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RESEARCH ARTICLE

PHYSICAL AND PHYTOCHEMICAL SCREENING OF MARKET SAMPLES OF ASHWAGANDHA [*Withaniasomnifera (Linn)*Dunal] IN KERALA.

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Abstract

Withania somnifera (Linn) Dunal (Solanaceae) is an ayurvedic herb widely distributed throughout India. The Ayurvedic Pharmacopoeia of India (API) mentions root of *Withania somnifera (Linn)* Dunal as the officinal part of *Ashwagandha*. In Kerala, it is used in a variety of formulations either as single or in combinations. But there is a huge difference between its demand and availability. So the study was undertaken to assess the genuineness of *Ashwagandha* available in herbal raw drug markets of Kerala. For this purpose, 28 commercial samples (one from urban and one from rural) of *Ashwagandha* was collected from each of 14 districts of Kerala and its characters were compared with the root collected from the genuine source plant of *Ashwagandha [Withania somnifera (Linn) Dunal*] and also with the standards mentioned for *Ashwagandha* in API. Comparisons were done with the aid of Physical and Phytochemical evaluations including estimation of Foreign matter, Total ash, Acid insololuble ash, Water insoluble ash, Alcohol soluble extractive, Water soluble extractive, Fibre content ,heavy metal analysis and HPTLC. The result got from each of these matched with that of genuine sample and the standards given in API. But there were slight variations. These slight variations in physicochemical and phytochemical results may be due to several factors such as different geographic condition, edaphic factors, environmental condition etc. So the samples got from each of the 14 districts of Kerala were thus found to be genuine

Introduction:-

The quality of medicine is of paramount importance in the Indian system of medicines. The organized drug industries today have replaced the traditional indigenous practices. Along with the mechanization, many unhealthy practices have also crept into the profession detrimentally affecting the credibility of the system of medicine.The adulteration and substitution of herbal drugs is a burning problem in the herbal industry. This can be traced to indiscriminate/unregulated harvesting practices being followed with no concern for the sustainability of the resources. This obviously leads to a supply crunch. Such a situation is already being experienced in *Ashwagandha*.
Ashwagandha (*Withania somnifera (Linn)* Dunal), is one of the essential medicinal herbs in Ayurveda used from time immemorial. From medieval period onwards, *Ashwagandha* emerged as an aphrodisiac agent. The annual demand is 9127.5 tons per annum in the year 2005[1] (SMPB Kerala, 2012). Based on the current trend the current

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production would be 8429 tons and demand of *Ashwagandha* per annum would be around 12500 tons[2, 3]. The plant is distributed throughout the drier subtropical regions of India. But it is not common in Kerala in spite of using in variety of such formulations and in spite of such huge demand. The mixing of cheap variety of *Ashwagandha* is reportedly rampant. Intentional mixing of lateral thin roots of same species by primary collectors was also reported in the market samples[4]. Although there exists reports of adulteration, no much detailed studies regarding the genuineness of *Ashwagandha* available in raw drug markets of Kerala have been carried out till date. A physical and phytochemical study is essential for ascertaining the quality of medicines available in market

Materials And Method:-

I) Sample Collection:-

a) Collection of genuine sample

The genuine sample of *Ashwagandha* [*Withania somnifera (Linn*) Dunal] was collected from the Bhopal in Madhya pradesh and authenticated by pharmacognosy unit, Ayurveda Research Institute, Poojappura.

b) Collection of market samples

Two market samples of *Ashwagandha* were collected from each of fourteen districts of Kerala, one from rural and one from urban area of each district. Thus a total of twenty eight market samples were collected for the study. The samples from the urban and rural areas of the same district were named as sample A and sample B respectively. They were stored in air tight polythene bags.

II) Determination of physico-chemical and Phytochemicl Parameters:-

Preliminary phytochemical evaluation is a part of chemical evaluation. Methanolic extract of plant was subjected to qualitative phytochemical tests. Comparisons were done with the aid of Physical and Phytochemical evaluations including estimation of foreign matter, Total ash, Acid insoluble ash, Water insoluble ash, Alcohol soluble extractive, Water soluble extractive, Fibre content, detection of steroids, phenols, alkaloids, flavanoids, glycosides, tannins and saponins.

Physico-chemical Analysis

a) Examination of total ash

Approximately 1 gm of powdered and accurately weighed drug was taken in a previously weighed dry silica crucible. It was then scattered in a fine even layer at the bottom of the crucible and incinerated in an electric Bunsen burner by gradually increasing the heat until freed from carbon. It was then allowed to cool slowly and weighed to a constant weight in an accurate digital weighing machine. The percentage of ash was calculated with reference to the air dried drug.

b) Determination of acid insoluble ash value

To the crucible containing total ash, added 25 ml of dil HCL and boiled gently for 5 minutes. The insoluble matter was collected on an ashless filter paper [Whatman 41] and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible, and ignited in Bunsen burner for half an hour. The residue was allowed to cool and weighed. The acid insoluble ash was calculated with reference to the air dried drug.

c) Determination of water insoluble ash

To the crucible containing total ash, added 25 ml of water and boiled for five minutes. The insoluble matter was collected o an ashless filter paper [Whatman 41] and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible, and ignited in Bunsen burner for half an hour. The residue was allowed to cool and weighed. The water insoluble ash was calculated with reference to the air dried drug.

d) Fibre content

Fibre content was calculated and observations were recorded.

e) Determination of extractive values

Water soluble extractives

Approximately 10 g of the drug was weighed and transferred into a round bottom flask. To this, 100ml of chloroform water was added. The content was shaken for about 24 hrs occasionally. After that it was filtered through ordinary filter paper. The filtrate was evaporated to dryness in a previously weighed beaker. Heating and weighing was continued till a constant weight was obtained. The percentage of water soluble extract was calculated.

Alcohol soluble extract

Approximately 5g of the drug was weighed and transferred into a round bottom flask. 100ml of 95% ethanol was added. The content was shaken for about 24hrs, occasionally. The filtrate was evaporated to dryness in a

previously weighed beaker. Heating and weighing was continued till a constant weight was obtained. The percentage of alcohol soluble extract was calculated

Qualitative analysis

The **alcoholic extractive** obtained was subjected to qualitative analysis for identification of various plant constituents.

a) Detection of steroids (Liebermann Burchard's test)

The steroid was determined by evaporating the extractive in a watch glass, and to the residue added acetic anhydride and conc. Sulphuric acid through the sides. A play of colours like yellow, green or brwn to black on the watch glass indicated the presence of steroids.

b) Detection of phenols

Take 2-3ml of the extract in a test tube and neutral ferric chloride was added. A deep blue or violet colour indicated the presence of phenols in the extractives.

c) Detection of alkaloids

Alkaloids were determined by evaporating the extractives in a small beaker and to all the residues, dilute hydrochloric acid was added separately and filtered. The filtrate obtained was transferred into test tubes and a few drops of Mayer's reagent were added. A white precipitate indicated the presence of alkaloids. With Dragendroff's reagent, it gave an orange brown precipitate.

d) Detection of flavonoids

To the extract add magnesium ribbon and add concentrated HCL and it was kept over water bath for a few minutes to boil. A reddish brown precipitate indicates the presence of flavonoids.

e) Detection of saponins

A few drops of sodium bicarbonate solution were added to the alcoholic extractive and was shaken well . A honey comb (frothy) appearance indicates the presence of saponins.

f) Detection of glycosides (Keller-Killani test)

Alcoholic extract taken in a test tube was treated with few drops of glacial acetic acid and crystals of ferric chloride were added to this and were mixed. Concentrated sulphuric acid was then added through the sides of test tube and formation of two layers were observed, lower reddish brown layer and upper acetic acid layer which turned bluish green indicated the presence of glycosides.

g) Determination of terpenoid (Salkowskis test)

2ml of chloroform and 1ml of conc.H2SO4 was added to 1mg of extract and reddish brown colour indicated the presence of terpenoid.

h)Test for tannin

To 1-2ml of alcoholic extract of the substance, 5% of ferric chloride solution was added. Brownish black precipitate indicates the presence of tannin

Table-1: Preliminary qualitative phytochemical analysis of *Withania somnifera* root.

