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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND THIN LAYER CHROMATOGRAPHY OF INDIAN *ASPARAGUS OFFICINALIS* LINN.

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Abstract

Asparagus officinalis Linn is a medicinal plant of temperate Himalayas belonging to the family Liliaceae. *Asparagus officinalis* is one of the most nutritionally well balanced vegetables in existence, and it possess a variety of biological properties. This is high in folic acid, thiamin, vitaminB6, rutin. Traditionally it is used as a powerful cardiac sedative, hepatoprotective activity and the roots of *Asparagus officinalis* is more diuretic than its shoots, it is recommended in dropsy, its roots have been used as a remedy for schistosomiasis and tuberculosis. The present study sought to perform successive solvent extraction by using non polar to polar solvents, and investigate the chemical constituents by preliminary phytochemical screening, followed by isolation and identification of constituents by means of thin layer chromatography. The various crude extracts of *Asparagus officinalis* is subjected to thin layer chromatography and separation of spots were observed under day light, shorter wavelength 254nm and longer wavelength 365nm by using different solvent systems, and the Rf values were compared with standard drugs of rutin and quercitin. TLC and qualitative phytochemical analysis revealed the presence of some active phytoconstituents. Such as flavanoids, glycosides, alkaloids, saponins. Among all the solvent extracts, alcoholic, methanolic and aqueous extracts showed well separation of spots. The retardation factor was found to be 0.35 and 0.98 which is similar to standard rutin and quercitin.

Introduction:-

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. The medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, saponins, flavonoids and phenolic compounds. This is high in folic acid, thiamin, vitaminB6, rutin. Traditionally it is used as a powerful cardiac sedative, hepatoprotective activity and the roots of *Asparagus officinalis* is more diuretic than its shoots, it is recommended in dropsy, its roots have been used as a remedy for schistosomiasis and tuberculosis. [3,4,5] steroidal saponins have been evaluated *in vitro* for activity against human and animal cancer cell lines.[6,7,8] Many of the indigenous medicinal plants are used as spices and food plants. They also sometimes added to foods as a nutritive

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substance. Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Different parts of the plants like bark, roots, stems, leaves, berries, spears etc. are used as per medicinal properties. *Asparagus officinalis* Linn is a dioecious, perennial herb with scale-like leaves and an erect, multi-branched stem that grows up to 3 m in height. Asparagus is native to Europe and Asia and is cultivated widely. The aerial stems or spears arising from rhizomes are consumed as a vegetable. The fleshy roots and, to a lesser degree, seeds have been used for medicinal purposes. [1,2]. In order to identify the bioactive compounds responsible for the above pharmacological activities, phytochemical studies have been carried out for various extracts. This study is based on its traditional medicinal use of *Asparagus officinalis*. Figure no-1.

Materials and Methods:-

Collection of plant:-

Asparagus officinalis Linn. Was commonly known as vegetable herb. It is indigenous to Africa, Asia and European countries. The plant material was collected from the region of Tirupathi in Andhra Pradesh, India in the month of October 2016 and it was authenticated by Dr. Madhava chetty professor of botany department, Andhra University, Andhra Pradesh. A herbarium is prepared and submitted for future reference in the department of pharmacognosy in Nalanda College of Pharmacy. Under the Vocher no: NCOP/Ph'cog/2016-2017/2550.

Figure no-1*:* *Asparagus officinalis* Linn

Preparation of plant extract:-

The plant material was thoroughly washed, shade dried, and coarsely powdered by using grinder then it was subjected to soxhlation for 34 hrs at 30˚ C by using petroleum ether, n-hexane, Toluene, ethyl acetate, chloroform, ethanol, methanol and water by successive solvent extraction method based on the increasing order of polarity of solvent. The thick mass was evaporated with the help of rotary vacuum evaporator and percentage yield was calculated. The extracted crude drug was subjected to the physiochemical screening and TLC profiling studies. The dried extract was properly stored in the desiccators for further experiment and analysis.

