# SCREENING OF SECONDARY METABOLITES OF *EULOPHIA NUDA* LINDL., HPTLC PROFILING, AND DETECTION OF ANTIOXIDANT COMPOUNDS USING TLC.

**Vikas Nanekar[1], Varsha Shriram[1] and Vinaykumar[2]**
**[1]Prof. Ramkrishna More A C S College, Akurdi, Pune 44; [2]Modern College, Ganeshkhind Pune.**

**Abstract:***Eulophia nuda* Lindl. (Orchidaceae), a medicinally important perennial orchid with underground tubers used against tumors, scrofulous glands of the neck, bronchitis, blood diseases, rheumatoid arthritis besides as blood purifier, appetizer, vermifuge by folklores. Antioxidant, DNA damage protecting activity and antiproliferative activity of this plant had been reported earlier. Present work deals with screening of secondary metabolite of *Eulophia nuda* Lindl., HPTLC profiling, and detection of antioxidant compounds using TLC. Results revealed most of secondary metabolites including phenols flavonoids, tannins and alkaloids etc were present in ethyl acetate extract followed by methanol. The DPPH reagent was used for the detection of active antioxidant molecules on thin layer chromatograph, developing an easy and useful technique for isolation of active molecules. Clear zone (white spots) against violet background of DPPH indicated and confirmed the presence of antioxidant molecules. Ethyl acetate extract was showing highest DPPH radical scavenging activity and clear white spot on TLC plate. Thus ethyl acetate extract can be used further for the isolation of antioxidant molecules.

**Keywords:** medicinal orchid, HPTLC profiling, secondary metabolites, DPPH

## 1. Introduction:

***Eulophia nuda* Lindl.** [Synonym: ***Eulophia spectabilis* (Dennst.) Suresh**] commonly known as '*Amarkand*' is highly medicinal perennial orchid with underground tubers. In India, this plant is found in tropical Himalayas (Uttarakhand, Arunachal Pradesh, Assam), Western Ghat (Maharashtra, Karnataka, Kerala) (Singh and Duggal 2009; Nanekar et al., 2014). Raw tubers are eaten for curing rheumatoid arthritis. Its rhizomes were reported to be used against tumors, scrofulous glands of the neck, bronchitis, blood diseases (Mali and Bhadane 2008; Patil and Mahajan 2013). It is also used as appetizer, tonic vermifuge, aphrodisiac drug and blood purifier. Nagulwar et al., 2017 reported antibacterial, antifungal and antiglycation effect of this plant. Some phenanthrene derivatives had been reported from this plant (Bhandari et al., 1985; Tuchinda et al., 1989). Earlier our group has reported anti-proliferative activities of a phenanthrene derivative compound 9, 10-dihydro-2, 5- dimethoxyphenanthrene-1, 7-diol isolated from this plant against human cancer cells (Shriram et al 2010). Further, Kumar et al., 2013 reported antioxidant activity and DNA damage protecting activity of this plant. Here we are first time HPTLC profiling and detection of antioxidant molecules on TLC plates.

## 2. Material and method:

### 2.1 Collection and Extraction of plant material:

The plant material collected in July – August from Belguam (India) and authenticated from Botanical Survey of India, Pune. Rhizomes of the plant were washed thoroughly with tap water. The tubers were allowed to be shade dried completely and were coarsely powdered and used for the further solvent based extraction procedure. Five hundred g of dry powder was extracted successively with petroleum ether (PE), ethyl acetate (EA), methanol (M) and aqueous methanol (AqM) by cold percolation method. All the obtained extracts were concentrated *in vacuo* using a rotary evaporator (Roteva, Equitron, India). Amount of all the extracts were measured and expressed as percentage yield. Concentrated extracts were transferred in glass vials and stored at 4ºC for the further use.

