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RESEARCH ARTICLE

QUALITATIVE AND QUANTITATIVE ESTIMATION AND CHARACTERIZATION OF SECONDARY METABOLITES OF *Typhonium trilobatum* AND *Trichosanthes dioica* (Less focussed edible herbal medicinal plants of Bengal) LEAF EXTRACT

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Abstract

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest particularly in edible plants has grown throughout the world. So the aim of the present study is to investigate the two undocumented edible medicinal herb leaf *Typhonium trilobatum* (kharkol) and *Trichosanthes dioica* (parwal) for their qualitative and quantitative analysis and characterization of secondary metabolites which involves phytochemical screening assays, Total polyphenol content, Total flavonoid content, DPPH radical scavenging assay, ABTS radical scavenging assay, Total antioxidant capacity, Total tannin content and chromatographic techniques such as Column chromatography, HPLC and, TLC. Result of phytochemical screening showed that, among different solvent extract most biologically active phytochemicals were present in 80% methanolic leaf extract of *Trichosanthes dioica* and *Typhonium trilobatum*, followed by aqueous extract irrespective of their content. Quantitative results revealed that both leaf extract were quite good enough in phytochemical and antioxidant potential but in comparison to Kharkol leaf Parwal leaf except total hydrolysable tannin, is a better source of polyphenol and flavonoids. Parwal leaf extract also exhibited more significant Total antioxidant capacity (387.84 µg/g dry extract), DPPH (50% inhibition at a concentration of 170.86 µg/ml) and ABTS (50% inhibition at a concentration of 140.64 µg/ml) radical scavenging potential than kharkol (170.01, 421.81 and 176.01 µg/ml respectively) leaf extract. HPLC analysis of purified fraction of both sample extracts documented that Quercetin, Gallic acid and in some cases Catechin was present as specific phytochemicals, which further support their disease treating potential in traditional medicine.

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Introduction:-

The nature provides remedies of majority of diseases to the mankind which has been a victim of diseases since the very beginning of their existence. So, medicinal plants are an important research area for novel and bioactive molecules for drug discovery. The heavy reliance on plant medicine is attributed to their relative accessibility, low prices, local availability, and acceptance by local communities and the low number of dispensaries and doctors for healthcare needs especially in rural areas. Some natural plants are being used extensively as food and health supplements, which help to combat diseases but due to lack of scientific evidence considerable part of them still

remain less focused. Medicinal plants are biosynthetic laboratory, not only for chemical compounds but for phytochemicals such as glycosides, alkaloids e.t.c which exert physiological and therapeutic effects (Dfeudis., 1991). The impressive and large chemical diversity expressed in the plant kingdom is due to the high capacity developed by plant genomes, namely the high diversity of genes able to codify many metabolic enzymes. A study shown that plants possess more genes than other living organisms, such as mammals and bacteria. Furthermore, given that only a small portion of over 400,000 worldwide plant species has been analyzed from a phytochemical point of view, (Yonekura et al., 2009; Macel et al., 2010). In modern civilization where the plant derived drug, life style modification and food based approach in combating the degenerative diseases become the agenda of modern research it is beneficial to fabricate as more as possible the less focused plant extract in medicine. (FAO & ILSI., 1997). Such type of less focused edible medicinal leafy vegetables are *Typhonium trilobatum* and *Trichosanthes dioica*, which are the subject of this study. Now in addition to quantitative measures, in modern pharmaceutical research chromatography achieves a great deal of contract for characterisation of bioactive compound. Scientific characterisation and isolation of specific bio potential compound is immensely important for new drug. Chromatography more specifically analytical high performance liquid chromatography is used as a valuable way for isolation, characterisation, and identification of specific bioactive compound. So in this study HPLC is selected to highlight the bioactive potential with scientific evidence of ignorant less focused medicinal herb.