Phytochemical Screening:-

Chemical tests were performed for the identification of bioactive chemical constituents like alkaloids, carbohydrates, glycosides, saponins, phenolic compounds, phytosterols, proteins, amino acids, flavonoids, and tannins. Phytochemical screening was carried out for various extracts by using standard procedure.

Thin layer chromatographic studies:-

Each solvent extract was subjected to thin layer chromatography. TLC was performed to analyze the variation in bioactive chemical constituents. Readymade TLC plates (coated with silica gel 60 F*254* on aluminum sheets) purchased from Merck Germany were used. The powdered sample was extracted with different procedures for the identification of each of the active constituents i.e. anthracene glycosides, arbutin, cardiac glycosides, flavonoids, bitter principles, saponins, coumarins and alkaloids. The mobile phase solvent systems used were ethyl acetate: methanol: water (100:13.5:10) for the detection of anthracene glycosides, arbutin, cardiac glycosides, bitter principles and alkaloids. The mobile phase ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26) was used for flavonoids identification and for the identification of saponin, the solvent system of chloroform: glacial acetic acid: methanol: water (64:32:12:8) was used while for the identification of coumarins, toluene: ethyl acetate (93:7) was used.[9] The developed chromatograms were analyzed for presence of drug constituents by spraying with an appropriate group reagent/s. The chromatograms were then observed under UV-254 nm and UV-365 nm light. Photos were taken with Nikon camera and the R*f* values were calculated with the following formula.

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Thin layer chromatography is a preliminary investigation of active constituents in plants was carried out by thin layer chromatography (TLC) technique. After applying specific spraying reagents for a particular active constituent, the results of TLC showed the presence of anthraglycosides, arbutin, flavonoids, cardiac glycosides and coumarins in alcoholic extract of *Asparagus officinalis.* Rf values were calculated for all the spots.

Results:

Percentage of yield extract:

The yield of sequential extracts (g) is shown in [Table 1]. The amount of crude extract obtained from petroleum ether, n-hexane, Toluene, ethyl acetate, chloroform, ethanol, methanol and water and the percentage yield was found to be 0.67, 06, 0.56, 0.9, 1.09, 1.10, 1.08 and 1.06.

Table no 1:- Percentage yield of extracts of *Asparagus officinalis* Linn.

S. No	Solvent Color of extract	Colour of the extract	Percentage yield(%w/w)
1	Petrolium ether	Brown	0.67
2	n-hexane	Brown	0.6
3	Toluene	Brown	0.56
4	Ethyl acetate	Brown	0.9
5	Choloroform	Green	1.09
6	Ethanol	Brown	1.10
7	Methanol	Green	1.08
8	Water	Brown	1.06

Phytochemical Screening:-

The present study carried out in the *Asparagus officinalis* Linn revealed the presence of medicinal active constituents. The phytochemical active compounds of *Asparagus officinalis* were qualitatively analysed and the results are presented in Table 2. In these screening process alkaloids, glycosides, flavonoids, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, proteins, amino acids, and vitamin c. Shows different types of results in different solvents extracts. Among these phytochemical screening, Alkaloids, glycosides, flavanoids Saponinis,steroids, phenolic, Tannins, carbohydrates, proteins & Amino acids, were present in alcoholic solvent extracts whereas steroids and saponins are present in almost all extracts.

Table no 2:- Phytochemical screening of *Asparagus officinalis* Linn.

Phyto-constituents	Petrolium ether	n-hexane	Toluene	Chloroform	Alcohol	Methanol	Water
Carbohydrates	-	-	-	-	+ve	+ve	+ve
Proteins	-	-	-	-	+ve	+ve	+ve
Amino acids	-	-	-	-	+ve	+ve	-
Fats	+ve	+ve	-	+ve	+ve	-	-
Alkaloids	-	-	-	-	+ve	+ve	+ve
Glycosides	-	-	-	-	+ve	+ve	+ve
Flavonoids	+ve	+ve	-	-	+ve	+ve	+ve
Tannins & Phenolic	-	-	-	-	+ve	+ve	+ve
Steroids	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Saponins	+ve	+ve	-	-	+ve	+ve	+ve
Vitamin-C	-	-	-	-	+ve	-	-