### 2.2 Qualitative analysis (Preliminary screening of secondary metabolites)

Preliminary screening of all the extracts including PEE, EAE, ME and AqME was carried out by standard qualitative tests for following secondary metabolites (Trease and Evans 1989; Kujur et al., 2010; Savithramma et al., 2011; Bhandary et al., 2012).

### 2.2.1 Phenols and tannins

Crude extract was treated with 2 ml of 2% solution of $FeCl_3$. A blue-green or black colouration indicated the presence of phenols and tannins.

### 2.2.2 Flavonoids

Crude extract was treated with 2 ml of 2% solution of sodium hydroxide. The dark yellow color was formed turned colorless after addition of 3 - 4 drops of diluted acid which indicated the presence of flavonoids.

### 2.2.3 Alkaloids

Crude extract was treated with 2 ml of 1% hydrochloric acid and heated lightly followed by addition of Mayer's and Wagner's reagents. Turbidity of the resulting precipitate was indicated presence of alkaloids.

### 2.2.4 Terpenoids

Two ml extract was added to 2 ml of acetic anhydride and concentrated of sulfuric acid resulted in blue - green rings formation which indicated the presence of terpenoids.

### 2.2.5 Steroids

One ml extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulfuric acid was added. The upper layer turns red and sulfuric acid layer showed yellow with green fluorescence indicated the presence of steroids.

### 2.2.6 Saponins

Five ml of extract was treated with 20 ml of distilled water and agitated for 15 min. Formation of foam indicated the presence of saponins.

### 2.2.7 Glycosides

Crude extract was treated with each of 2 ml of chloroform and 2 ml of acetic acid and mixture was cooled by keeping in ice. Concentrated sulfuric acid was added. A color change from violet to blue to green indicated the presence of glycoside.

### 2.2.8 Coumarins

Three ml of 10% sodium hydroxide was added to 2 ml of aqueous extract formation of yellow color indicates the presence of coumarins.

### 2.3 High performance thin layer chromatography (HPTLC) profiling of crude extract

HPTLC was performed on silica gel 60 f 254, HPTLC plates (Merck, Germany). Ethyl acetate: methanol: formic acid: water [20:2.5:0.5:2 (v/v)] used as a mobile phase. Five µl of each extract (1 mg $ml^{-1}$) were applied to the plates with 10 mm bands, sample application with CAMAG - Linomat IV automated spray on band applicator equipped with a 100 µl syringe and operated with settings as follows: application rate 10 s $\mu l^{-1}$, distance between bands 4 mm, band width 10 mm, distance from the plate side edge 15 mm and 20 mm from the bottom of the plate. CAMAG TLC Scanner 3 was used for scanning. The scanner operating parameters were: (Mode: absorption / reflection; Slit dimension; 5 x 0.1 mm; scanning rate: 20 mm $s^{-1}$ and monochromator band width: 20 nm at an optimized wavelength 254, 366 nm and in visible range).The plate was scanned at 254 nm and 366 nm before spraying (derivatization) and at 600 nm after spraying with detection reagent (Anisaldehyde sulfuric acid reagent and plate was heated at 110 °C for 5 min).

### 2.4 Determination of antioxidant molecules from extract on TLC by DPPH reagent:

All the extract were subjected to TLC on silica gel 60 f 254, 20X10 cm plates (Merck, Germany-#5642), with ethyl acetate: methanol: formic acid: water [20:2.5:0.5:2 (v/v)] as a mobile phase. Anti-oxidant compounds from extracts were visualised on TLC plate by dipping TLC plate in 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) reagent and antioxidant compounds were identified which showed clear zone against violet background (Nile et al 2014).

## 3. Results and discussion:

The yield of extracts in percentage was calculated with respect to the initial dry material (Table 1). Substantial variation was observed in terms of yield of each extract, obtained using solvents of different polarities. Highest yield was observed in ME (2.98%), whereas the least polar solvent PE yielded only 0.24% extract, indicating solvent polarity-dependent yield. The second best yield was attained in EAE (2.31%), followed by AqME (1.12%).