Taxonomic Hierarchy of *Typhonium trilobatum*:-

Kingdom:	Plantae
Phylum:	Magnoliophyta
Class:	Liliopsida
Order:	Arales
Family:	Araceae
Genus:	<i>Typhonium</i>
Species:	<i>Typhonium trilobatum</i>

Typhonium trilobatum belongs to the Araceae family and it is small to moderate sized perennial herb, commonly known as Bengal arum, Ghatkanchu or Ghatkol or Kharkol(WHO., 1990). It has been valued in Ayurveda and Unani system of medicine for possessing variety of therapeutic properties. The rhizome of *Typhonium trilobatum* has been used for the treatment of vomiting, cough, asthma, excessive expectoration, pyrogenic sore throat, headache, gastric ulcer, abscess, snake bite (Halder et al., 2011; Kandhasamy et al., 2008), diarrhoea and dysentery(Shanmugam et al., 2011), stimulant and menstrual troubles (Biswas et al., 2010). Leaves are cooked as vegetables and given to the patient suffering from piles and rheumatism(Das et al., 2009; Rahmatullah., 2010).

Taxonomic Hierarchy of *Trichosanthes dioica*:-

Kingdom	Plantae-	Plants
Division	Magnoliophyta-	Flowering plants
Class	Magnoliophyta-	Dicotyledons
Subclass-		Dilleniidae
Order-		Violales
Family	Cucurbitaceae-	cucumber family
Genus	<i>Trichosanthes</i> L.-	<i>trichosanthes</i> P

Species *Trichosanthes dioica* Roxb:-

The plants in Cucurbitaceae family are an annual or perennial herb distributed in tropical Asia, Polynesia and Australia. Over 20 species are recorded in India of which two, namely *T. anguina* and *T. dioica*, are cultivated as vegetables. *Trichosanthes dioica*, commonly known as parwal(Nadkarni., 1982) has a promising place in Ayurvedic system of medicine from ancient time. According to Ayurveda, leaves of the plant are used as antipyretic, diuretic, cardi tonic, laxative, antiulcer, diabetes mellitus etc(Khare., 2004). In Charaka Samhita, leaves and fruits are used for treating alcoholism, jaundice, edema and alopecia (Kumar et al., 2014).

In spite of having potential antioxidant activity, the edible plant leaf remain less focused in our daily diet due to lack of scientific popularity but the use of these plants as traditional medicine confirms that they may possess some important biological activities. Thus, the unfocussed part of *Trichosanthes dioica* and *Typhonium trilobatum* leaves insisted the authors to look out for assay of their antioxidant property though characterisation, which might be beneficial to ameliorate diseases of the present & future generations.

Materials and method:-**Sample Collection:-**

The plant *Typhonium trilobatum* and *Tychosanthes dioica* were collected from different district of west Bengal as well as from different local market.

Identification of sample:-

Whole plant of *Typhonium trilobatum* (L.) and *Tychosanthes dioica* were collected during July, 2014, from three different district as well as local market of kolkata. Then the plant samples were submitted to the Herbarium of Calcutta University, Kolkata. One week later its voucher specimen was collected after its identification (Accession No. 20012 and 20013 respectively) which was identified and authenticated by taxonomist of the Calcutta University Herbarium, Kolkata.

Preparation of the Sample:-

The leaves were air-dried for easy powdering. The dried leaves were ground into fine powder and then weighed.

Phytochemical Screening:-

The following tests were carried out on crude extracts and solvent-solvent extracts (i.e. ethylacetate, diethyl ether, benzene, petroleum ether, ethanol, 80% methanol, aqueous extracts) of two samples in order to ascertain the presence of these phytochemicals: flavonoids, polyphenol, tannins, glycosides, saponins, steroid and alkaloid.

Test for Flavonoids:-

1cm³ of 10% NaOH was added to 3cm³ of the extract. A yellow colouration indicates the presence of flavonoid.

Test for Tannins:-

1ml of freshly prepared 10% KOH was added to the extract, a dirty white precipitate indicates the presence of tannins.

Tests for Phenolic compounds:-

- **Ferric chloride test:** Extract solution gives blue-green color with few drops of FeCl₃.

Test for Glycosides:-

To 1ml of the extract in the test tube, 10ml of 50% H₂SO₄ was added. The mixture was heated in boiling water for 15minutes. 10ml of fehling's solution was added, a brick red precipitate indicates the presence of glycosides.