No	Phytochemical	Test	Observation
1	Alkaloids	Dragendroff's test Mayer's test	(+++) (++)
2	Carbohydrate and glycosides	Keller-Killani test	(++)
3	Saponins	Foam test	(-)
4	Tannins	Ferric chloride test	(+++)
5	Flavanoids	Shinoda test	(++)
6	Steroids	Liebermann-Burchard	(+)
7	Terpenoids	Salkowskis test	(++)
8	Phenols	Ferric chloride test	(++)
(-):- No presence, (+):- Less presence, (++):- Moderate presence, (+++):- High presence			

Comparisons were done with the aid of Physical and Phytochemical evaluations including estimation of foreign matter, Total ash, Acid insololuble ash, Water insoluble ash, Alcohol soluble extractive, Water soluble extractive, Fibre content, detection of steroids, phenols, alkaloids, flavanoids, glycosides, tannins and saponins.

III) HPTLC (High Performance Thin Layer Chromatography)

Ethanolic extract of the root of genuine *Ashwagandha (Withania somnifera (Linn)* Dunal*)* and market samples of *Ashwagandha* were centrifuged at 3000 rpm for 5 minutes. Supernatant was used as test solution for HPTLC analysis. 2µl of each ethanolic extract solution was applied as 8 mm band length in the 10 X 200 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples applied plate was kept in TLC twin through developing chamber (after saturated with solvent vapour) with respective mobile phase Toluene: Ethylacetate: Formic acid (5:5:1) up to 70 mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG TLC SCANNER 3) and scanning was done at UV 254 nm and UV 366 nm mode using photo-documentation (CAMAG REPROSTAR 3) chamber. Before, derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254 nm and UV 366 nm. The peak table and peak densiogram for each profile were noted. The software used was WinCATS 1.3.4 version. HPLC grade reagents were used.

Test solution- 3gm of accurately weighed coarsely powdered sample was taken in a round bottom flask . 100ml of 99% methanol was added to it. It was then condensed with water condenser and boiled for one hour in electric mantle, allowed to stand for some time and then filtered.

Solvent system

Toluene: Ethyl acetate: Formic acid: 5:5:1.

IV) Atomic Absorption Spectroscopy (AAS)

Determination of Lead, Cadmium, Nickel, Copper, Iron and Zinc in ppm levels in test drug were carried out using standard procedure at DSU, Govt Ayurveda College, Thiruvananthapuram

V) Statistical Analysis

The collected data on phytochemical evaluation which includes Foreign matter, Alcohol soluble extractives, Water soluble extractives, Moisture content, Volatile oil, Total ash, Acid insoluble ash, Water soluble ash, Fibre content, Total sugar content and Reducing sugar of the root of genuine *Ashwagandha* (*Withania somnifera (Linn*) Dunal) and market samples of *Ashwagandha* collected from all the districts of Kerala were subjected to statistical analysis using appropriate statistical techniques. The statistical techniques used include:Descriptive and Inferential Statitics. The significant difference between the genuine sample and market sample mean values is tested using one-sample t test with the test value of the difference is set at zero (market sample and genuine sample are equivalent). A calculated P value less than 0.05 is considered to be statistically significant. The results are as follows:

Result:-

A)Physico- chemical analysis of genuine root and market samples

Table 2. Physico -chemical evaluation of genuine sample and market samples

SAMPLES	Foreign matter (%)	Total ash (%)	Water insoluble ash (%)	Acid insoluble ash (%)	Cold water soluble extractive (%)	Alcohol soluble extractive (%)	Fibre content (%)
API	**1.29**	**Not >7%**	**-**	**Not >1%**	**-**	**Not <15%**	**-**
Genuine root	1.32	5.8	0.69	0.29	9.00	22.0	25.2
TVM A	1.30	3.45	2.32	.412	10.65	7.9	14.5
TVM B	1.42	4.2	1.93	.390	11.23	8.1	13.72
Kollam A	1.38	4.2	2.08	.380	12.11	19.59	17.32
Kollam B	1.5	3.91	2.01	.365	13.01	18.5	16.89
Alappuzha A	1.23	4.2	2.103	.452	9.1	11.4	16.7
Alappuzha B	1.21	4.3	1.99	.412	9.23	10.98	16.49
EKM A	1.82	3.46	1.232	.523	11.81	12	13.44

EKM B	1.4	3.2	1.265	.477	11.02	13.15	13.65
PTA A	1.2	3.65	1.9	.4	11.5	7.8	14.7
PTA B	1.45	3.72	1.87	.425	9.4	7.65	13.91
Kottayam A	1.2	4.2	2.39	.395	8.80	14.0	19.83
Kottayam B	1.25	4.4	2.60	.412	10.19	13.31	19.54
Thrissur A	1.3	5.6	2.2	.2002	8.7	11.8	15.72
Thrissur B	1.78	5.42	2.43	.382	10.53	12.42	16.37
Kozhikode A	1.24	3.5	1.24	.512	13.91	14.63	16.1
Kozhikode B	1.23	4.2	1.65	.476	13.45	14.12	16.34
Kannur A	1.7	4.2	2.13	.909	.410	7.9	17.9
Kannur B	1.83	4.1	1.12	2.43	.398	8.4	16.73
MLP A	1.05	4.4	2.11	.299	10.9	17.19	16.20
MLP B	1.12	3.9	1.92	.372	12.2	17.42	15.2
Wyanad A	1.5	5.4	2.4	.690	10.00	13.89	17.85
Wyanad B	1.29	4.9	2.13	.542	11.45	14.00	15.57
Palakkad A	1.34	3.8	1.29	.562	8.5	9.64	14.8
Palakkad B	1.5	3.9	1.36	.432	9.2	10.11	13.4
Idukki A	1.92	4.15	2.30	.300	14.4	9.7	17.13
Idukki B	1.67	4.02	1.99	.362	12.97	14.40	16.76
Kasargod A	1.47	3.86	2.43	.436	10.70	13.24	14.42
Kasargod B	1.49	4.5	2.07	.478	10.91	13.12	13.56

- TVM- Thiruvananthapuram, EKM- Ernakulam, PTA- Pathanamthitta, MLP- Malappuram

B)Statistical comparison of foreign matter mean in genuine root and market samples:-

