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PHYTOCHEMICAL SCREENING AND THIN LAYER CHROMATOGRAPHIC STUDIES OF ELAEOCARPUS *GANITRUS* SEED THE MAGICAL ELECTROMAGNETIC BEAD (RUDRAKSHA)

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ABSTRACT

Elaeocarpus Ganitrus seed commonly known as Rudraksha is famous for its electromagnetic property. It is proved in modern scientific lab that this seed heals many diseases chronically due to its natural electromagnetic property. The present study deals with the Phytochemical screening and thin layer chromatographic study of Elaeocarpus Ganitrus seed extract belonging to family Elaeocarpaceae. Phytochemical screening determination by some chemical test and thin layer chromatographic study (TLC) was carried out by using solvent system of various polarities of hexane, chloroform, ethyl acetate, acetone and methanol extracts. Phytochemical screening reflects presence of Alkaloids, Glycoside, phytosterols, carbohydrate, Tannin, Flavanoid, amino acid, saponin; tepenoids shows different types of result in different solvent extracts. Thin layer chromatographic studies of Elaeocarpus Ganitrus seed extracts constituted different colored Phytochemical compounds with different R_f value. The chloroform and methanol extracts of drug is carried out to establish the biomarker compound. The result obtained in present study indicated Elaeocarpus Ganitrus seed as rich source of alkaloids, Flavanoids, phytosterols, tannins, carbohydrate and protein.

KEY WORDS

Elaeocarpus Ganitrus, seed extract, phytochemical screening, R_f value, TLC studies

I. INTRODUCTION

Plants are universally recognized as a vital component of the world's biological diversity and an essential resource for the planet. The art of healing has its origin in the antiquity of human civilization. The medicinal value of the plant lies in some of its chemical substances that produce a definite physiological action on human body. The most precious bioactive constituents of plants are alkaloids, tannins, Flavanoids and phenolic compounds In Hindu mythology; *Elaeocarpus Ganitrus* seed (Rudraksha) beads bear a great spiritual, religions and materialistic significance. Due to its natural electromagnetic property it also heals many diseases chronically due to its electromagnetic property. As per Ayurvedic system of medicine, wearing Rudraksha beads relieves strain, insomnia, anxiety, lack of concentration, depression, palpitation, hypertension, rheumatism, infertility and asthma and It has al so anti- aging effect[1-3].Rudraksha is a natural tranquilizer. Wearing rosary of its beads relieves stress, insomnia, anxiety, depression and lack of concentration. It also calm mind and cool down body temperature[4-5].

II MATERIAL AND METHODS

Collection of plant

Genuine Five faced Elaeocarpus Ganitrus bead (Rudrakasha) were collected from online seller CHINTAN JOSHI 92/3, Bank Colony, Brahmeshwar Patna, Bhubaneswar (Orissa) Pin-751018 through EBay India in 2016 and further authenticated by X-Ray, water dipping technique in Maharana Pratap College of Pharmacy Lab.

Preparation of plant extract

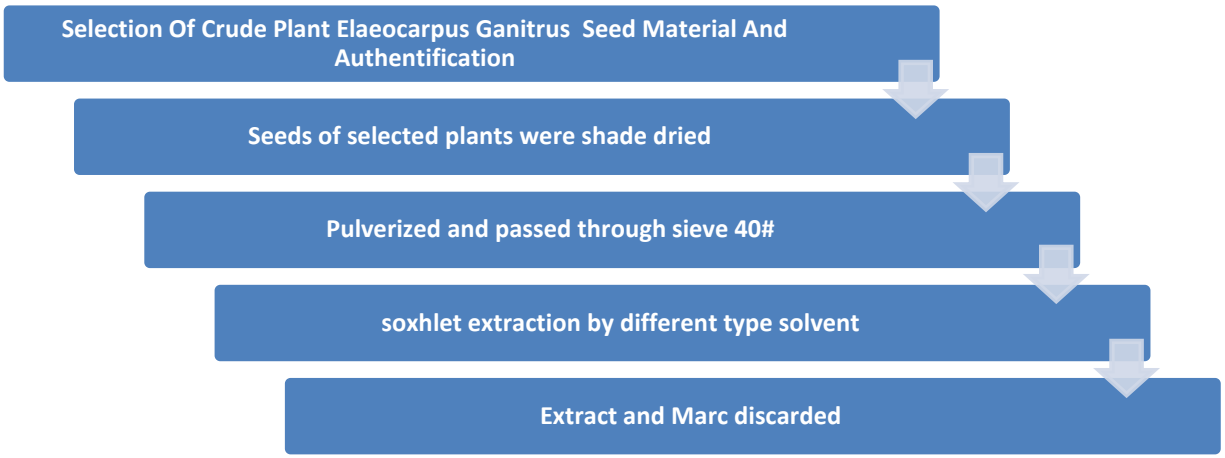
Seeds were collected in bulk and washed then dried in shade, macerated and extracted with hexane, chloroform, ethyl acetate, acetone, and methanol.

