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Comparative Study on Extraction Isolation and Phytochemical Screening of Leaves and Stems of Euphorbia Heterophylla with Their *In-Vitro* Antioxidant Potential

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Abstract: *In recent years, the search for biologically active compounds from Euphorbia heterophylla in the treatment of different diseases has always been of great interest to researchers. In this present study, we investigated the effect of the aqueous leaf and stem extract of euphorbia heterophylla. Phytochemical studies revealed the presence of tannins, anthraquinones, alkaloids, flavonoids and phenols in hexane, acetone and water extracts containing only phenol.The plant extracts demonstrated antimicrobial activity against both the test bacteria and fungi with water extract demonstrating the highest activity followed by the acetone extract. The plant has been presented for further isolation, identification and purification of these phyto-constituents. The result of the study showed that the crude plant material contained some secondary metabolites such as saponins, flavonoids and tannins. The phytochemical investigation led to the isolation of four known compounds stigmasterol, - stigmasterol glucoside, benzoic acid and 4 – hydroxyl benzoic acid. The four compounds exhibited good activity against the xanthine oxidase enzymes while the 4-hydroxybenzoic acid showed a marked activity. The isolation of the compounds from the leaves of E. heterophylla, which inhibited the xanthine oxidase enzyme. The aqueous leaf extract of the plant indicated the presence of carbohydrates, saponins, tannins, flavonoids, alkaloids, terpenoids and steroids, but anthracene derivatives were absent. The results obtained in this study, therefore, justify the traditional use of the plant for food and medicinal purposes respectively.*

Key words: *Euphorbia heterophylla, euphorbiaceae, Phytochemicals, In vitro antioxidant activity, leaf extract, stem extract.*

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I. Introduction

Modern new medicines has make use of various plant derived compounds as the basis of evidence based pharmaceutical drugs. Herbal medicines are also known as Phytomedicine, Phytotherapy, Paraherbalism. These are the pseudoscientific methods of using unrefined plants or animal extracts , as they supposed to have medicines and health promoting agents.[1]

There is an archeological evidence which indicates that uses of medicinal palnts dates back to the approximately 60,000 years ago. Herbs are also commonly featured in the traditional system of medicine of ancient India and the principal treatment for the disease was diet. Greek physician Pedanius Dioscorides , has described several plants of medicinal importance in the book " The Materia Medica" . It was estimated by the World Health Organisation that the 80% of population of some of the asian and African countries are using herbal medicines for their primary health care. [2]

Medicinal plants such as Turmeric, Ginger, Aloe, Tulsi, Neem, Fenugreek seeds can cure several common ailments and these are also considered as home remedies in several parts of the country.

Now a day' s various medicinal herbs are very important sources for manufucturing of different pharmaceutical products which can be used for the different ailments like diabetes, hypertension, constipation, weak penile erection, menstrual disorders, leucorrhea, cancer, fever, diarrhoea, constipation etc. [3]

There are some herbs which also have antibiotic properties in them for example turmeric which is very useful in inhibiting the growth of harmful microbes. It can also be used to heal cuts and wounds as a home remedy.

PLANT PROFILE

The Fresh leaves and stems of the Euphorbia heterophylla were collected from local area of Rajawala selaqui and it has been authenticated by Mr. Kumar Ambrish from botanical survey of India Northern Regional Centre 192, Kaulagarh Road , Dehradun -248195 Uttarakhand and it has been identified as Euphorbia heterophylla L. (milkweed) belonging to family Euphorbiaceae, Account No. 118606.