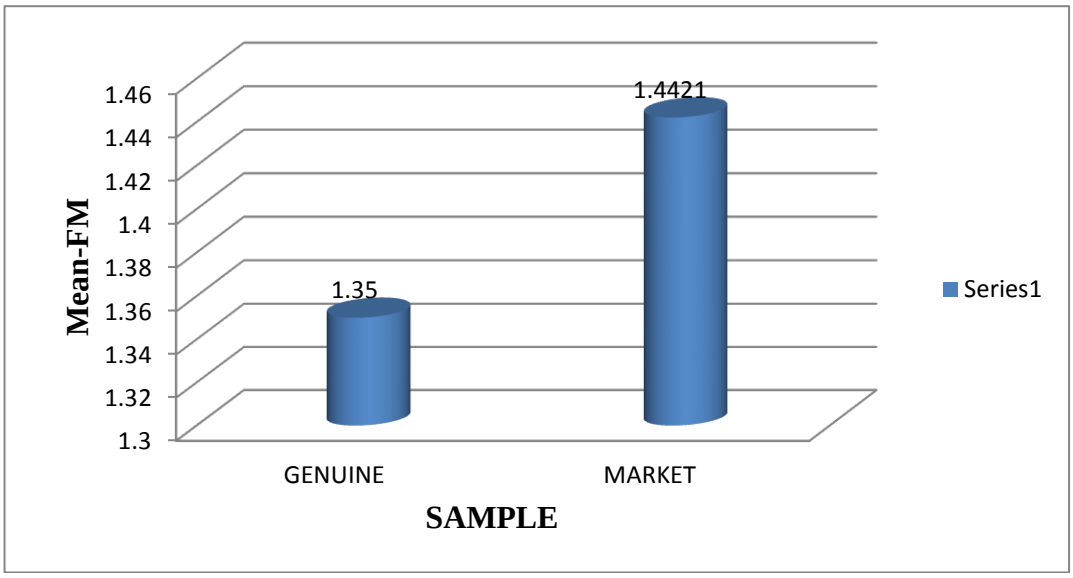
Table 2:-Data (mean+ SE) and test of significance (one sample t test) of foreign matter in market and genuine sample

Variable	Genuine sample value	Market sample mean value	CI	t	df	P
FM	1.350+0.032	1.442+0.047	1.345 to 1.54	1.942	27	0.000***

***significant at .1% level (p<0.001)

From Table, it is seen that the mean + SE of the genuine sample was 1.350+0.032 percent and the mean + SE value of the foreign matter in the market sample was 1.442+0.047 percent. Thus there exists significant increase in foreign matter in the market sample. The 95% CI for mean FM ranges from 1.345% to 1.54%. The comparative bar diagram is shown in graph 1.

Graph 1:-Comparative bar diagram of foreign matter in genuine root and market samples.



C) Statistical comparison of total ash mean in genuine root and market samples

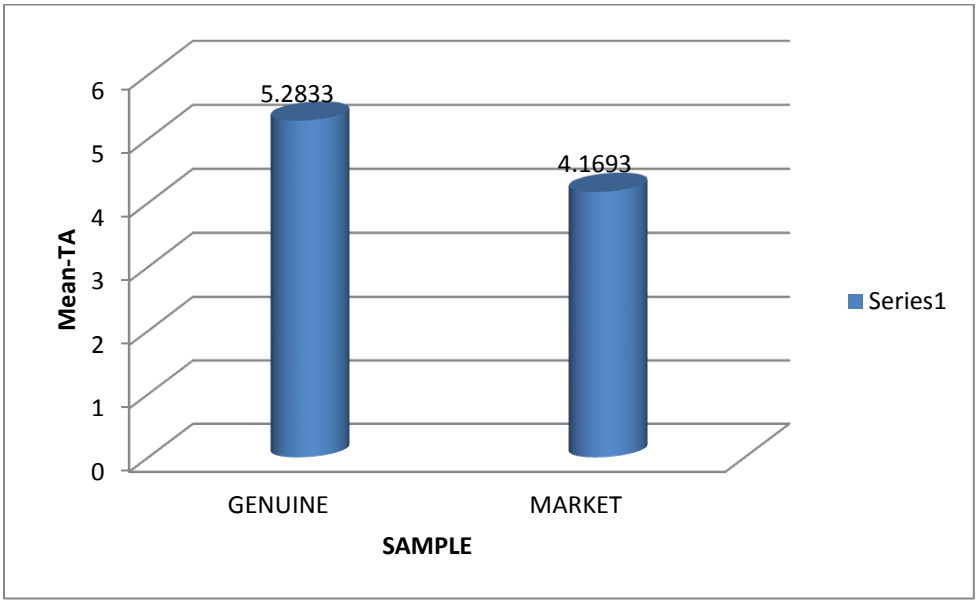
Table 3: Data (mean± SE) and test of significance (one sample t test) of total ash in market and genuine sample

Variable	Genuine sample value	Market sample mean value	CI	t	df	P
TA	5.283±0.294	4.1964±0.116	3.9 to 4.39	10.04	27	0.000***

Significant at .1% level (p<0.001)

From Table, it is seen that the mean ± SE of the genuine sample was5.283±0.294 percent and the mean ± SE value of the total ash in the market sample was 4.196±0.116percent. Thus there exists significant decrease in total ash in the market sample. The 95% CI for mean TA ranges from 3.94% to 4.39%. The comparative bar diagram is shown in graph 2.

Graph 2: Comparative bar diagram of total ash in genuine root and market samples.



D) Statistical comparison of acid insoluble ash mean in genuine root and market samples

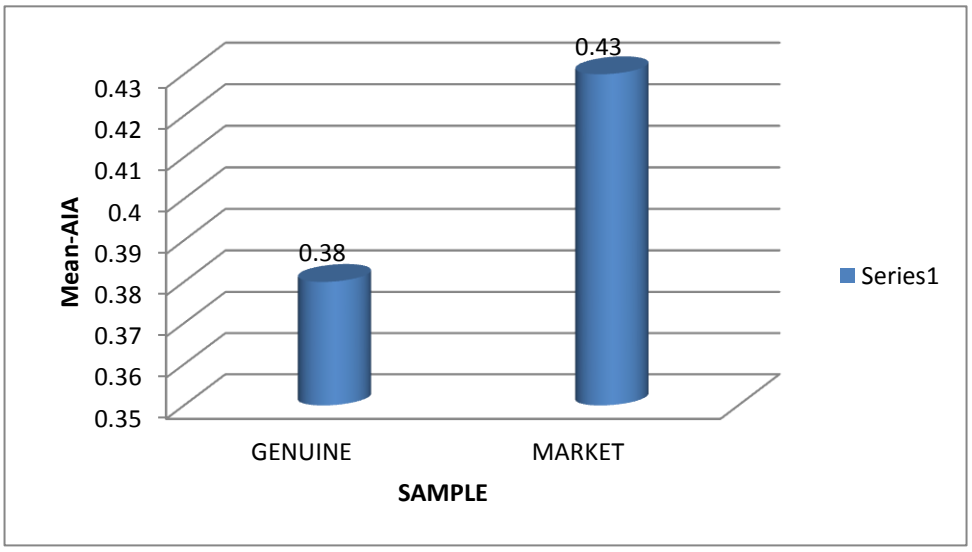
Table 4: Data (mean± SE) and test of significance (one sample t test) of acid insoluble ash in market and genuine sample

Variable	Genuine sample value	Market sample mean value	CI	t	df	P
AIA	.380±.062	.425±.017	0.39 to 0.46	2.572	27	0.008***

***significant at 1% level (p<0.01)

From Table, it is seen that the mean ± SE of the genuine sample was 0.380±0.062 percent and the mean ± SE value of the acid insoluble ash in the market sample was 0.425±0.017 percent. Thus there exists significant increase in acid insoluble ash in the market sample. The 95% CI for mean AIA ranges from 0.39% to 0.46%. The comparative bar diagram is shown in graph 3.