The extract was filtered and it was at last dried at low room temperature under pressure in a rotary vacuum evaporator. The extracts were concentrated, percentage yield calculated and then subjected to phytochemical screening and TLC profiling studies. The dried extract was properly stored in the desiccators for further experiment and analysis[6].

Phytochemical Screening

The phyto extract were further processed for Chemical tests for the screening and identification of bioactive chemical constituents of alkaloids, carbohydrates, glycosides, saponins, phenolic compounds, phytosterols, proteins, amino acids, Flavanoids, and tannins,. The screening procedure the medicinal plants under study were carried out in extracts by using standard procedure[7]. All the plant seed materials obtained were shade dried, made into coarse powder and passed through sieve#40, were successively extracted with different solvent like Hexane, Ethyl acetate and Ethanol by Soxhlet extraction method

Figure 1: Schematic representation of extraction



Thin layer chromatographic studies (TLC)

Thin layer chromatographic technique is a very precise, specific and chromatographic versatile method. For this study Each solvent extract was subjected to thin layer chromatography (TLC) as per traditional one dimensional ascending method by means of silica gel 60F254, 7X6 cm (Merck) were slice with ordinary household scissors. Markings of TLC plate were made with soft pencil[8-9]. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro liter by means of capillary at space of 1 cm at 5 tracks. In the twin trough chamber with different solvent system like Solvent -1 Hexane: Acetic acid (9:1) , In solvent system II we use Hexane: Ethyl acetate :Acetic acid (5:4:1) for solvent system III we selected Hexane: Ethyl acetate: Acetic acid (4:4:2), In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1) and at last In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1) were used. After pre-saturation with mobile phase for Almost 30 min for development were used. After applying Silica gel slurry we left it dried and the run plates are dried and we sprayed newly prepared iodine reagents to detect the bands on the TLC plates[10-11]. The movement of the active compound was articulated by its retention factor (R_f), values were calculated for different samples

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front TLC plates}}$$

The plates were dried and visualized under normal day light, ultraviolet light (254nm & 366nm) for detection of spot.

III.EXPERIMENT AND RESULT

Total Ash value

About 40gm of powdered drug was measured accurately into a tarred silica crucible and incinerated at 450ºC in muffle furnace until we make it free from carbon. The crucible was cooled to room temperature and weighted. Percentage of ash was calculated with reference to air dried substance [12-13].

- **Acid soluble Ash:** Ash obtained from total ash was boiled with 25ml of 2N HCl for few minutes and filtered through an ash less filter Paper. The filter paper was then transferred into a tarred silica crucible and incinerated at 650º C in muffle furnace until free from carbon. The crucible was cooled and weighted. Percentage of acid insoluble ash was calculated with reference to air dried material.
- **Water soluble Ash:** Ash obtained from total ash was boiled in 25 ml of distilled hot water for few minutes and filtered through an ash less filter paper. The filter paper was transferred to a tarred silica crucible then incinerated at 450ºC in muffle furnace until getting 0% carbon. The crucible was allowed to be cool and weighted. Percentage of water soluble ash was calculated with reference to air dried substance.
- **Alcohol soluble extractive values:** 5gm of powdered drug was refluxed with 100ml of alcohol for at least 2hrs and filtered through whattman filter paper. 10ml of filtrate was evaporated in a tarred dish at 105ºC and weighed. Alcohol soluble extractive values were premeditated.
- **Water soluble extractive values:** 5gm of powdered plant seed material was treated with 100 ml water at in a stopper flask with frequent trembling during 6 hrs using electrical shaker and allowed to stand for one day . Temperature was kept constant at 45°c during whole process. Extract was filtered and 10ml of filtrate was evaporated in a tarred dish at 105ºc and weighed. Water soluble extractive values were calculated.
- **Ether soluble extractive values:** 5gm drug was refluxed with 100 ml of petroleum ether for 2hrs and filtered through whattman filter paper. 10 ml of the filtrate was evaporated in a tarred dish at 105ºC and measured. Ether soluble extractive values were measured.