Test for Saponins:-

Emulsion test: 5 drops of olive oil was added to 3ml of the extract in a test tube, the mixture was vigorously shaken. A stable emulsion indicates the presence of saponins.

Test for Steroid:-

Salkowski test; 5 drops of concentrated H₂SO₄ was added to 1ml of the extract. Red colouration indicates the presence of steroids in the extract.

Test for Alkaloids:-

To 1ml of the extract, 2 drops of Maeyer's reagent was added. A creamy precipitate indicates the presence of alkaloids in the extract.

Determination of Phytochemicals content:-**Determination of Total Polyphenol Content (TPC):-**

Total polyphenol content of the leaf extracts was determined by the method described by Matthaus 2002 with some modification. Briefly, 0.2 ml of different concentrations of the extracts were taken, to which 1ml of Folin-ciocalteu reagent (diluted to 10-folds) and 0.8 ml of 2% Na₂CO₃ were added, and the volume was made up to 10 ml with methanol:water (6:4). After 30 min the absorbance was read at 740 nm wavelength against a standard calibration curve. The results were expressed as Gallic acid equivalents per gram of extract.

Determination of Total Flavonoids Content (TFC):-

Total flavonoid content was measured by the aluminium chloride colorimetric assay (Ebrahinzadeh et al., 2008). An aliquot of extracts (100 µg/ml) or the standard solution of quercetin (20 to 100 µg) were added to a 10 ml volumetric flask containing 4 ml of distilled water. 0.3 ml 5% NaNO₂ was added. After 5 min, 0.3 ml 10% AlCl₃ was added. At 6th min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with water. The solution was mixed well, and the absorbance was measured against prepared reagent blank at 510 nm.

Determination of Total Hydrolysable Tannin Content(HTC):-

Content of tannins was determined by Folin-Denis reagent (Parimala et al., 2011) based on colorimetric estimation of tannins (measurement of blue colour formed by the reduction of phosphotungstomolybdic acid by tannins in alkaline medium 0.5gm of dry sample was added to 75 ml of distilled water, heated gently at first and then boiled for 30 minutes. Then it was centrifuged at 2000 rpm for 20 minutes. The supernatant liquid was collected and made up to the volume of 100ml (volumetric flask) by distilled water. After that 0.1ml of this solution was taken in a test tube and 7.5ml of water, 0.5ml of Folin-Denis reagent and 1ml of 35% Na₂CO₃ were added to it. The volume was made up to 10ml (volumetric flask) by using distilled water and shaken well. The test tubes were incubated for 30 minutes at room temperature and the absorbance of the solution was measured at 700nm wavelength against a standard calibration curve. Tannic acid was used as a standard to prepare the calibration curve in the range of 20µg-100µg.

In vitro Antioxidant activity:P-**1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay:-**

The DPPH radical scavenging method was used to evaluate the antioxidant property. Different concentrations of the plant extracts were used to scavenge DPPH. The antioxidant activity of each sample was expressed in terms of IC₅₀, and was calculated from the graph after plotting inhibition percentage against extract concentration. DPPH assay was carried out after making some modifications in the standard protocol (Hsu et al., 2007). 3 ml of 0.1 mM DPPH solution was mixed with 1 ml of various concentrations (100 to 300 µg/ml) of leaf extract. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm by a spectrophotometer. The solution without any extract and with DPPH and methanol was used as control. The experiment was replicated in three independent assays. Inhibition of DPPH free radical in percentage was calculated by the formula:

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{test}}) / A_{\text{control}}] \times 100$$

Where A_{control} is the absorbance of the control and A_{test} is the absorbance of reaction mixture samples (in the presence of sample). All tests were run in triplicates (n=3), and average values were calculated.

IC50 value:-

Inhibition Concentration (IC₅₀) parameter was used (Brand-Williams et al., 1995) for the interpretation of the results from DPPH method. The discoloration of sample was plotted against the sample concentration in order to calculate the IC₅₀ value. It is defined as the amount of sample necessary to decrease the absorbance of DPPH by 50 %.