Graph 3: Comparative bar diagram of acid insoluble ash in genuine root and market samples.



E) Statistical comparison of water insoluble ash mean in genuine root and market samples

Table 5: Data (mean± SE) and test of significance (one sample t test) of water insoluble ash in market and genuine sample

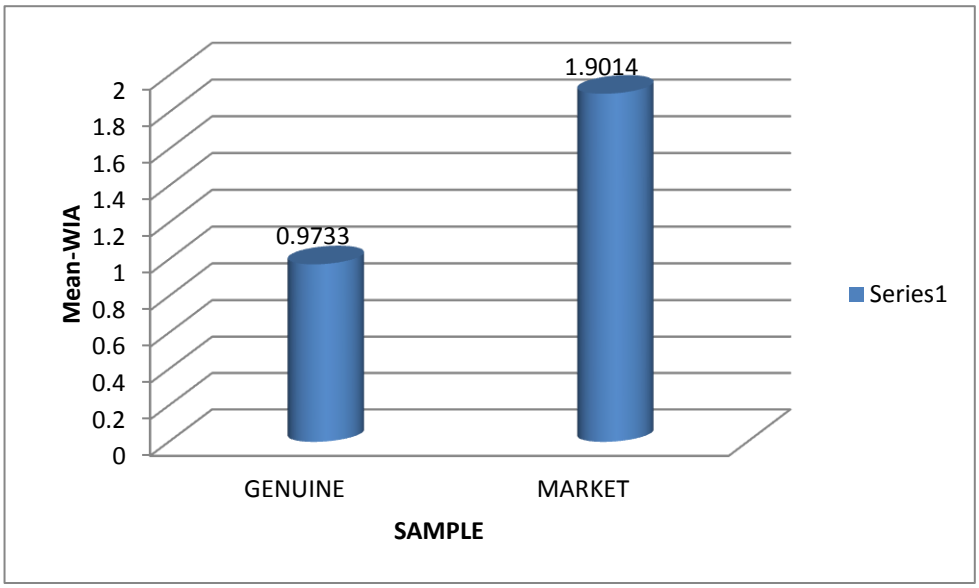
Variable	Genuine sample value	Market sample mean value	CI	t	df	P
WIA	0.973+0.159	1.901+0.087	1.72 to 2.08	10.634	27	0.000***

***significant at.1% level (p<0.001)

From Table, it is seen that the mean ± SE of the genuine sample was 0.937±0.159 percent and the mean ± SE value of the water insoluble ash in the market sample was 1.90±0.087 percent. Thus there exists significant increase in

water insoluble ash in the market sample. The 95% CI for mean WIA ranges from 1.72% to 2.08%. The comparative bar diagram is shown in graph 4.

Graph 4: Comparative bar diagram of water insoluble ash in genuine root and market samples.



F) Statistical comparison of alcohol soluble extractive mean in genuine root and market samples

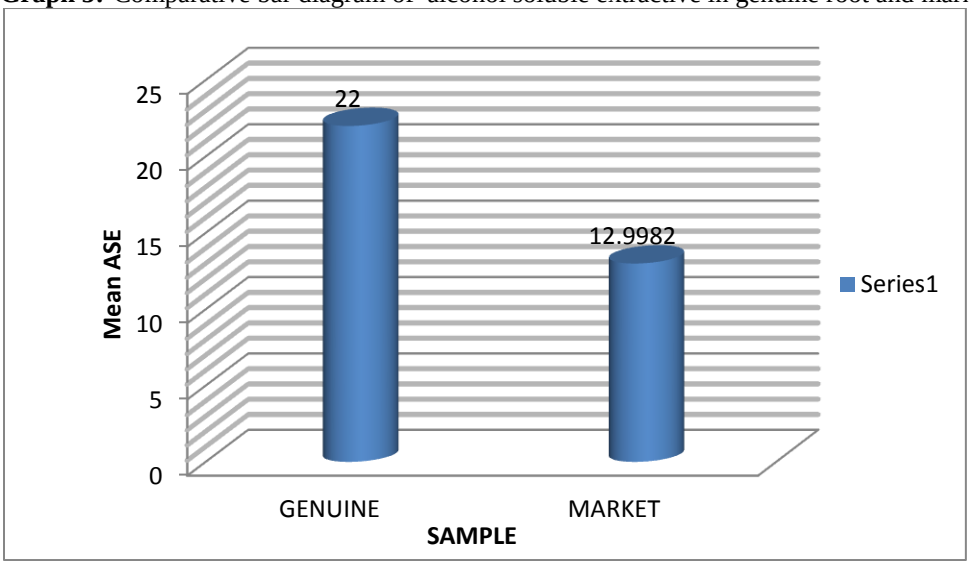
Table 6:-Data (mean+ SE) and test of significance (one sample t test) of water alcohol soluble extractive in market and genuine sample

Variable	Genuine sample value	Market sample mean value	CI	t	Df	P
ASE	22.0+1.154	12.998+.5424	11.88 to 14.11	16.598	27	0.000***

***significant at .1% level (p<0.001)

From Table, it is seen that the mean + SE of the genuine sample was 22.0+1.154 percent and the mean + SE value of the alcohol soluble extractive in the market sample was 12.998+0.54 percent. Thus there exists significant decrease in alcohol soluble extractive in the market sample. The 95% CI for mean ASE ranges from 11.88 %to 14.11%. The comparative bar diagram is shown in graph 5.

Graph 5:-Comparative bar diagram of alcohol soluble extractive in genuine root and market samples.