Table.1Physicochemical Parameters

Parameters	Seed extract %
Ash value	4.6
Acid insoluble Ash	1.7
Water soluble Ash	0.85
Alcohol soluble extractive value	5.1
Water soluble extractive Value	9.23
Ether soluble Extractive Value	2.8
Loss on drying	**15.5**

Qualitative Phytochemical Investigations

Extraction of plant material

The authenticated fresh whole plant were dehydrated under shade and used for the research of extract. These entire plants were coarsely powdered with the assist of mechanical grinder. The powder was stored in an airtight container for further use[14-15].

Preparation of extracts

Method of extraction

Uninterrupted hot percolation (successive solvent extraction) procedure by using Soxhlet apparatus and cold maceration technique.

Materials

1. Soxhlet apparatus
2. n-hexane
3. Ethanol
4. Distilled water
5. Shade dried coarse leaves powder of *Elaeocarpus ganitrus*.

Extraction procedure

Hexane extract

The shade dried coarsely powdered whole plant *of Elaeocarpus ganitrus* (50 gm) was extracted with hexane until the extraction was completed. After achievement of extraction, the solvent was removed by distillation. Dark brown color residue was obtained. The residue was stored in dessictor.

Ethanol extract

The marc left after hexane extraction was dried up and then extracted with ethanol 95%v/v, until the extraction was finished. After completion of extraction, the solvent was removed.

Aqueous extract

The marc remains after ethanol extraction was dried and then extracted with aqueous medium by cold maceration process for 7 days. At the end of 7th days, it was filtered through muslin cloth and filter was concentrated. The residual solution was evaporated by heating on water bath. The brown color filtrate was obtained. The residue was then stored in dessictor.

Preliminary Phytochemical tests-

All the extracts of *Elaeocarpus ganitrus* were subjected to qualitative tests for the identification of various active constituents[16-18].

1) Detection of Alkaloids

Solvent free extract was stirred with few ml of dilute hydrochloric acid and clean. The filtrate was tested watchfully with a range of alkaloidal regents as follows.

- **Mayer's Test:** To a few ml of filtrate, one to a couple of drops of Mayer's reagent was added by the side of the test tube. A white or creamy precipitate indicated the test as positive.
- **Wagner's test:** To a few ml of filtrate, couples of Wagner's reagent were added by the side of the test tube. A brick red precipitate confirmed the test as positive.
- **Hager's test:** To a few ml of filtrate 1 or 2 ml of Hager's reagent added (saturated aqueous solution of picric acid) were added. An outstanding yellow precipitate indicated the test as positive.
- **Dragendorff's test:** To a few ml of filtrate, 1 to 2 ml of Dragendorff's reagent was added. An outstanding brown precipitate indicated the test as positive.

2) Detection of Carbohydrates

The extract was dissolved in 5 ml of water and filtered. The filtrate was subjected to the following tests.

- **Molish's test:** To 2 ml of filtrate, a couple of drops of alcoholic solution of naphthol were added, the mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the incidence of carbohydrates.
- **Fehling's test:** One ml of filtrate was boiled on water bath with 1 ml each of Fehling solutions A and B. A red precipitate indicated the occurrence of sugar.
- **Barfoed's test:** To 1 ml of filtrate add1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 min. Red precipitate indicated presence of sugar.

3) Detection of Glycosides

The extract was hydrolyses with concentrated hydrochloric acid for 2 hrs on water both, filtered and the hydrolysis was subjected to the following tests.

- **Borntrager test:** To 2 ml of filtered hydrolysis, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it pink color indicated the occurrence of glycosides.

4) Detection of Saponins by Foam Test

The filtrated was diluted by means of distilled water and made up to 20 ml. The suspension was traumatized in a graduated cylinder for 15 min. A couple of cm layer of foam indicated the incidence of saponins.

5) Detection of Proteins and Amino Acids

The filtrated was dissolved in 10 ml of distilled water and filtered through whatman No.1 filter paper and the filtrate was entering to tests for proteins and amino acids.

- **Million's test:** To 2 ml of filtrate couple of drops of Million's reagent was added. A white precipitate indicated the presence of proteins.
- **Biuret test:** An aliquot of 2 ml of filtrate was treated with one drop of 2 % copper sulphate solution. To this, 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets, pink color in the Ethanolic layer show the presence of proteins.

6) Detection of Phytosterols

- **Salkowski's reagent Test:** The extract was treated with Salkowski's reagent. The yellowish color with green fluorescence appearance indicates the presence of phytosterols in it.