The *Euphorbia heterophylla* L. is the botanical name of the plant and it is now named as *Euphorbia cyathophora* Murraya and now the name *Euphorbia heterophylla* is considered as synonym of *E.geniculata* in which the bracts of the plant are green in colour. [4] *Euphorbia heterophylla* is widely distributed to tropical and subtropical America. It has been introduced in south and southeast Asia as an ornamental plant and it has become a weed in India and Thailand and there it has invaded cotton fields and other agriculture terrains. It is also cultivated in manila and in larger towns as an ornamental plant. [5]


Plant name :- painted euphorbia
Synonym :- Fireplant, milkweed, japanese poinsettia, painted leaf, kaliko plant.
Botanical name :- *Euphorbia heterophylla*
Family :- Euphorbiaceae.
Subfamily :- Euphorbioideae
Kingdom :- plantae
Clade :- Angiosperms
Tribe :- Euphorbieae
Subtribe :- Euphorbiinae
Genus :- Euphorbia
Species :- E.*heterophylla*

Morphology

The leaves at the upper end of the stalk, close to the cyathium, have a striking, scarlet red coloration. Leaves are mainly 2-4 lobed and 4– 7 cm long by 1.5– 3 cm wide. Leaves are ovate in shape with obtuse apex and cuneate base. [6,7] The leaf margin was found to be undulate, the leaves has prominent petiole and stipule. The stalk exudes a toxic milky white latex. Regarding the leaf venation, the multicoasted divergent reticulate type observed in Euphorbia heterophylla.

Preparation of Plant Extract

The leaves of the plants are always harvested freshly for preparation of the extract for the fourteen days that the animals are given the extract. The leaves are weighed and macerated using mortar and pestle.[8] A specific quantity of water is added to ensure proper maceration. Thereafter, the solution is filtered using filter paper. [9]

Extraction

100 g of the ground part is macerated successively for three days (with occasional shaking) using cold maceration technique. 1000 ml of distilled water, methanol, and chloroform and petroleum ether are used as extraction solvents respectively. The macerated samples are sieved with muslin cloth and evaporated to dryness using a steam bath. The dried extracts are weighed and stored in sterile sample bottles and kept in the refrigerator for further studies. [10,11]

ISOLATION

There are different techniques for the isolation of the bioactive compound obtained from plants. Techniques used for isolation are TLC, HPLC, column chromatography, flash chromatography, these techniques should be used to obtain pure compound. [12]

These compounds after isolation can be used for the structural determination and biological activity. For this fourier transform infrared spectroscopy (FTIR), Phytochemical screening assay is useful to obtain identification of bioactive compounds. [12,13]

CHEMICAL CONSTITUENTS:- have a very goo
It contains saponins , tannins, flavonoids, alkaloids. It contains steroid known as stigmasterol, stigmasterol glucoside and it also has benzoic acid , 4-hydroxy benzoic acid. All these four compounds d activity against an enzyme known as xanthine oxidase. [14]

Phytochemical Screeningleum ether, N-hexane, chloroform, methanol and water are screened for phytochemical screening for the presence of its active chemic
Extract obtained from Petro al constituents using standard method of analysis.

Thin Layer Chromatography

The extract of Petroleum ether, N-hexane, chloroform, methanol and water are used for the TLC and are subjected to the thin layer chromatographic analysis, to find the presence of number of active chemical constituent for chemical test. The Analytical plates of TLC are prepared by pouring the slurry of silica gel G on glass TLC plates. [15] Activation of the TLC plates are done by drying them in air for 30 minutes and then in an oven at 110°C for 30 minutes. About 2 cm above the spot of the of sample extract was applied in a row along one side of plate by using capillary tubes. The sample solution volume range is controlled by spreading not more than 0.5 cm . The prepared plates are placed in the pre-prepared saturated TLC chamber with the mobile phase. The chromatographic conditions are given in the table 3. The RF values are compared with the
RF values are calculated by the given Formula:-

RF= Distance travelled by the solute/ distance travelled by the solvent.