Thin layer chromatographic studies:-

Thin layer chromatographic analysis provides a chromatographic drug fingerprints. It is therefore suitable for monitoring the identity and purity of drugs and for detecting adulterants and substitutions. With aid of appropriate separation procedures, TLC can be used to analyze drug combinations and phytochemical preparations. In thin layer

chromatographic analytical method a large number of solvent systems were tried to achieve a good resolution. Finally, the solvents system Toluene: Ethyl acetate (93:7) and Ethyl acetate: Methanol: Water (100:13.5:10). It was shown the good resolution in alcoholic and chloroform extracts. Visibility of spots in long wavelength, shorter wavelength and visible light was examined separately. The Number of spots, colour of the spots, and calculated *Rf* values are tabulated in Table: 3, and related figures of thin layer chromatography are in figure no: 2

Table no 3: Thin Layer Chromatography *Asparagus officinalis* Linn

Type of extract	Solvent system & its ratio	Wave length (nm)	Spots	Colour of spot	*Rf* Values
Alcohol	Toluene: Ethyl acetate(93:7)	365nm(Longer Wave length)	9	Reddish Yellow Blue Reddish Blue Reddish Blue Reddish Blue	0.72 0.70 0.69 0.67 0.65 0.5 0.50 0.45 0.34
Alcohol	Toluene: Ethyl acetate(93:7)	254nm (Shorter Wave length)	3	Greenish yellow Greenish yellow Greenish yellow	0.50 0.45 0.34
Alcohol	Toluene: Ethyl acetate(93:7)	Visible Light	3	Greenish yellow Greenish yellow Greenish yellow	0.50 0.45 0.34
Alcohol	Ethyl acetate: Formic acid: Glacial acetic acid: Water (100: 11 :11:26)	365nm(Longer Wave length)	2	Orange Blue	0.96 0.86
Alcohol	Ethyl acetate: Formic acid: Glacial acetic acid: Water (100: 11 :11:26)	254nm (Shorter Wave length)	1	Dark brown	0.96
Alcohol	Ethyl acetate: Formic acid: Glacial acetic acid: Water (100: 11 :11:26)	Visible Light	1	Dark Green	0.96
Alcohol	CHCL3: Glacial acetic acid: Methanol: H20 (64:32:12:8)	365nm(Longer Wave length)	4	Orange Blue Yellow Pink	0.98 0.96 0.94 0.92
Alcohol	CHCL3: Glacial acetic acid: Methanol: H20 (64:32:12:8)	254nm(Shorter Wave length)	1	Dark Green	0.98
Alcohol	CHCL3: Glacial acetic acid: Methanol: H20 (64:32:12:8)	Visible Light	1	Green	0.98
Aqueous	CHCL3: Glacial acetic acid: Methanol: H20 (64:32:12:8)	365nm(Longer Wave length)	1	Blue quenching	0.96
Aqueous	CHCL3: Glacial acetic acid: Methanol: H20 (64:32:12:8)	254nm(Shorter Wave length)	1	Brown tailing	0.96
Aqueous	CHCL3: Glacial acetic acid: Methanol: H20 (64:32:12:8)	Visible	1	Brown tailing	0.96
Alcohol	Ethyl acetate: Methanol: Water (100:13.5:10)	365nm(Longer Wave length)	1	Reddish-orange	0.96
Alcohol	Ethyl acetate: Methanol: Water (100:13.5:10)	254nm (Shoter Wave length)	1	Dark Green	0.96
Alcohol	Ethyl acetate: Methanol: Water (100:13.5:10)	Visible	1	Dark Green	0.96
CHCL3	Toluene: Ethyl acetate(93:7)	365nm(Longer Wave length)	7	Pink Yellow Blue Pink	0.49 0.47 0.45 0.33