7) **Detection of Fixed Oils and Fats A. Spot test**

A small quantity of filtrate was pushed between couples of filter papers. Oil stain on the paper indicated the attendance of fixed oil.

- **Saponification test:** A few drop of 0.5 N alcoholic potassium hydroxide solutions were added to a small quantity of extract along with a drop of phenolphthalein. The combination was heated on water bath almost for 2hrs. Formation of soap or partial Neutralization of alkali shows the presence of fixed oils and fats.

8) Detection of phenolic compounds and Tannins A. Ferric chloride test

The extract was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green apple color indicated the presence of phenolic compounds.

- **Lead acetates test:** The extract was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the incidence of phenol compounds.

9) Detection of Gum and Mucilage :

The extract was dissolved in 10 ml of distilled water and to this; 25 ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilage's.

III. RESULT

Percentage of yield extract

The yield of chronological extracts (g) is shown in [Table 2]. The quantity obtained from different solvent extract hexane, chloroform, ethyl acetate, acetone and methanol extracts are 4.010 gm, 3.090 gm, 3.730 gm, 2.720 gm, and 4.150 gm respectively.

Table 2: The percentage yield of different extracts of *Elaeocarpus Ganitrus* seed

S. No	Solvent	Color of extract	Yield of the extract (in gm)	Percentage yield (%w/w)
1	Hexane	Light grey	4.010	2.51%
2	Chloroform	Light brown	3.090	2.04%
3	Ethyl acetate	dark brown	3.350	1.37%
4	Acetone	Light brown	2.720	0.86%
5	Methanol	Light black	4.150	1.85%

Qualitative Phytochemical Screening

The present study carried out in the Elaeocarpus Ganitrus seed extract revealed the presence of medicinal active constituents[19]. The Phytochemical active compounds of *Elaeocarpus Ganitrus* were qualitatively analyzed for seeds and the results are presented in Table 3. In these screening procedure alkaloids, glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, proteins, amino acids, Flavanoids, quinines and tepenoids shows different types of results in different solvents extracts. Among these Phytochemical screening, Alkaloids, Saponins, Tannins, Amino acids, Flavanoids and Tepenoids were present in all solvent extracts where as Phytosterols are present all extracts except methanol, Phenolic compounds are in Ethyl acetate and methanol extracts, proteins and carbohydrates were present in ethyl acetate and methanol extracts, Quinones were found in hexane, acetone, and methanol extracts, Glycosides are absent in all solvent extracts.

Table:-2 Phytochemical constitute of the seed extract of *Elaeocarpus Ganitrus*

S.No	Phytoconstituents	Tests	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol
1	Alkaloids	Mayer's test	+++	++	++	+	++
2	Glycosides	Borntrager's test	-	+	-	-	-
3	Saponins	Froth forming test	+	-	-	+	-
4	Phenolic compounds	Lead acetate test	++	+	+	-	+
5	Tannins	$Fecl_3$ test	+	-	-	+	-
6	Phytosterols	Libermann-Buchard test	+	++	+	-	-
7	Corbohydrates	Fehilings test	-	+	-	+	-
8	Protiens	Biuret test	-	+	-	+	+
9	Aminoacids	Ninhydrin test	-	+	+	+	+
10	Flavanoids	Alkaline test	+	+	-	+	+
11	Quinones	Quinone test	+	++	-	+	-
12	Terpenoids	Terpenoid test	+	-	+	-	+

+++ = Very strongly show positivity in test
++ = strongly show positivity in test
+ = Show positivity in test
- = Show positivity in test