Evaluation of Biological Activity
Antioxidant activity

An antioxidant is defined as a substance which inhibits the oxidation and which reduces damage due to the oxygen. Antioxidant especially used to inhibit the deterioration of stored food products due to oxidation. [15]

An oxidation is a chemical reaction which produce free radicals, and leads to chain reactions that may damage cells. Antioxidants such as vitamin E, Ascorbic acid, β-carotene, selenium, lycopene, lutein are some examples of antioxidants which are capable of terminating the damaging effects of oxidation. Molecules which are called as free radicals contributes to the aging process, to balance this process of formation of free radicals our body uses antioxidants. [16.17]

Free radicals which are produced due to oxidation may contribute to chronic disease from cancer to heart disease and Alzheimer' s disease to vision loss but it is not proven that antioxidants have substantial impact on heart disease. [16]

Antioxidants are generally classified into two main categories on the basis of whether they are soluble in water(hydrophilic) or in lipids (lipophillic). Lipid soluble antioxidants generally act to protect cell membrane from lipid peroxidation and water-soluble antioxidants mainly react with oxidants in the cell cytosol and also with blood plasma.

II. Method

DPPH (2,2-diphenyl-1picryl-hydrazyl-hydrate) Free radical method.

DPPH (2,2-diphenyl-1picryl-hydrazyl-hydrate) free radical method is an antioxidant assay which is used to study In-Vitro antioxidant activity which is based on electron transfer that produces a violet solution in ethanol. The free radical scavenging activity of different extract was determined by using a solution of 0.135mM DPPH in methanol which was taken from the Himalayan Institute Of Pharmacy and Rsearch Dehradun. Samples solutions having different concentrations are prepared (1000, 500, 250, 100 $\mu g.ml^{-1}$). As appositive control Ascorbic acid was used at different concentrations of 100,50, 25, and 10 $\mu g.ml^{-1.}$ Firstly blank samples are run using 1ml methanol in place of test solution. 1 ml of 0.2 mM DPPH in methanol was taken and added to 1 ml of test solution or standard and taken 1ml methanol for dilution and then it is allowed to stand at a room temperature in a dark chamber for about 30 minutes. The change in colour which is from deeo violet to yellow was measured at 517 nm. The ability of sample to scavenge DPPH radicals was calculated by the following equation. [16, 17, 22]

$$\textbf{DPPH radical scavenging activity(\%)} = [(Abs_{control} - Abs_{sample})/ (Abs_{control})]\times 100$$

Where, $Abs_{control}$ is the absorbance of DPPH radical + methanol

Abs_{sample} is the absorbance of DPPH radical + sample extract / reference

Table 4:-Total tannin content in *E.heterophylla:*

Concentration (mg/ml)	Standard drug absorbance of tannic acid	Absorbance of sample (y)=mx+c	Concentration of sample (x)=y-c/m	Total tannins= (x)/weight of drug*100)
0.1	0.0069	0.0042	0.028	1.65%
0.2	0.0142			
0.3	0.0236			
0.4	0.0325			
0.5	0.0421			

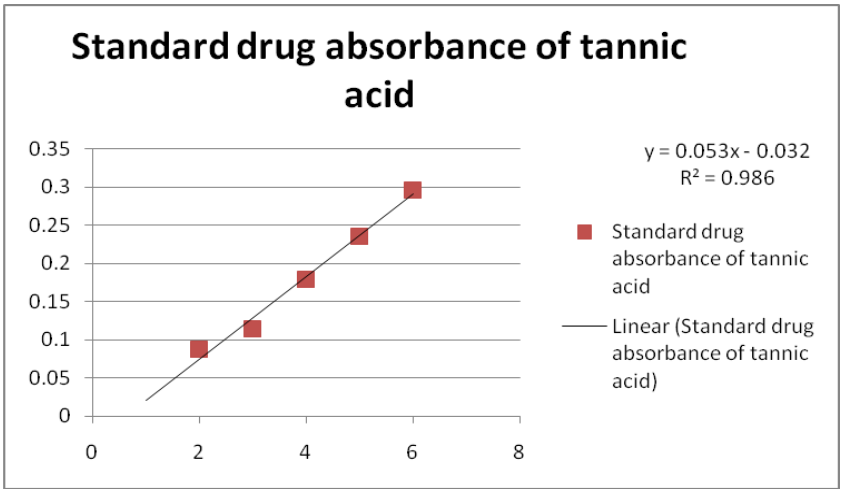

Fig 8:- Absorbance vs. concentration graph of standard curve for total tannin content in tannic acid