G) Statistical comparison of water soluble extractive mean in genuine root and market samples

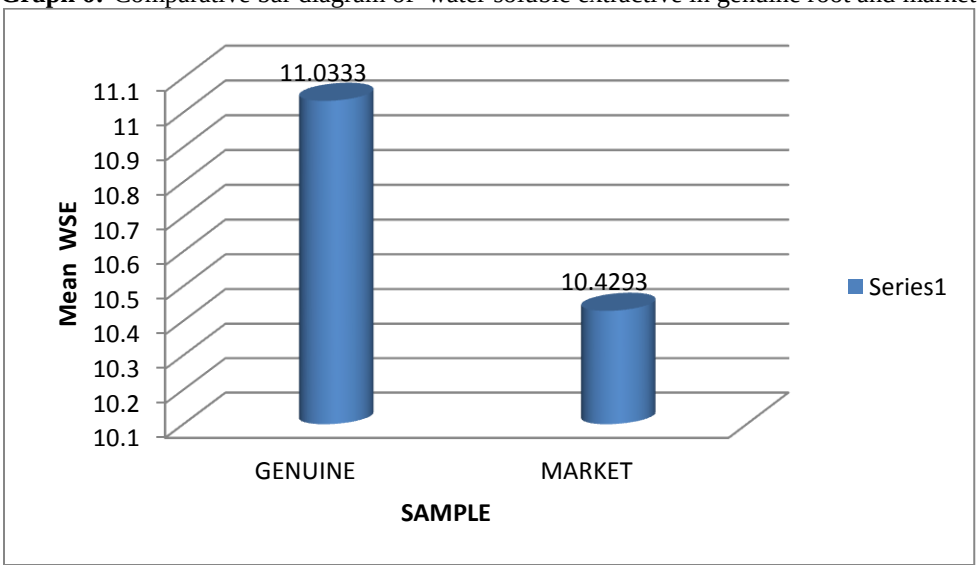
Table 7:-Data (mean+ SE) and test of significance (one sample t test) of water soluble extractive in market and genuine sample

Variable	Genuine sample value	Market sample mean value	CI	t	df	P
WSE	11.03+1.155	10.429+0.335	9.742 to 11.12	1.793	27	0.042*

*significant at 5% level ($p<0.05$)

From Table, it is seen that the mean + SE of the genuine sample was 11.03+1.155percent and the mean + SE value of the water soluble extractive in the market sample was 10.429+0.335 percent. Thus there exists significant decrease in water soluble extractive in the market sample. The 95% CI for mean WSE ranges from 9.742% to 11.12%. The comparative bar diagram is shown in graph 6.

Graph 6:-Comparative bar diagram of water soluble extractive in genuine root and market samples.



H) Statistical comparison of fibre content mean in genuine root and market samples

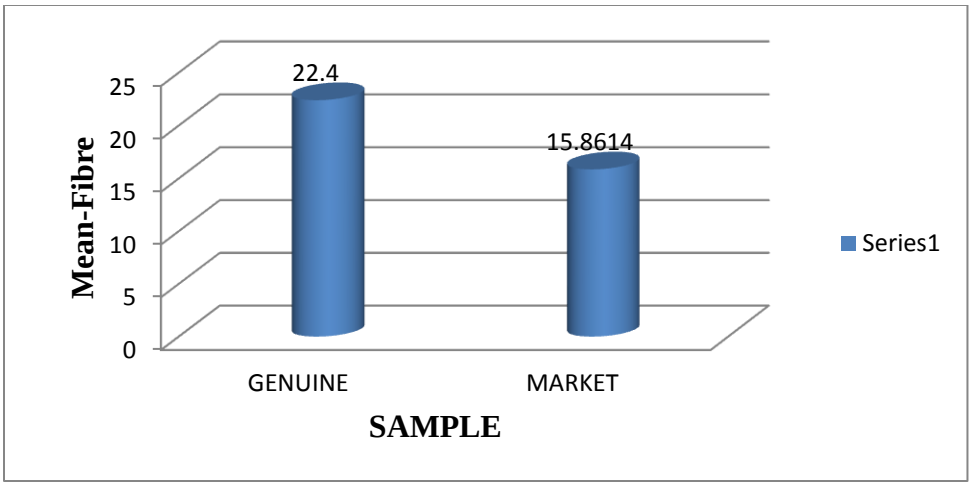
Table 8:-Data (mean+ SE) and test of significance (one sample t test) of fibre content in market and genuine sample

Variable	Genuine sample value	Market sample mean value	CI	t	Df	P
FC	22.40+1.56	15.86+0.335	15.729 to 16.55	19.485	27	0.000***

***significant at .1% level ($p<0.001$)

From Table, it is seen that the mean + SE of the genuine sample was 22.40 + 1.56 percent and the mean + SE value of the fibre content in the market sample was 15.86+0.335 percent. Thus there exists significant decrease in fibre content in the market sample. The 95% CI for mean FC ranges from 15.729% to 16.55%. The comparative bar diagram is shown in graph 7.

Graph 7: Comparative bar diagram of fibre content in genuine root and market samples.



I) Results of Qualitative tests done in genuine and Market samples of Ashwagandha

The ethanolic extracts of root of genuine *Ashwagandha [Withania somnifera (Linn*) Dunal] and market samples showed following constituents:

Table 9:-Qualitative analysis of genuine and Market samples of *Ashwagandha*

SAMPLES	Steroid	Flavanoid	Terpenoid	Alkaloid	Tannin	Saponin	Cardiac Glycoside	Phenol
Genuine root	+	++	+++	+++	++	-	++	++
28 Market samples	+	-	+++	+	++	-	++	++

+++ (Appreciable amount), ++ (Moderate amount), + (Traces)

Market samples-TVM A, TVM B, Kollam A, Kollam B, Alappuzha A, Alappuzha B, Ernakulam A, Ernakulam B, Pathanamthitta A, Pathanamthitta B, Kottayam A, Kottayam B, Thrissur A, Thrissur B, Kozhikode A, Kozhikode B, Kannur A, Kannur B, Malappuram A, Malappuram B, Wyanad A, Wyanad B, Palakkad A, Palakkad B, Idukki A, Idukki B, Kasargod A, Kasargod B

J)Inference from Qualitative Analysis:-

Ethanolic extract of genuine and market samples showed the presence of flavanoids, tannins, cardiac glycosides, terpenoids, alkaloids, phenols etc. Saponins were absent both in genuine and market samples.All similar constituents were found in genuine and market samples. But the amount of some was less comared to genuine sample**.**Alkaloids were present in trace amounts in market samples, while there was appreciable amount of it in the genuine sample.Flavanoids were moderately present in genuine sample, but it was completely absent in all the market samples. Terpenoids were present in appreciable amount in both the market and genuine samples. Steroids were present in trace amounts in both samples. All other chemical constituents like cardiac glycosides, tannin, phenol were present in moderate amount in both the samples.

K) Inference from Qualitative Analysis Of flavanoids

Fig 1: Qualitative analysis of Flavonoids of genuine and market samples.