Thin layer chromatographic studies

A huge number of solvent systems were checked to achieve a good resolution. Finally, the solvents hexane: ethyl acetate: acetic acid was used[19]. Thin layer chromatographic studies of the hexane extract of *Elaeocarpus Ganitrus* . Solvent system I Hexane: Acetic acid (9:1), 3 spots were visible R_f values 0.24, 0.37 and 0.58. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 1 spot detected R_f value 0.87. In solvent system III Hexane: Ethyl acetate: Acetic acid(4:4:2), 1 spot detected R_f value 0.84. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible R_f values 0.08 and 0.92. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 3 spots were obtained having R_f of 0.09, 0.81 and 0.94. TLC studies of the Chloroform extract of *Elaeocarpus Ganitrus.* Solvent system I Hexane: Acetic acid (9:1), 2 spots were visible R_f values 0.18 and 0.45. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 3 spots were detected R_f values 0.12, 0.84 and 0.93. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 2 spots were detected R_f values 0.07 and 0.92. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible R_f values 0.08 and 0.77. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 2 spots were obtained having R_f of 0.17 and 0.95. TLC studies of the Ethyl acetate extract of *Elaeocarpus Ganitrus* . Solvent system I Hexane: Acetic acid (9:1), 2 spots were visible R_f values 0.15 0.44. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 2 spots were detected R_f values 0.83 and 0.92. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 1 spot detected R_f value 0.87. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible R_f values 0.06 and 0.84. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 2 spots were obtained having R_f of 0.05 and 0.91. TLC studies of the Acetone extract of *Elaeocarpus Ganitrus.* Solvent system I Hexane: Acetic acid (9:1), 2 spots were visible R_f values 0.18 and 0.46. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 1 spot detected R_f f value 0.85. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 2 spots were detected R_f values 0.07 and0.93. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible R_f values 0.04 and 0.86. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 2 spots were obtained having R_f of 0.09 and 0.88. TLC

studies of the Methanol extract of *Elaeocarpus Ganitrus* . Solvent system I Hexane: Acetic acid (9:1), 1 spot detected R_f value 0.18. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 1 spot detected R_f value 0.97. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 4 spots were detected R_f values 0.11, 0.24, 081 and 0.87. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible R_f values 0.12 and 0.76. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 2 spots were obtained having R_f of 0.19 and 0.76 (Table 3).

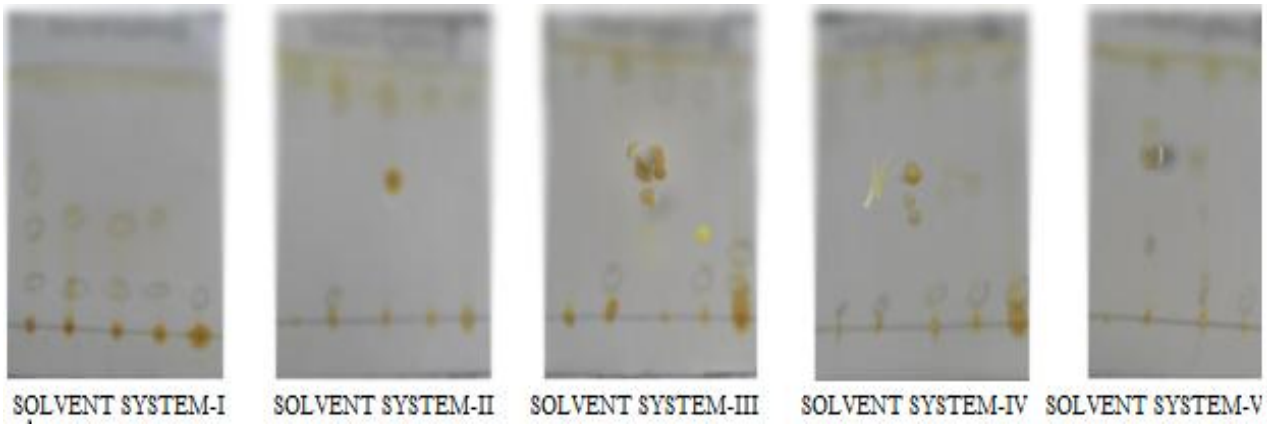
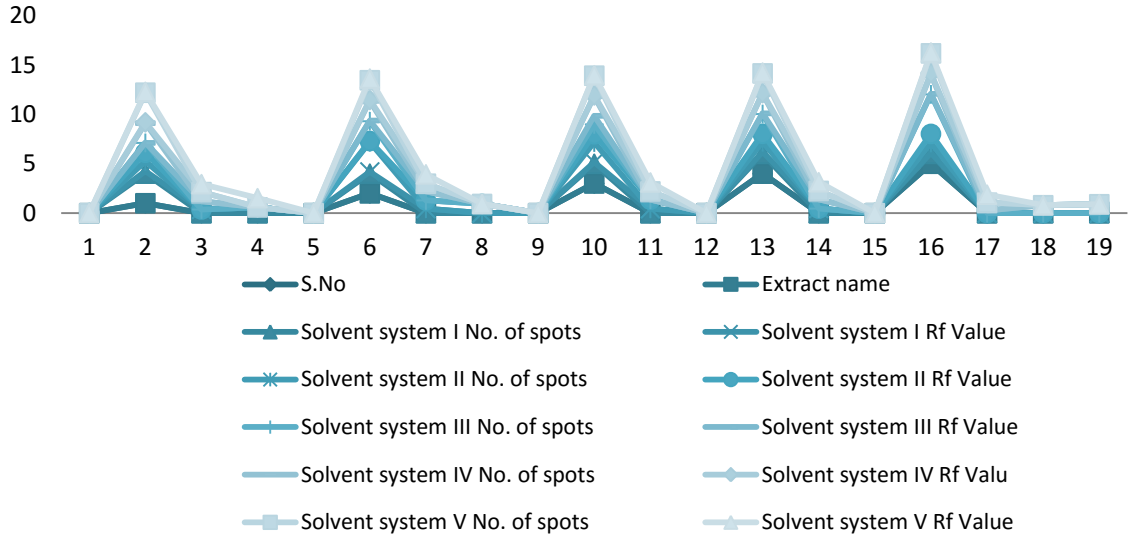