				Blue Blue Pink	0.18 0.16 0.15
CHCL3	Ethyl acetate: Formic acid :Glacial acetic acid :water(100:11:11:26)	365nm(Longer Wave length)	2	Pink Blue	0.96 0.86
CHCL3	Ethyl acetate:Formic acid :Glacial acetic acid :water(gly)	254nm (Shoter Wave length)	2	Light Green Quenching	0.96 0.86
CHCL3	CHCL3: Glacial acetic acid: Methanol: H20 (64:32:12:8)	365nm(Longer Wave length)	2	Pink Pink	0.98 0.56
CHCL3	CHCL3: Glacial acetic acid: Methanol: H20 (64:32:12:8)	254nm (Shoter Wave length)	2	Dark Green Dark Green	0.98 0.56
CHCL3	CHCL3: Glacial acetic acid: Methanol: H20 (64:32:12:8)	Visible	2	Green Green	0.98 0.56
CHCL3	Ethyl acetate: Methanol : Water (100:13.5:10)	365nm(Longer Wave length)	3	Yellow fluorescent Blue Orange-Yellow	0.94 0.95 0.86 0.76
CHCL3	Ethyl acetate: Methanol : Water (100:13.5:10)	254nm (Shoter Wave length)	3	Light Yellowish Light Yellowish Light Yellowish	0.94 0.86 0.76
Ethyl Acetate	Toluene: Ethyl acetate(93:7)	365nm(Longer Wave length)	2	Pink Blue	0.58 0.56
Ethyl Acetate	Ethyl acetate:Formic acid:Methanol: Water(100:11:11:26)	365nm(Longer Wave length)	2	Pink Blue fluroscence	0.98 0.9
Ethyl Acetate	Ethyl acetate: formic acid: Methanol: Water(100:11:11:26)	254nm (Shoter Wave length)	2	Green	0.98 0.9
	Ethyl acetate: Formic acid:Methanol: Water(100:11:11:26)	Visible	2	Green	0.98 0.9
Ethyl Acetate	Ethyl acetate: Methanol : Water (100:13.5:10)	365nm(Longer Wave length)	2	Pink Blue	0.86 0.84
Ethyl Acetate	Ethyl acetate: Methanol : Water (100:13.5:10)	Visible	2	Green Green	0.86 0.84

Discussion:

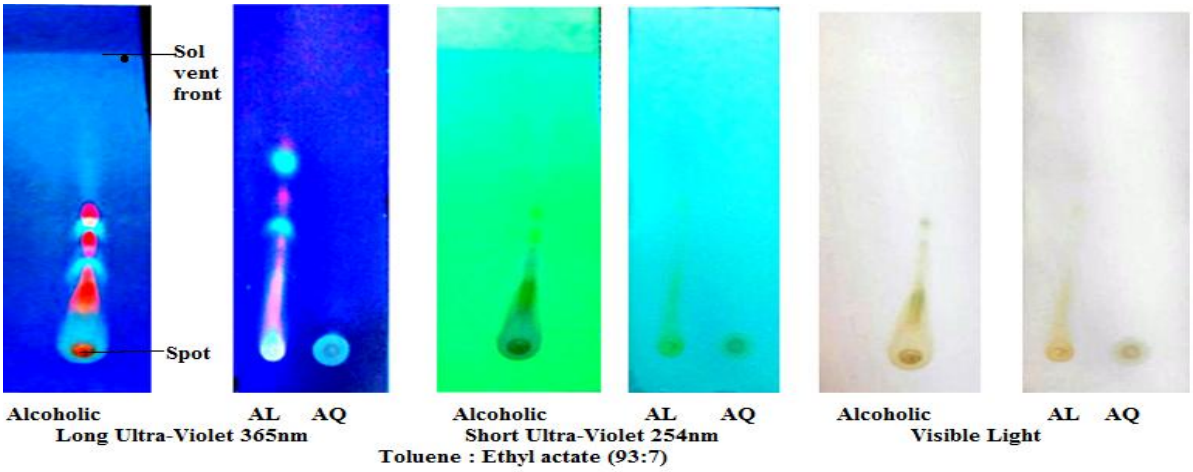
The pharmacological importance of a drug is attributed to the various secondary metabolites present in it and a particular compound might possess a clinical significance. Therefore it is essential to separate the compounds present in the plants with an appropriate chromatographic method. TLC technique has proved its worth as a simple, inexpensive and reproducible method for the chemical and biological screening of plant extracts. It provides a basic idea of polarity of a particular chemical constituent. Development of TLC plates with appropriate group reagents indicates the presence of anthraglycosides, arbutin, flavonoids, cardiac glycosides and coumarins in *Asparagus officinalis* powder. The pattern of bands on TLC plates provides fundamental data and is used to demonstrate the consistency and stability of herbal components. It is a potent and rapid way to distinguish between chemical classes which may not be fulfilled by macroscopic and microscopic analysis. TLC is most recommended technique to create the fingerprints of herbal medicines because of its simplicity, versatility, specific sensitivity and easy sample preparation. Thus, TLC is a convenient method of determining the quality and purity. To detect the possible adulteration of herbal drugs.