Determination of ABTS+ scavenging activity:-

For ABTS assay, the procedure followed was the method of (Dimitrina et al. 2010 ; Roberta et al., 1999) with some modifications. ABTS was dissolved in water to make a concentration of 7 mmol/L. ABTS+ was produced by reacting the ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. For the test of samples, the ABTS+ stock solution was diluted with 80% methanol to an absorbance of 0.70 ± 0.02 at 734 nm. After the addition of 4.85 ml of diluted ABTS+ to 0.15 ml of stock samples solution (final concentration 1mg/ml), the absorbance reading was taken 6 min after the initial mixing. The activities of the samples were evaluated by comparison with a control (containing 4.85 ml of ABTS solution and 0.15 ml of 80% Methanol). Each sample was measured in triplicate and averaged. This activity is given as percentage ABTS+ scavenging that is calculated by the following formula:

$$\text{ABTS+ scavenging activity (\%)} = (A_c - A_s) / A_c \times 100$$

Where, A_c is the absorbance value of the control and A_s is the absorbance value of the added samples test solution. The antioxidant capacity of test compounds was expressed as EC₅₀, the concentration necessary for 50% reduction of ABTS+.

Extraction of the Sample for characterisation:-

Solvent-solid extraction was carried out on the weighed, air-dried and pulverized leaves of typhonium trilobatum and Tychosanthes dioica. The weighed 50 gm of each sample were soaked with 500ml of 80% methanol for one day. The separation of the residue from filtrate was done by using filter paper. Final solvent extract was concentrated separately under reduced pressure (Harborne, 1998).

Fractionation of extracts:-

The dried methanol extract was redissolved in 100ml of 80 % aqueous methanol. The 80% aqueous extract was taken in a separating funnel, 100 ml of petroleum ether was added to the separating funnel, the funnel was shaken for few minutes and allowed for phase separation, the organic petroleum ether phase was separated, the lower methanolic phase was collected. Methanolic aqueous phase was taken again in separating funnel 100 ml of chloroform was added and the funnel was shaken for few minutes and allowed for phase separation. The lower chloroform phase was collected. 10-20 ml of water was added to the aqueous phase and taken in a separating funnel, 100 ml of diethyl ether was added in the separating funnel and shaken for few minutes. Diethyl ether phase was collected and the remaining methanolic fraction was further re-extracted with ethyl acetate and the residual phase is treated as aqueous phase. All the fractions were collected and dried using rotary evaporator and subjected for chromatography analysis.

Chromatography:-

We use two types of chromatography methods to separate the constituents that were present in the leaves extract i.e. column chromatography and thin-layer chromatography.

Column Chromatography:-

This was done to isolate and purify the constituents present in the extracts.

Packing of Column:-

Dried glass column was held in place by retort stand and was sealed with glass-wool.

The column was packed with petroleum ether and silica gel as adsorbent and the column was tapped in order to avoid air-bubbles. 5ml of the extract was introduced into column then solvent mixture (eluent) in proper ratio was added into the column. Several fractions were obtained, concentrated and their purity was determined by using thin-layer chromatography. The impure fractions were further re-chromatography using a different solvent mixture.

Thin-Layer Chromatography (TLC):-

TLC was used to ascertain the number of constituents present in the extract and to determine their purity and to select most active fraction extract. TLC was also used to determine the solvent mixture that will affect the separation of the components.

HPLC Analysis:-

Due to the fact that plant extracts usually occur as a combination of various type of bioactive compounds or phytochemicals with different polarities, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds. For characterisation of these bioactive compounds a number of different separation techniques such as TLC, column chromatography and HPLC, should be used to obtain pure compounds. In this study the qualitative and quantitative analysis of the phytochemicals of the Trichosanthes dioica and Typhonium trilobatum were performed by HPLC with a Perkin- Elmer 200 Series HPLC with UV-VIS detector (operated at 262 nm for quercetin, 257 nm for gallic acid and 315nm for Catechin) and injection valve with 20- μ L sample loop. Compounds were separated on a 4.6 mm \times 250 mm. Brownlee analytical PR-C18 column protected by a guard column containing the same packing. The flow rate were 1.2 ml/minutes for quercetin and 1.0 ml/minutes for gallic acid and 0.65ml/minutes for catechin. Data were integrated by total chrom navigator software (version-6.3.2.0646) and results were obtained by comparison with standards. Simple mobile phase was used as control for identification of blank peaks.