- **I[st]sample –Genuine, II[cnd]sample- Market sample**

L) HPTLC analysis of Root of genuine Ashwagandha [*Withania somnifera (Linn)* Dunal]

HPTLC profiling of ethanolic extract of root of genuine Ashwagandha and market samples were done in solvent system Toluene: Ethyl acetate: Formic acid in propotion 5:5:1. The number of peaks as well as the Rf values were noted and the observation were as follows:

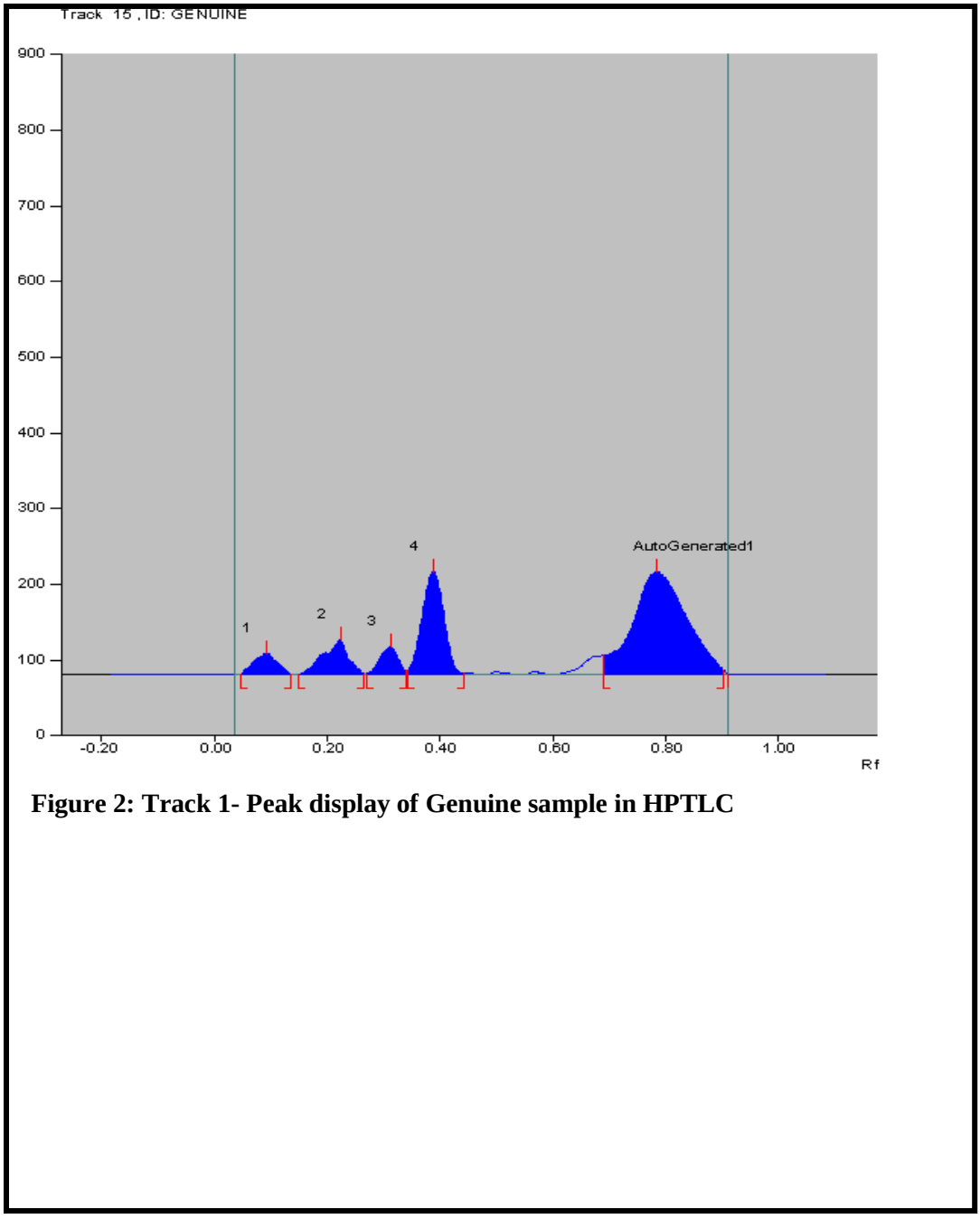
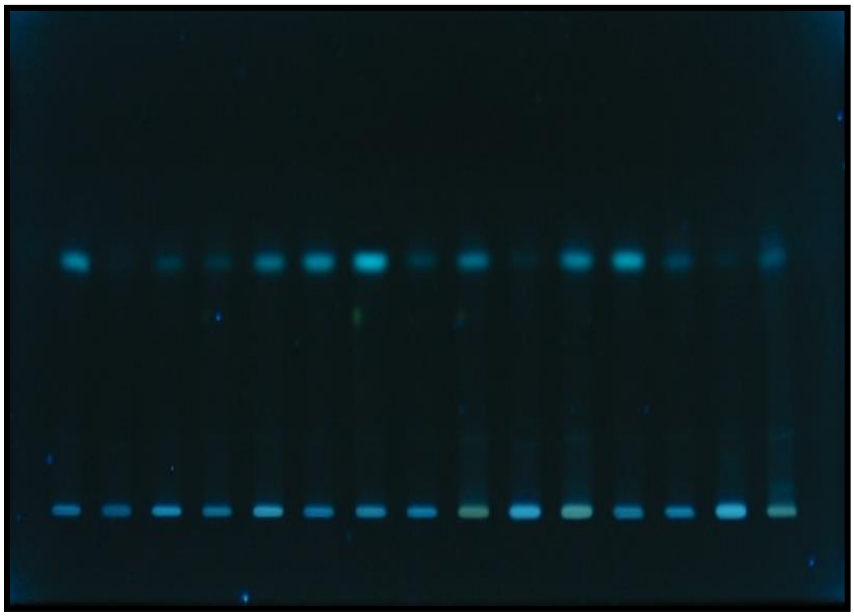


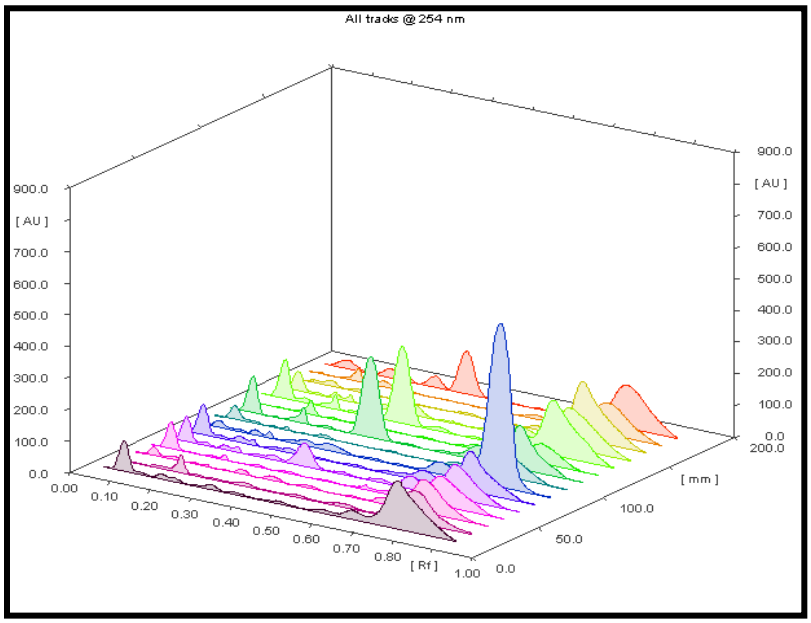
Figure 2: Track 1- Peak display of Genuine sample in HPTLC



Track	SAMPLE
1	TVM A
2	Kollam A
3	Alappuzha A
4	Ernakulam A
5	Pathanamthitta A
6	Kottayam A
7	Thrissur A
8	Kozhikode A
9	Kannur A
10	Malappuram A
11	Wyanad A
12	Palakkad A
13	Idukki A
14	Kasargod A
15	Genuine root