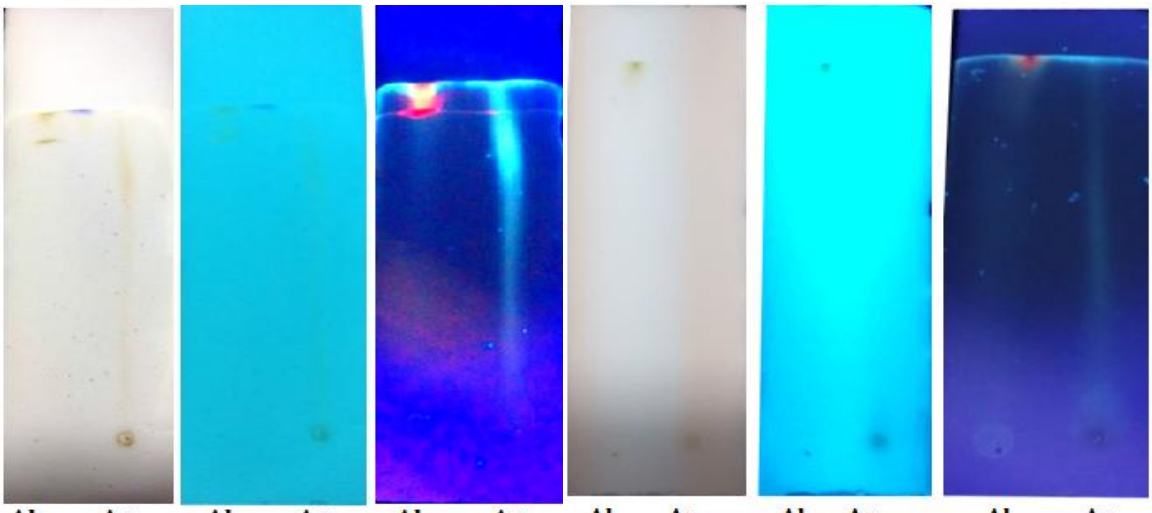
A large number of plants produce secondary metabolites such as alkaloids, flavanoids, phenols, terpenoids, steroids and quinines that are used in pharmaceuticals, cosmetics and pesticide industries. Thus the present study confirms the traditional medical practice and previous pharmacological observations and supplement treatment for other health problems such as allergic reactions, arthritis, some malignancies, and diseases resulting from hormone deficiencies or abnormal production etc[5,6]. In the present study, phytochemical screening for all five extracts

showed significant indication about the presence of metabolites. Alkaloids, Saponinis, Tannins, Amino acids, Flavanoids and Terpenoids, were found to be present in the all the sequential extracts of *Asparagus officinalis*. The results of the present study also supplement the folkloric usage of the studied plants which possess several known and unknown bioactive compounds with bio-activity. By isolating and identifying these bioactive compounds new drugs can be formulated to treat various diseases and disorders. TLC profiling of all 5 extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals gives different Rf values in different solvent system. This variation in *Rf* values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the *Rf* values of compounds in different solvent system. Different *Rf* values of the compound also reflect an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

Conclusion:

The plant screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. These findings suggested that asparagus could be a potential source of natural of natural antioxidant having great importance as therapeutic agent and preventing oxidative stress related degenerative diseases. The crude extract of *Asparagus officinalis* can provide lead molecules which could be useful substrate for the synthesis of new drugs for the treatment of dropsy, hepatic diseases and heart related diseases. Further purification, identification and characterization of the active compounds would be our priority in future studies.