Table:-3 R_f values of TLC solvent systems for different extract of *Elaeocarpus Ganitrusseed*

S. No	Extract name	Solvent system I		Solvent system II		Solvent system III		Solvent system IV		Solvent system V	
		No. of spots detected	R_f Value	No. of spots detected	R_f Value	No. of spots detected	R_f Value	No. of spots detected	R_f Value	No. of spots detected	R_f Value
1	Hexane extract	3	0.24 0.37 0.58	1	0.87	1	0.84	2	0.08 0.92	3	0.09 0.81 0.94
2	Chloroform extract	2	0.18 0.45	3	0.12 0.84 0.93	2	0.07 0.92	2	0.08 0.77	2	0.17 0.95
3	Ethyl acetate extract	2	0.15 0.44	2	0.83 0.92	1	0.87	2	0.06 0.84	2	0.05 0.91
4	Acetone extract	2	0.18 0.46	1	0.85	2	0.07 0.93	2	0.04 0.86	2	0.09 0.88
5	Methanol extract	1	0.10	1	0.92	4	0.05 0.25 0.80 0.90	2	0.10 0.81	2	0.09 0.81



IV DISCUSSION

Plants are known for its metabolites such as alkaloids, glycosides, Flavanoids, phenols, tepenoids, steroids, Tannin, Phytosterols quinines and other derivatives which are use for Pharmaceuticals, cosmetics, biochemistry, Medicinal , chemical and pesticide industries. And different therapeutic property produced by different plant is also due to different type chemicals present in plant which we are called above plant metabolite. The result of preliminary Phytochemical screening of the crude Elaeocarpus ganitrus seed extract exposed the presence of all the constituents tested including alkaloids, Flavanoids, saponins, tannins, carbohydrates, phenols, glycosides, Quinones the fractions of protein and phenolic compound. In the present study, Phytochemical screening for all six extracts showed significant indication about the presence of metabolites. Alkaloids, Phenolic compound, Phytosterols, Amino acids, Flavanoids and Tepenoids, were found to be present in the all the chronological extracts of *Elaeocarpus Ganitrus* seed. Therefore the present study confirms the traditional medical practice and previous pharmacological observations and supplement action for other health problems such as allergic reactions, Antiageing, hepatoprotective, immunomodulatory property and diseases resulting from hormone deficiencies or abnormal production *etc*: In the present study, Phytochemical screening for all six extracts showed significant indication about the presence of metabolites. Alkaloids, Phenolic compound, Phytosterols, Amino acids, Flavanoids and Tepenoids, were found to be present in the all the sequential extracts of *Elaeocarpus Ganitrus* seed. 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 five extracts gives a remarkable result that directing towards the presence of number of Phytochemical. Various Phytochemical metabolites give different R_f values in different solvent system. This variation in R_f values of the Phytochemical show a very important clue in understanding of their polarity and also shoot some light in selection of appropriate solvent system for partition of pure compounds by open column chromatography. Combination of solvents with changeable polarity in dissimilar ratio can be used for separation of unpolluted compound from plant extract. Proper selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the R_f values of plant constituent in different solvent system. Different type of R_f values of the compound also reproduce an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

V. 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 *Elaeocarpus Ganitrus* seed could be a potential source of natural antioxidant having great importance as therapeutic mediator and preventing oxidative stress and immune modulators property. The seed of *Elaeocarpus Ganitrus* seed provide lead molecules which could be useful substrate for the synthesis of new broad spectrum antibiotics for the treatment of infections caused by the organisms. Further purification, identification and characterization of the active compounds would be our priority in future studies.

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