Table 10: Districts represented by each spot

Fig 3: HPTLC profile of ethanoic extract of Withania somnifera (Linn) Dunal root and market samples (A) of all districts (UV 366nm)



Track	SAMPLE
1	TVM A
2	Kollam A
3	Alappuzha A
4	Ernakulam A
5	Pathanamthitta A
6	Kottayam A
7	Thrissur A
8	Kozhikode A
9	Kannur A
10	Malappuram A
11	Wyanad A
12	Palakkad A
13	Idukki A
14	Kasargod A
15	Genuine root

Fig 4: 3D Display of Peaks of Genuine root and Market Samples of *Ashwagandha*

Table 11: Heavy metal content comparison in market and genuine sample

Sample Name	Heavy metal Concentration in ppm		
	Iron	Cadmium	Nickel

Trivandrum-A	7.17	0.0126	.1278
Trivandrum-B	7.77	.029	.158
Kollam- A	12.99	.0209	.2986
Kollam- B	10.38	.046	.192
Alappuzha-A	2.22	.0165	.1443
Alappuzha- B	3.16	.032	.178
Ernakulam- A	8.00	.0192	.1280
Ernakulam –B	5.89	.056	.203
Pathanamthitta- A	4.55	4.42	.0988
Pathanamthitta- B	3.87	3.94	.0191
Kottayam- A	3.30	.0166	.1166
Kottayam- B	4.44	.025	.1243
Thrissur- A	5.21	.049	.1304
Thrissur- B	5.65	.048	.055
Kozhikode- A	8.24	.041	1.1772
Kozhikode- B	7.65	.052	1.83
Kannur- A	5.33	.0173	.2102
Kannur- B	5.12	.028	.059
Malappuram- A	4.42	.0167	.0988
Malappuram- B	3.90	.033	.0191
Wyanad- A	4.58	.045	.1492
Wyanad- B	4.72	.044	.152
Palakkad-A	2.43	.052	.1231
Palakkad-B	3.45	.060	.263
Idukki- A	10.16	.046	.0953
Idukki- B	4.91	.039	.124
Kasargod- A	5.63	.0201	.0936
Kasargod- B	4.11	.012	.0878
Genuine	8.23	.036	.1576

Discussion:-

The physical and phytochemical evaluation is a very sensitive aspect in the process of standardization and quality control. The mean value of acid insoluble ash, water insoluble ash foreign matter, was higher than that of genuine sample. Whereas the mean value of total ash, alcohol soluble extractive, water soluble extractive, fibre content, were lower than that of genuine sample. All the values except alcohol soluble extractive were within the limits prescribed in API. Only there existed slight variations between genuine and market sample values. The slight variation in physicochemical and phytochemical results may be due to several factors such as different geographic condition, edaphic factors, environmental condition, period of cultivation and harvesting, method of collection, source of irrigation and fertilizers, age of the plant, powdering method and extraction method (Santhosh *et al.*, 2005)[4].

Foreign matter was comparatively high in market samples than genuine sample. It mainly consisted of visible moulds on the external surface. The increased value of foreign matter implies reduced purity of raw drugs. Herbal drugs should be made from stated part of the plant and be devoid of other parts of the same plant or other plants. They should be entirely free from moulds or insects, including excreta and visible contaminant such as sand and stones, poisonous and harmful foreign matter and chemical residues. Animal matter such as insects and "invisible" microbial contaminants, which can produce toxins, are also among the potential contaminants of all medicines (WHO,2004,2003;EMEA,2002) (Folashade *et al.*, 2012)[5].Such drugs should be rejected even though the percentage of other foreign matter are less in them. Special care should be taken to avoid the formation of moulds, since they may produce aflatoxins as per the study of Roy et al[6].

Total ash value represents the presence of inorganic salts like calcium oxalate crystals found naturally in the drug (Natural/physiological ash),as well as inorganic matter derived from external sources like sand(Non physiological ash). Incineration of herbal drugs produces ash which constitutes inorganic matter. Treatment of ash

with hydrochloric acid results in acid-insoluble ash which measures the amount of silica present, especially in the form of sand and siliceous earth.
The extractive value plays an important role in evaluation of crude drug. Extractive values indicate the nature of chemical constituents present in the drug. Water soluble extractive value is applied for the drugs which contain constituents such as sugars, and mucilage etc. Alcohol soluble extractive value is applied for the drugs which contain alcohol soluble constituents such as tannins, resins and alkaloids. (Kokate *et al.*,2014). Alcohol soluble extractive was found lower than genuine sample. The alcohol soluble extractive value proved to be higher than water soluble extractive value. This shows that the constituents of the drug are more extracted and soluble in alcohol compared to water.

Fibre content estimates the residue that remains after treatment with acid and alkali. The crude fibre contains cellulose, hemicelluloses, lignin etc components of the plant body. The fibre content of market samples were comparatively lower than genuine sample. As per Singh et al, High fibre content noted in the root was attributed to the delayed harvesting of plants. Fibre content may also increase with age because of the synthesis of cellulose, hemicelluloses, lignins etc that constitue the plant fibre increase with age[7.] Further, the authors were of the opinion that the root with less fibre content were mostly preferred and exploited for commercial purpose.

In next step qualitative analysis of genuine and market samples were done to determine the physiologically active chemical constituents in it. The study has got supreme importance since the therapeutic properties of the drug mainly depends on the chemical constituents. The result of qualitative analysis showed the presence of alkaloids, tannins, terpenoids, steroids etc. Saponins were absent in both genuine and market samples. Also alkaloids were present in fewer amounts compared to the genuine sample in all the market samples. Flavanoids were present in genuine sample, but absent in all the market samples.

The alkaloid content of the root was reported to vary between 0.13 to 0.31 but sometimes yield upto 4.3%(Singh & Kumar1998)[8].The wide variation in the yield of alkaloid may be due to several factors such as method of isolation used, genotype variability in species and genotype environmental interaction (Srivastava et al at 1960;Scharting et al 1963)[9].Ontogenic changes in alkaloid and withanolide content was reported by Kumar et al (2001)[10] that total alkaloid and withanolide content gradually deacrease with the advancement of crop age. The production of secondary metabolites is organ/ stage specific and depends largely on the physiological stage of the plant (Acamovic and Brooker 2005)[11]. The ontogeny or development stages could not be the only cause for increased production of secondary metabolites of *Withaniasomnifera (Linn)* Dunal. It is widely known that plant induce the production of secondary metabolites during low temperature as a part of their defence mechanism (Janskaet al.2010; Zhu et al.2007)[12]. Also, plants have higher content of secondary metabolites in tender and nutrient-rich young tissues as they attract herbivores and pathogens (Liu et al.1998; Van Dam and Bhairo – Marhe 1992)[13].