Solvent used:- Methanol: acetonitrile: water (60:20:20) for quercetin and Methanol: water: acetic acid (45:54:1) for gallic acid and Acetone: water: acetic acid (70: 29.5: .5) for catechin

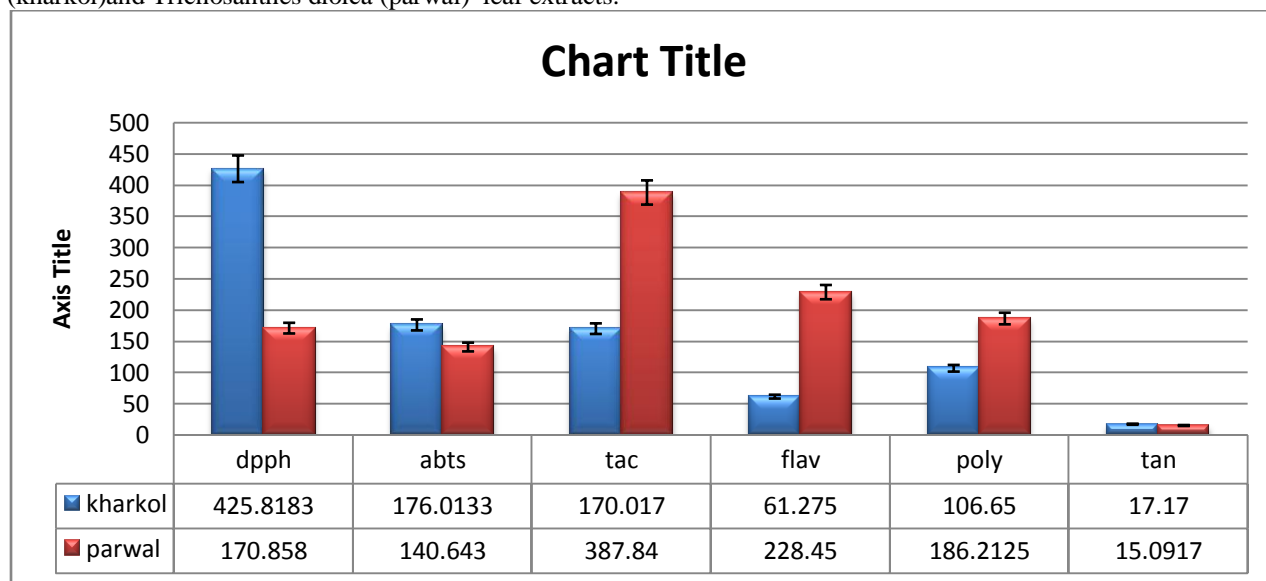
Isolated compound was dissolved uniformly in 100% methanol and 10 μ l of sample taken and made up to 1ml in HPLC grade methanol and injected (20 μ l) in the HPLC system.

Result:-**Table 1:-** Phytochemical screening of *Typhonium trilobatum*.

Extract	Pheno-lics	Flavo-noids	Tan-nin	Anthra-Quinine	Glyco-sides	Sapo-nin	Ster-Oid/Terp enoids	Alka-loids
Ethyle acetate	+	+	+	-	-	-	+	-
Diethyle ether	+	+	-	-	-	-	-	-
Benzene	-	-	-	-	-	-	+	-
Petrolium ether	-	-	-	-	-	-	+	-
Chloroform	-	-	-	-	-	-	+	-
Ethanol	++	+	-	-	-	-	+	-
80% Methanol	+++	++	+	-	+	-	+	+
Aqueous	++	++	+++	-	+	-	-	+

Table 2:- Phytochemical screening of *Trichosanthes dioica*.