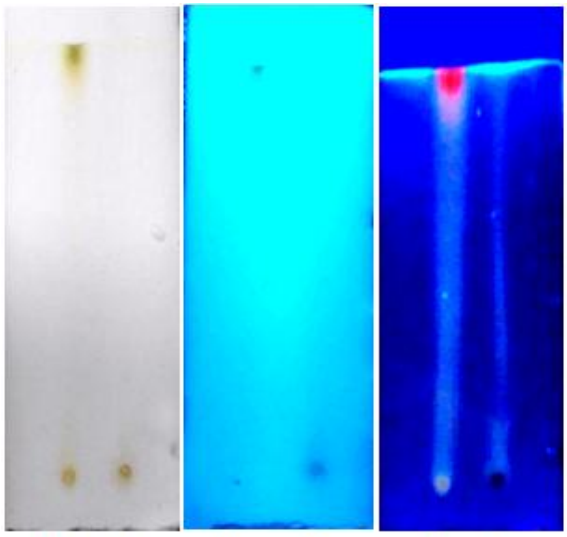




Al Aq Al Aq Al Aq Al Aq Al Aq Al Aq

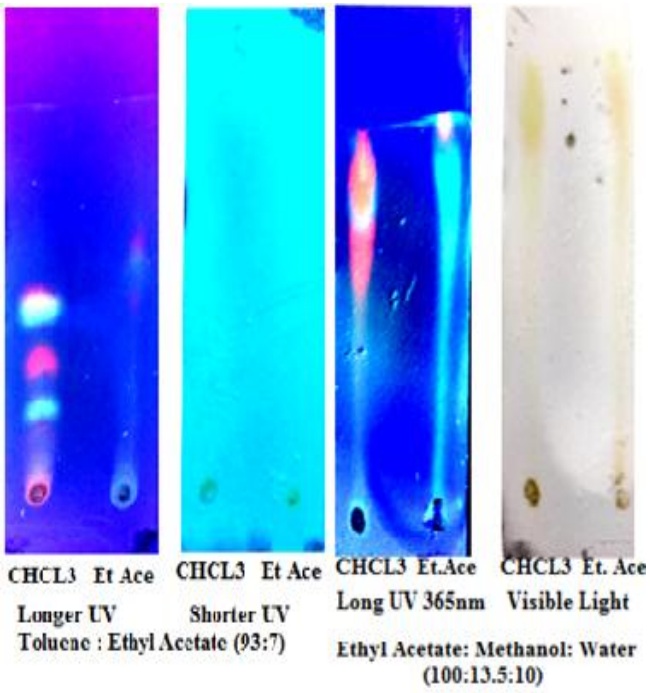
Solvent system:
CHCL3: GAA: Methanol: Water
(64:32:12:8)

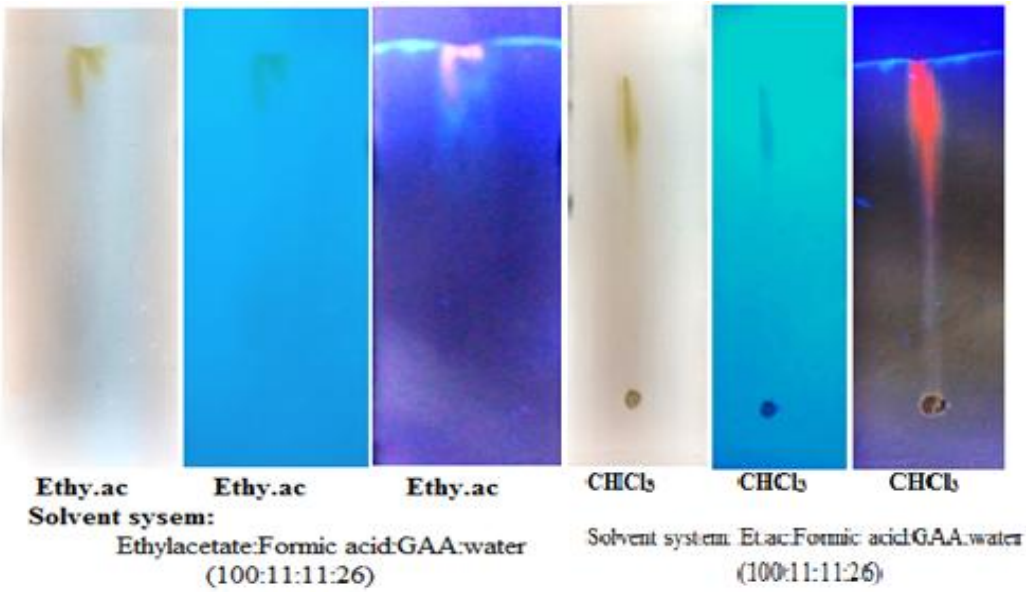
Solvent system:
Ethylacetate:Formic acid:Glacial Acetic Acid:Water
(100:11:11:26)