Most flavanoids are plastically produced as an acclimation process to environmental stressors (Manetas,2006; Albert et al, 2011, Anderson et al,2013; Hectors et al, 2014)[14]. Individuals in different populations exposed to varying environmental conditions usually show variable accumulation of flavonoids (Jaakola and Hahtola, 2010)[15]. Environmental factors (temperature, precipitation, solar radiation, etc.) in populations throughout the species distribution area are often subjected to latitudinal, longitudinal, or altitudinal gradients (Narbonaet al.2010, Arista et al, 2013; Prendeville et al., 2013)[16]; thus, flavonoid accumulation may show geographical clines. The plant can react to elevated and low temperatures by altering flavonoid synthesis in a species –specific way. In general too high a temperature can inhibit biosynthesis and cause degradation of flavonoids. A low temperature can increase flavanoid production, although the accumulation of flavonoids in cold temperatures is light dependent. There appears to be some evidence that cooler temperatures favour the production of flavonoids with a higher hydroxylation level[17]. The flavonoid synthesis also increases in plant during fruiting, flowering and colouration of flower stages[18].Phenol compounds are secreated to improve resistance when plant undergoes any type of external stressor response[19]. But in the present study the amount of phenol showed no such variations in both market and genuine sample.

Studies by Anna et al suggest ecological conditions like insect feeding; microbial infections may affect secondary metabolite and in turn chemical composition of the plant. Also different parts of same plant contain different concentration of chemical constituents. At the same time diurnal variations and seasonal changes also account for variability in herbal medicines. The therapeutic or toxic components of plant vary depending on the part of plant used as well as stages of ripeness ripening (Drew and Myers, 1997) [20]. Shelf-life also has obvious impact on availability of active principle.

For further authentification and identification of chemical constituents TLC and HPTLC analysis of genuine root and market samples were carried out. It was found that the no of peaks in the market samples varied from one to eight compared to five peaks in the genuine sample. Although slight variations were seen in TLC and HPTLC profile of root of *Withania somnifera (Linn)* Dunal, the similarities suggested the presence of similar chemical constituents in it. The variation may be due to the microbial contamination of market samples. The chemical constituents in a plant may vary depending on the stage of collection, parts of plant collected, harvest seasons, plant origin (regional status), drying processes, storage conditions and other factors (Kamboj,2012) [21].

The presence of various heavy metals was also screened in the genuine and market samples and it was found out to be in the permissible limits in both market and genuine samples. The concentration `of iron was high in genuine root samples and some market samples but was in permissible limits described in API.

Quality is the sum of all the factors which contribute directly or indirectly to the safety efficacy and acceptability of the product. So care should be taken in the storage of herbal drugs to avoid microbial contamination and also to get maximum concentration of active principles. Also care should be taken to harvest *Ashwagandha* at proper time in order to get complete therapeutic effect. Although, standardization of herbal products is not an easy task, manufactures must ensure proper testing of raw materials for better product development. Bodies like AYUSH should issue standards for presence of active principles in finished Ayurvedic products.

Conclusion:-

The result of preliminary physico- phytochemical evalution of market samples were identical to that of the genuine sample and also the standards mentioned in Ayurvedic Pharmacopoeia of India.The market samples of *Ashwagandha* collected from the raw drug markets of the fourteen districts of Kerala are genuine

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Reference:-

1.Annual demand of 32 priorized medicinal plants of India,2012.page1-3

2. Atul Kumar Shrivastava PKS. Economics of yield and production of alkaloid of Withania somnifera (Dunal) Linn. American Journal of Plant Sciences. 2013;4:2023-3

3.Shinda Ashastri et al.Yield and Phytochemical evaluation of wild and cultivated samples of Ashwagandha. Journal of Biological and Scientific Opinion. Volume2(2).2014;P153

4.Santhosh MK, Shaila D, Chandrakumar T. Physicochemical and Phytochemical examination of medicinal plants used in indigenous system of medicine, vol 2. - journal of Chemistry. 2005; pp 142-151. Available from : http://www.e-journals.net

5. Kunle, Folashade O, Egharevba, Omoregie H, Ahmadu, Ochogu P.Standardization of herbal medicines-A review.International Journal of Biodiversity and Conservation. 2012; 4(3): 101-12

6. De Freitas Araujo MG, Bauab TM. Microbial Quality of Medicinal Plant Materials: INTECH Open Access Publisher; 2012.

7. Studies on Some Medicinal Plants used in Ayurvedic Formulations 1994-2001; Availble from: http://shodhganga.inflibnet.ac.in/bitstream/10603/6813/9/09 chapter%201.pdf.

8. Singh S, Kumar S. Chemical constituents of Ashwagandha (W. somni-fera). Singh S, Kumar S, editor, Withania somnifera The Indian Ginseng Ashwagandha, Central Institute of Medicinal and Aromatic Plants, Lucknow, ‘ India, 1998; pp 293 .

9. Srivastava SK, Iyer SS, Ray GK. Estimation of the total alkaloids of Withania somnifera (Dunal). Indian Journal of Pharmacy 22,1960; pp 94-95 .

10. Kumar A, Kaul BL, Verma HK. Phenological observations on root yield and chemical composition in different morphotypes of Withania somnifera. J Med Aro Plant Sci 23, 2001; p 21–23.

11.Acamovic T, Brooker JD. Biochemistry of plant secondary metabolites and their effect in animals. Proc Nutr Soc 64, 2005; p 403–412.

12. Janska´ A, Marsˇka P, Zelenkova´ S, Ovesnaˇ J. Cold stress and acclimation—what is important for metabolic adjustment? Plant Biol 12, 2010; p 395–405.

13. Liu Z, Carpenter SB, Bourgeois WJ, Yu Y, Constantin RJ, Falcon MJ, Adams JC. Variations in the secondary metabolite camptothecin in relation to tissue age and season in Camptotheca acuminata. Tree Physiol 18, 1998; p 265–270.
14. Manetas Y. Why some leaves are anthocyanic and why most anthocynic leaves are red? 2006 ; Flora 201, 163 - 177. 10.1016/j.flora.2005.06.010 [Cross Ref].
15. Jakoola L, Maatta- Riihinen K, Karenlampi S, Hohtola. Activation of flavonoid biosynthesis by solar radiation in bilberry (Vaccinum myrtillus L) leaves Planta 218, 2004; p 721-728.10.1007/s00425-003-1161-x [PubMed][Cross Ref].
16 . Narbona E, Guzman B, Arroyo J, Vargas P. Why are fruits of Citrus ladanifer (Cistaceae) so variable: a multi-level study across the western Mediterranean region. Perspect. Plant Ecol Evol Syst, 12. 2010; p 305 -315. 10.1016/j.ppees.2010.06.001
17. Laura J, Anja H. Effect of latitude on flavonoid biosynthesis in plants. Plant , Cell and Environment ,2010; 33: p 1239-1247.
18. Lattanzio V, Lattanzio VM, Cardinali A, Role of phenolics in the resistance mechanism of plant against fungal pathogens and insects. Phytochemistry: Advances in research 2006; p 23- 67. ISBN 81-308-0034-9.
19. Andersen OM, Markham KR, editors. Flavanoids Chemistry, Biochemistry and Applications. Boca Raton, London, New York, FL Part of the Taylor & Francis imprint, a member of the Taylor & Francis Group, the Academic Division of T & F informa Plc, 2006;
20. Drew AK, Myers SP. Safety issues in herbal medicine: implications for the health professions. The Medical journal of Australia. 1997; 166(10): 538-4.
21. Kamboj A. Analytical evaluation of herbal drugs: INTECH Open Access Publisher; 2012.