Table 5:-Total Flavonoid content in *E.heterophylla*

Concentration (mg/ml)	Standard drug absorbance (Rutin)	Absorbance of sample y=mx+c	Concentation of Sample (x)=y-c/m	Total Flavonoid= (x)/weight of drug *100
0.2	0.2998	0.2567	0.1856	3.48%
0.4	0.3258			
0.6	0.3468			
0.8	0.4453			
1.0	0.5546			

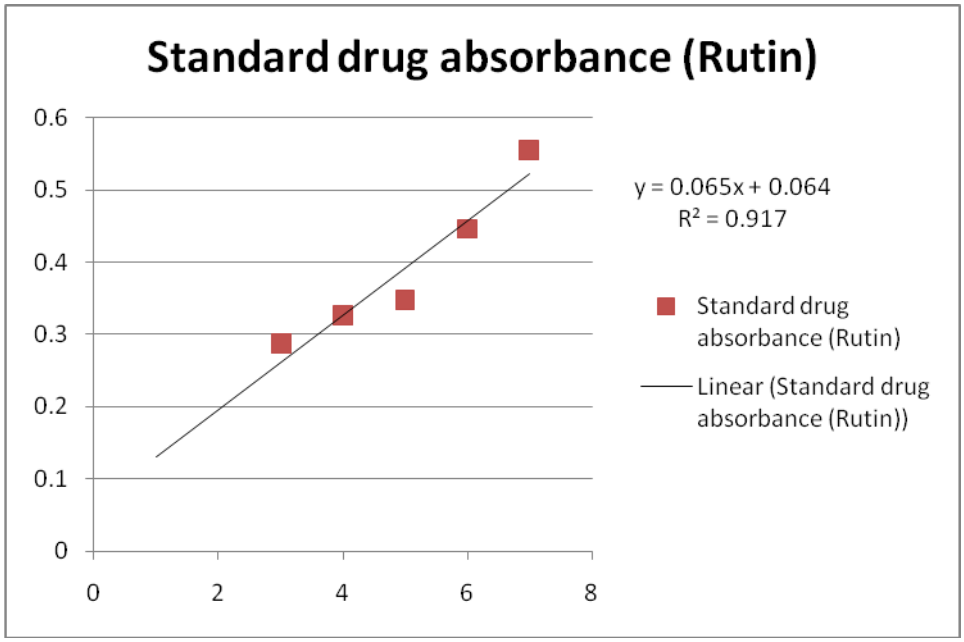

Fig 9:- Absorbance vs. concentration graph of standard curve for total flavonoids content of rutin

6. Percentage loss on Drying (%LOD):

Table 6.A: % LOD of *Euphorbia heterophylla* leaf:

S.No.	Weight of powder taken (gm)	%LOD weight (w/w)	Mean Value
1.	1	0.21	
2.	1	0.135	
3.	1	0.101	
4.	1	0.101	
			0.1367%

Table 6.b: % LOD of E.*heterophylla* stem:

S.no.	Weight of powder taken (gm)	%LOD weight (w/w)	Mean Value
1.	1	0.14	
2.	1	0.12	
3.	1	0.085	
4.	1	0.079	
			0.106%

7. Total Ash Value

Table 7.A Total Ash Value for *E.heterophylla leaf* :

S.no.	Weight of powdered drug taken (gm)	Weight of ash obtained (gm)	%w/w total ash	Mean value
1.	2	0.0235	0.63	
2.	2	0.0328	0.638	
3.	2	0.0266	0.644	
				0.636%