Al Aq Al Aq Al Aq

Solvent system: Ethyl acetate: Methanol: Water
(100:13.5:10)


CHCL3 Et Ace
CHCL3 Et Ace
CHCL3 Et.Ace
CHCL3 Et. Ace
Longer UV
Shorter UV
Long UV 365nm
Visible Light
Toluene : Ethyl Acetate (93:7)
Ethyl Acetate: Methanol: Water
(100:13.5:10)


Ethy.ac
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CHCl3
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Ethylacetate:Formic acid:GAA:water
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(100:11:11:26)

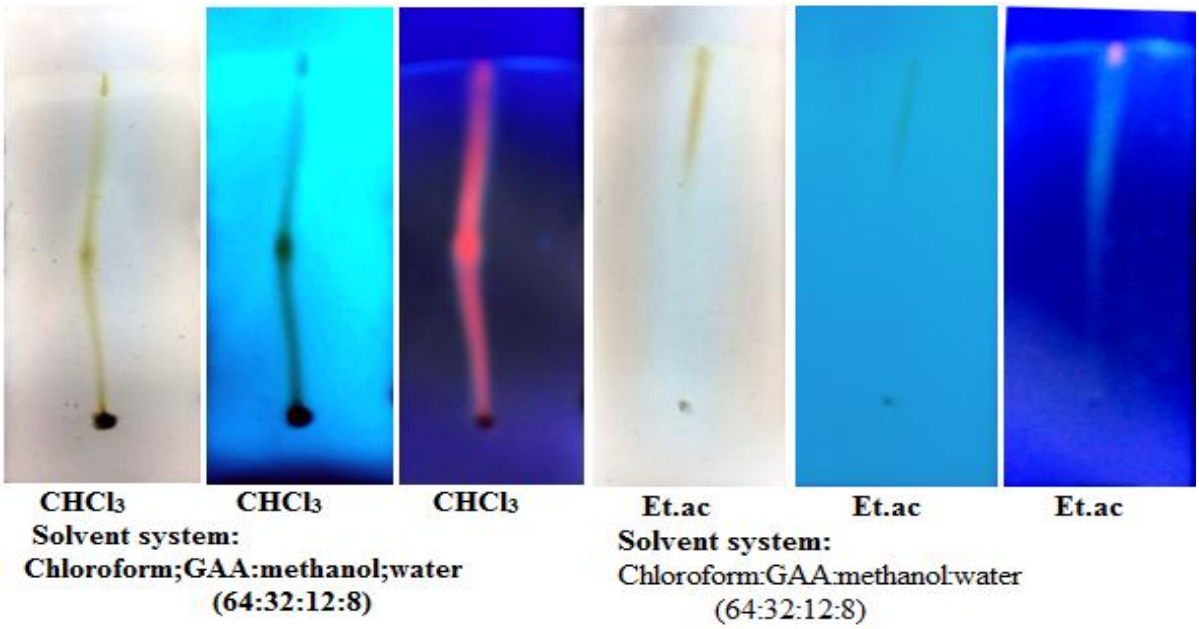

Figure no 2:- Thin Layer Chromatography.

Acknowledgements:-

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