Extract	Pheno-lics	Flavo-noids	Tan-nin	Anthra-quinone	Glyco-sides	Sapo-nin	Ster-Oid/Terp enoids	Alka-loids
Ethyle acetate	+	+	-	-	-	-	+	-
Diethyle ether	+	+	-	-	-	-	-	-
Benzene	-	-	-	-	-	-	-	-
Petrolium ether	-	-	-	-	-	-	+	-
Chloroform	-	-	-	-	+	-	+	-
Ethanol	++	+	-	-	-	-	+	+
80% Methanol	+++	+++	+	-	-	-	+	+
Aqueous	++	+	+++	-	+	-	-	+

Figure 1:- Comparisive result of phytochemical content and in vitro antioxidant assay of *Typhonium trilobatum* (kharkol) and *Trichosanthes dioica* (parwal) leaf extracts.

Values are presented as mean \pm SD (n=3). Values are significantly different at $P < 0.01$ by one way anova.

1. Standards	Retention time(RT)	Peak Area (uV Sec)	Peak Height (uV)	Major component
A. Gallic acid	2.973	145073.35	15708.17	
B. Quercetin	2.508	1578552.66	95546.77	
C. Catechin	6.249	25300267.13	925016.64	
2. Sample Diethyle ether fraction				
2.a. Ethyleacetate:Petroleum ether (1:1)eluates	i. 2.528 ii. 6.05	1333538.18	92783.10	i.Quercetin ii.Catechin
2.b. Ethyl acetate:Methanol (90:10)	i. 2.487 ii. 1.60	3408271.73	243509.46	i.Quercetin ii.Probly Gallic acid
2.c. 10% Methanol	i. 2.458 ii. 1.7	445872.73	39248.17	i. Quercetin ii.Probably Gallic acid
3. Ethyl acetate fraction				
3.a. 10% Methanol	2.972	1407589.46	136264.01	Gallic acid
4. Aqueous Fraction				
4.a. 10% Methanol	2.996	6952053.28	633426.92	Gallic acid

Table 3:- The HPLC analysis of Typhonium trilobatum.**Table 4:-** HPLC analysis of Trichosanthes dioica.

1. Standards	Retention time(RT)	Peak Area(uV Sec)	Peak Height (uV)	Major component
A. Gallic acid	2.973	145073.35	15708.17	
B. Quercetin	2.508	1578552.66	95546.77	
C. Catechin	6.249	25300267.13	925016.64	
2. Sample Diethyl ether fraction				
a. 100% ethyle acetate	i. 2.495 ii. 2.960	15001898.57	1.04e+06	i. Quercetin ii. Gallic acid
b. Ethyle acetate: Petroleum ether(3:1)	iii.5.75 i.2.960			iii.Probably Catechin i.Gallic acid
c. 10% Methanol	ii. 5.8			iii.Probably Catechin
d. 5% Methanol	i. 2.437 ii. 2.960 iii.5.85	1393208.49	107286.51	i. Quercetin ii. Gallic acid iii.ProbablyCatechin
	i. 2.472 ii. 2.90 iii.6.5	6674483.70	317601.15	i. Quercetin ii Gallic acid iii.Probably Catechin
3. Ethyle acetate fraction				
a. Ethyle acetate:Petroleum ether (1:1)	2.975	31175.91	15137.33	Gallic acid
b. 10% Methanol	2.988	1108611.79	132834.83	Gallic acid
4. Aqueous fraction				
a. 10% Methanol	i. 2.973 ii. 6.15	5367669.25	364494.23	i. Gallic acid ii.Probably Catechin
b. Pure Methanol	i. 2.950 ii. 2.5	5605153.86	443057.36	i. Gallic acid ii.Probably Quercetin

Discussion:-

The present study encompasses the Qualitative and quantitative evaluation of phytochemical content and antioxidant activity through the characterization of *T. Dioica* and *T. Trilobatum* leaves.

Preliminary Phytochemical screening:-

Results are represented in Table 1. And Table 2. In the qualitative phytochemical screening using various solvent extracts of both samples, it was found that most of the biologically active phytochemicals were present in 80% methanolic leaf extract of *Typhonium trilobatum* and *trichosanthes dioica*. In case of *Typhonium trilobatum* the presence of secondary metabolites in different solvent followed the order: 80% methanol > Aqueous > Ethyle acetate > Ethanol > Diethyl ether > Chloroform \geq Petroleum ether \geq Benzene. But the content of three important metabolites namely phenolics, Flavonoids, Tannin are greater in aqueous part than 80% methanolic part. The secondary metabolites in *trichosanthes dioica* followed the order : 80% methanol > Aqueous > Ethanol > Ethyle acetate > Diethyl ether > Chloroform \geq Petroleum ether \geq Benzene. This screening confirms the presence of different phytochemicals in these leaf samples which were further analysed both quantitatively and qualitatively.