7.b Total ash value for *E.heterophylla* Stem:

S.No.	Weight of powdered drug taken (gm)	Weight of ash obtained (gm)	%w/w Total ash	Mean value
1.	3	0.0289	0.66	
2.	3	0.0322	0.64	
3.	3	0.0320	0.64	
				0.64%

8. Water soluble Ash

Table 8.A Water soluble Ash values for *E.heterophylla* leaf:

S.no.	Weight of powdered drug taken (gm)	Weight of water soluble ash (gm)	%w/w Water soluble ash	Mean Value
1.	2	0.0125	0.62	
2.	2	0.0124	0.61	
3.	2	0.0124	0.61	
				0.61%

Table 8.b Water soluble Ash values for *E.heterophylla* Stem:

S.no.	Weight of powdered drug taken (gm)	Weight of water soluble ash (gm	%w/w Water soluble ash	Mean Value
1.	2	0.0185	0.92	
2.	2	0.0195	0.95	
3.	2	0.0194	0.965	
				0.945%

9.Acid Insoluble Ash:

Table 9.A Acid Insoluble Ash values for *E.heterophylla* leaf:

S.No	Weight of powdered drug taken (gm)	Weight of water soluble ash (gm	%w/w Water soluble ash	Mean Value
1.	2	0.0248	1.28	
2.	2	0.0251	1.275	
3.	2	0.0247	1.24	
				1.265%

Table 9.b Acid Insoluble Ash Values for *E.heterophylla* Stem:

S.No	Weight of powdered drug taken (gm)	Weight of water soluble ash (gm	%w/w Water soluble ash	Mean Value
1.	2	0.321	1.65	
2.	2	0.334	1.67	
3.	2	0.326	1.62	
				1.64%

10.Extractive values

Table 10.A Extractive values for *E.heterophylla* leaf:

S.no.	Solvent	Initial Weight (gm)	Amount of solvent (ml)	Final wt. of sample(gm)	Extractive values
1.	Ethanol	10	150	12.20	11
2.	Water	10	150	13.48	15.8

Table 10.B Extractive values for *E.heterophylla* Stem:

S.No.	Solvent	Initial Weight (gm)	Amount of solvent (ml)	Final wt. of sample(gm)	Extractive values
1.	Ethanol	10	150	10.20	16.6
2.	Water	10	150	11.18	10

Phytochemical Investigation:

Table 11: Colour of extracts of Leaf and Stem of *E.heterophylla* from different solvents:

Solvents	Color of Extracts	
	Leaf	**Stem**
Petroleum Ether	Dark green	Yellowish green
N-hexane	Yellowish green	Light green
Chloroform	Dark green	Light yellow
Methanol	Green	Creamy yellow
Ethanol	Light green	-

Table 12: Phytochemical screening of stems and leaves of *E.heterophylla*

Phytoconstituents	Test	Petroleum ether extract	n-hexane extract	Chloroform extract	Methanol extract	Ethanol extract
		Leaf extract				**Stem**
Alkaloid	Hager test	-	+	+	+	+
	Wagner test	+	+	+	+	+
Glycosides	Killer killani test	-	+	+	+	-
Flavonoids	Lead acetate test	-	+	+	+	-
Tannins	Fereric chloride test	-	+	+	+	-
	Lead acetate test	-	+	+	+	+
Steroids	Salkowski reaction	+	-	-	-	-
	Liberman burchard reaction	+	-	-	-	-
Saponins	Foam test					
Proteins	Xoanthproteic test	-	-	-	+	-
Amino acid	Ninhydrin test	-	-	-	+	-

Table 13:- Chromatographic Condition of different Extracts of *E.heterophylla*

Extracts	Mobile phase	Spraying Reagent
Petroleum ether leaf extracts	Toluene and ethyl acetate	Liberman burchard reagent
N-hexane leaf extract	n-hexane and toluene	UV at 254nm
Chloroform leaf extract	Ethyl acetate, methyl acohol water and toluene	UV at 254nm
Methanol leaf extract	n-butanol and glacial acetic acid	UV at 254 nm
Ethanol stem extract	Ethyl alcohol and toluene	UV at 254 nm
Proteins	Chloroform and methyl alcohol	Ninhydrine

Table 14:- Thin layer chromatography of Leaf and Stem extracts of *E.heterophylla*.

