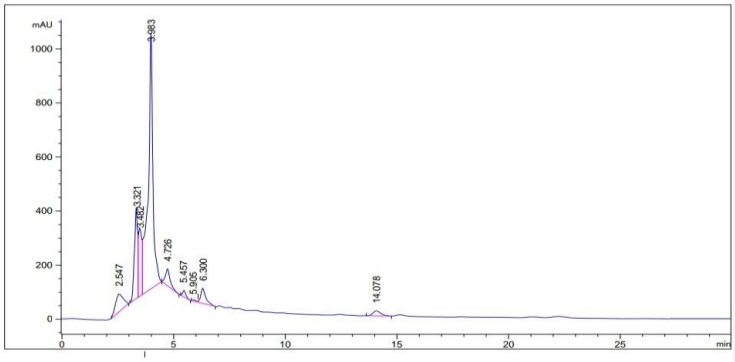
Totals : 2.66346e4 1767.18

*** End of Report ***

From above graph the highest peak value is 3 which shows that 65.33 % purity

For 4th Run

The following graph is absorbance v/s time, we get



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.547	BB	0.3429	1568.94006	67.06570	6.4390
2	3.321	BV	0.1319	3286.75049	336.56213	13.4890
3	3.482	VV	0.1508	2616.77368	252.78697	10.7394
4	3.983	VB	0.2076	1.43619e4	943.62604	58.9422
5	4.726	BB	0.2072	920.92017	63.50115	3.7795
6	5.457	BB	0.1490	233.03325	24.06755	0.9564
7	5.905	BV	0.1863	57.46835	4.34583	0.2359
8	6.300	VB	0.2241	866.97345	56.79498	3.5581
9	14.078	BB	0.3778	453.32718	18.37867	1.8605
Totals :				2.43661e4	1767.12902	

*** End of Report ***

From above graph the highest peak value is 4 which shows that 58.94 % purity

CONCLUSION

Based on the experiments performed and the results obtained we can conclude

While using solvent we conclude that high polarity solvent gives high yield in extraction solvent. The polarity of the solvent plays an important role in the extraction process Ursolic Acid is polar in nature, and the solubility of Ursolic Acid increases with an increase in polarity of the solvents. The process effectiveness in extraction was studied by considering three parameter solvent, solid-solvent ration, agitation speed, time by studying above parameters we came to conclusion that higher solvent ratio and agitation speed with perfect time gives higher concentration yield.

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