Phytochemicals (Polyphenol, Flavonoid, Tannin):-

Phenolic compounds and flavonoids are very important plant secondary metabolites. phenolics are responsible for the variation in the antioxidant activity of plants (Cai et al., 2004). They exhibit antioxidant activity by deactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals, (Pokorny et al., 2001; Pitchaon et al., 2007) or chelate metal ions and protect against pathogens and predators (Balasundram et al., 2006). The most frequently encountered flavonoids are flavonols, quercetin, flavanols. The concentrations of phenolics in the above discussed leaf extracts were expressed as micrograms of gallic acid equivalents per gram of extract. More or less near about double polyphenol content and triple flavonoids content of *T. dioica* suggested it more wealthy in phytochemicals content in comparison to *T. trilobatum*. But in case of tannin the result is reversed.

In vitro Antioxidant Assay:-**Total Antioxidant Capacity:-**

This method is based on the reduction of molybdenum (VI) to molybdenum (V) by the antioxidants and the subsequent formation of a green phosphate Mo(V) complex at acidic pH values. Electron transfer occurs in this assay which depends on the structure of the antioxidant (Prieto et al., 1999). The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, tocopherols and carotenoids. Ascorbic acid was used as standard to prepare the calibration curve. Better activity recommend *T.dioica* as a better antioxidant capacity holder in comparison to *T. trilobatum*.

DPPH and ABTS free radical scavenging activity:-

From result it is depicted that both samples were active in quenching free radicals like DPPH as well as ABTS but lower IC_{50} value of *T. Dioica* than *T. Trilobatum* suggested that former is more efficient in scavenging the free radical at a lower concentration than the other.

HPLC Analysis:-

For the identification of metabolites showing antioxidant potentials the fractions are subjected to HPLC. Quercetin, catechin and gallic acid are used as standard metabolites. After fractionation of 80% methanolic leaf extracts with different solvent of increasing polarity Thin layer chromatography was performed to select two or three most active fraction extract. From TLC result it is observed that among five different fraction Diethyl ether, ethyl acetate and aqueous fraction are active for both sample extracts. The residue of Diethyl ether extract of *kharkol* was resolved over a column of silica gel (Mesh size 100-200 μ) by elution with solvent and solvent mixtures of increasing polarity. The HPLC analysis of *T. dioica* and *T. trilobatum* were represented in Table 3. And Table 4. Ethyl acetate – petroleum ether (1:1) eluates revealed the presence of quercetin as major component, as the retention time of the major peak was compared with that of standard quercetin and the ethyl acetate and aqueous fraction contain gallic acid as major component. Diethyl ether fraction of *T. dioica* contains both Quercetin and gallic acid whereas the ethyl acetate fraction contains only Gallic acid as major component. Aqueous fraction contains Gallic acid and probably catechin and Quercetin.

Conclusion:-

In the present study, qualitative and quantitative analysis of leaf extract of *Trichosanthes dioica* and *Typhonium trilobatum* showed the presence of phenolic compound with high in vitro antioxidant potential. HPLC analysis for the different fraction of MeOH extract revealed the presence of Gallic acid, quercetin and catechin as specific phytochemicals which have potent radical scavenging as well as disease preventing potential. Moreover, the three different in vitro antioxidant activity assays, including FRAP assay, DPPH radical scavenging activity, ABTS radical scavenging assay and Total antioxidant capacity assay revealed that methanolic extract have potent antioxidant activity and could be employed as a new easily accessible source of natural antioxidants. They can be a possible to be used as dietary supplement in therapeutics and food. Moreover these leaves should get a place in our daily diet like other green leafy vegetables. Further investigation for antioxidant activities will be performed in future studies

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