S.no.	Solvent system	Spraying reagent	RF value
1.	Toluene[7]:ethyl acetate [3]	Liberman burchard reagent	0.58
2.	n-hexane[7]:toluene[3]	UV at 254nm	0.77
3.	Ethyl acetate[2.5]: methyl acohol[3]: water[3] and toluene[0.5]	Uv at 254nm	0.68
4.	n-butanol[7]: glacial acetic acid[3]	Uv at 254 nm	0.65
5.	Ethyl alcohol[6]: and toluene[4]	UV at 254 nm	0.63
6.	Chloroform and methyl alcohol	Ninhydrine	0.66

Table 15:-Absorbance of Leaf and stem extracts of *E.heterophylla* with ascorbic acid at 517 nm by UV visible spectrophotometer (DPPH scavenging assay method).

S.no.	Conc.	Absorbance	Leaf Extracts			Stem Extract
		Ascorbic acid (abs)	Petroleum ether	n-hexane (abs)	Methanol (abs)	Ethanol (abs)
1.	01	0.093	0.213	0.0139	0.0673	0.1789
2.	0.2	0.095	0.367	0.0228	0.1178	0.1898
3.	0.3	0.123	0.645	0.0298	0.1389	0.2043
4.	0.4	0.125	0.747	0.0326	0.1536	0.2354
5.	0.5	0.127	0.821	0.0438	0.158	0.2635
6.	0.6	0.136	0.922	0.0589	0.179	0.2763

Table 16:-% Inhibition of Leaf and Stems extracts of *E.heterophylla* with ascorbic acid using DPPH assay method.

S.no.	Conc.	%Inhibition	Leaf Extracts			Stem Extract
		Ascorbic acid (%inhibition)	Petroleum ether (%inhibition)	n-hexane (%inhibition)	Methanol (%inhibition)	Ethanol (%inhibition)
1.	01	93.64	84.75	99.01	95.68	87.58
2.	0.2	93.53	85.23	98.75	95.35	84.65
3.	0.3	91.47	73.55	98.39	91.75	83.84
4.	0.4	91.15	42.51	97.68	88.67	82.64
5.	0.5	90.46	39.67	96.83	85.68	81.23
6.	0.6	90.25	34.35	95.46	87.11	80.46