Table 1: Yield obtained and percentage of extract from dry powder using different solvents.

| Sr. No. | Extract | Yield obtained (g) | Yield percentage |
|---|---|---|---|
| 1. | Pet ether (PE) | 1.19 | 0.24 |
| 2. | Ethyl acetate (EaE) | 11.5 | 2.30 |
| 3. | Methanol (ME) | 29.9 | 5.98 |
| 4. | Aqueous methanol (AqME) | 11.2 | 2.24 |
| 5. | Aqueous (AqE) | 10.6 | 2.12 |

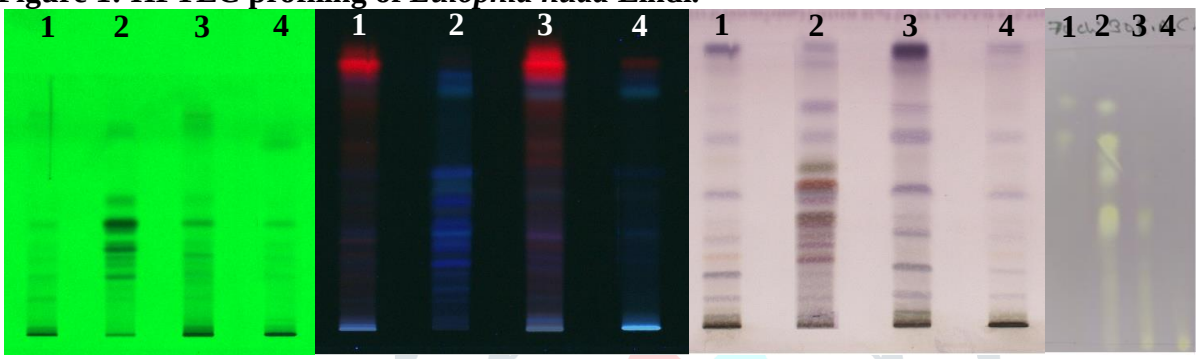
Crude plant extracts namely petroleum ether extract (PEE), ethyl acetate extract (EAE), methanol extract (ME) and aqueous methanol extract (AqME) were obtained from authenticated plant material via cold percolation method. Qualitative tests HPTLC profiling (Figure 1) revealed that most of secondary metabolites were detected in ethyl acetate extract followed by methanol.

**Table 2: Qualitative analysis of secondary phyto-chemicals in different extract**

| Extract | Phenols | Flavonoids | Tannins | Alkaloids | Terpenoids | Steriods | Saponin | glycosides | Coumarins |
|---|---|---|---|---|---|---|---|---|---|
| PE | + | + | - | - | + | - | - | + | +++ |
| EAE | +++ | +++ | ++ | +++ | +++ | +++ | + | ++ | ++ |
| ME | ++ | ++ | ++ | +++ | +++ | + | + | ++ | + |
| AqME | - | + | + | + | ++ | - | +++ | ++ | + |

- Not detected, + + slight presence; ++ medium presence; +++ heavy presence.

**Figure 1: HPTLC profiling of *Eulophia nuda* Lindl.**




Visualization at 254 nm 366 nm ansaldehyde reagent DPPH reagent

Lane 1: PEE, 2: EAE, 3: ME, 4: AqME.

HPTLC profiling of all the extract showed maximum bands in ethyl acetate extract followed by methanol extract which confirmed that most of the secondary metabolites were present in ethyl acetate extract followed by methanol extract.

Clear zones (white spots) against violet backgounnd indicate and confirm the radical scavenging activity. Ethyl acetate extract is showing highest DPPH radical scavenging activity and clear white spot on TLC plate. These antioxidant compounds can be isolated for the further work.

## 4. Conclusion:

Preliminary phychemical screening and HPTLC profiling of *E. nuda* were reported first time. Ethyl acetate extract showed presence of most of secondary metabolites. Further it contained antioxidant molecules which scavenged DPPH radicals on TLC showing white spot against blue background which can be isolated.

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