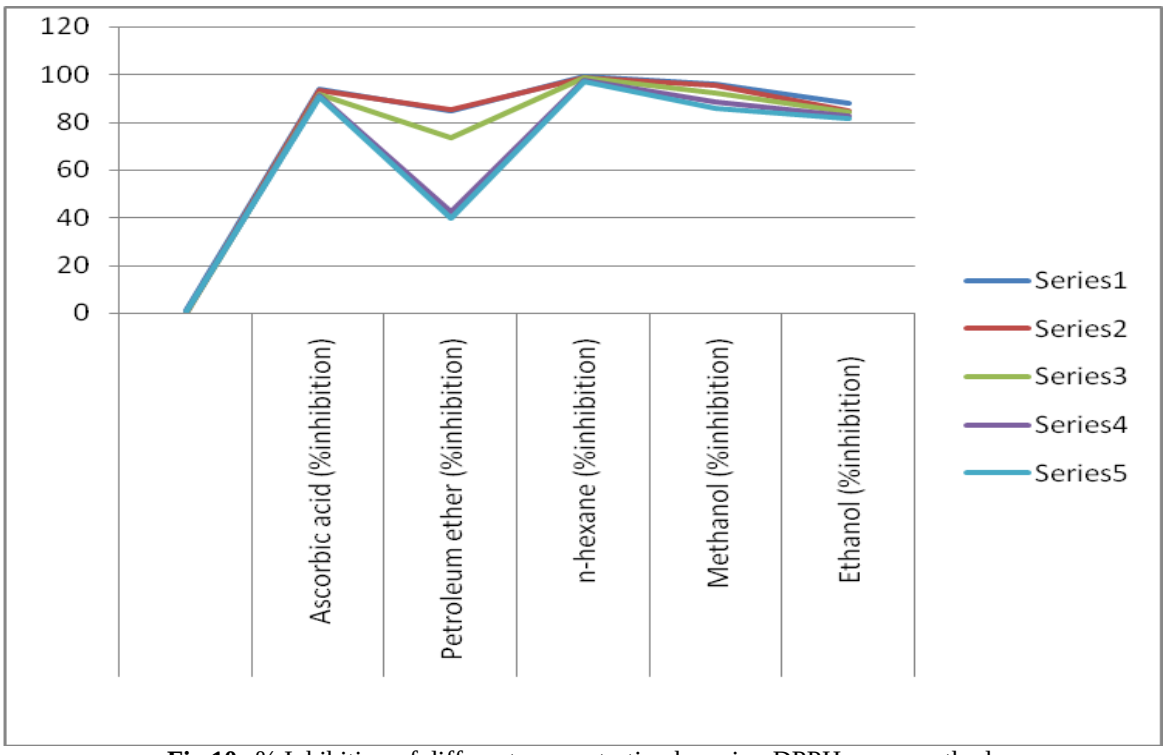

Fig 10:-% Inhibition of different concentration by using DPPH assay method.

III. Discussion

After Performing the phytochemical Screening of *Euphorbia heterophylla* leaves and stems of various extracts has shown total flavonoids content was 3.48% and total tannin content was 1.65% , Loss on drying of leaves was 0.1367% and loss on drying of stem was 0.106%. Total ash values for leaves was 0.636% w/w and for stem was 0.64% w/w . Water soluble ash values for leaves was 0.61% w/w and for stem it was 0.945%. Acid soluble ash values for stem was 1.265% w/w and for stem was 1.64%. Extractive values of leaves by using ethanol solvent was 11% and by using water as a solvent was 15.8% and the extractive values for stem using ethanol as a solvent was 16.6% and water was 10.%

Thin layer chromatography was done to determined by retardation factor . The rf values for Different extract was petroleum ether= 0.52, n-hexane= 0.75, chloroform= 0.78, methanol= 0.65, ethanol= 0.67.

The DPPH method was used to determine anti-oxidant activity of different extyracts ocf leaves and the stems of *Euphorbia heterophylla* with the standard ascorbic acid and it has revealed that maximum absorbance

has shown by Petroleum ether extract at 0.1mg/ml concentration [0.922] with minimum % inhibition [39.67%] whereas minimum absorbance was shown by n-hexane extract [0.0139] and maximum inhibition action [99.01%] followed by methanolic extract [95.68%] in the same concentrations.

It shows that the absorbance and percentage inhibitions are inversely proportional to each other as when there is increase in absorbance, the % inhibitory action of DPPH decreases and there is increase in % inhibition the absorbance decreases.

IV. Conclusion

The phytochemical investigation of leaves and stems of *Euphorbia heterophylla* has shown the presence of alkaloids, glycosides, flavonoids, saponins,steroids, tannins. The extract of n-hexane contained tannins and flavonoids therefore, it has shown maximum anti-oxidant activity due to the presence of polyphenolic groupand presence of free radicals. Therefore, these extracts can be further used to cure many disease which can undergo free radical mechanism through its inhibitory action.

The presence of major phyto-constituent in leaves extract has shown the maximum antioxidant activity then the stems of *Euphorbia heterophylla.*

Conflict of interest: Nil

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