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Estimation of Primary Metabolites and Antioxidant Activity of Tylophora Indica l. and nyctanthes-arbor-tritis

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ABSTRACT

The present study was aimed for isolation and quantification of Primary Metabolites from selected plant species. Antioxidant activity of plants was also carried out by using FRAP, Catalase, Lipid Peroxidation and Peroxidase assay. The maximum amount of primary metabolite was protein and the minimum was total soluble sugars in leaves of *Tylophora indica* whereas in the case of *Nyctanthes arbor tritis*. Maximum amount of primary metabolite was protein in roots and the minimum starch was found in stem. Thus leaves of *Tylophora indica*. and the roots of *Nyctanthes arbor tritis* were the rich source of protein. Stem of *Tylophora indica* was found to be better antioxidant as compared to leaves when observed by various antioxidant assays.. In the case of *Nyctanthes-arbor-tritis* different plant parts showed potent activity when assayed by different methods. The present results showed potential of this medicinal plant which can be used as herbal drug as therapeutic ventures in future aspects.

Introduction

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. The plants provided food, clothing, shelter and medicine. Recent estimates suggest that over 9,000 plants have known for medicinal applications in all cultures of various countries and this is without having conducted comprehensive research amongst several indigenous and other communities (Farnsworth, 1966). The potential of these plants depends upon the presence of phytochemicals inside those may be primary metabolites or secondary metabolites that are secreted by plants during life.

The present study was aimed for isolation and quantification of Primary Metabolites (Carbohyrates, Proteins, Lipids, and Phenols) from selected plant species and to find out antioxidant activity of these plants by using FRAP, Catalase, Lipid Peroxidation and Peroxidase assays.

Primary metabolites are those organic substances which are synthesised during photosynthesis and these organic compounds are essential for plant life, growth and development (Sayeed et al., 1999). These are widely distributed in nature, occurring in one form or another in virtually all organisms. They are like chlorophyll, amino acids, nucleotides and carbohydrates have a key role in metabolic processes such as photosynthesis, respiration and nutrient assimilation. They are used as industrial raw material and food additives (Rao et al., 2012). Tylophora indica and Nyctanthes-arbor-tritis have been evaluated for their composition of primary metabolites.

Antioxidants may be defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Antioxidants can also protect the human body from free radicals and ROS (Reactive Oxygen Species) effects (Gulcin et al., 2010). The FRAP assay is based on the ability of plant extract to reduce Fe3+ to Fe2+ ions (Benzie et al., 1999). Peroxidases are group of enzymes containing phorphyrin in their structure and catalyzing redox reactions. They belong to one of the most significant enzymes in bioanalytical chemistry with broad possibility of application in the both detection and as supporting enzymes (Martinkova and Miroslav, 2015). Catalase is a ubiquitous enzyme found in aerobic organisms. It efficiently catalyzes the decomposition of hydrogen peroxide to oxygen and water together with other enzyme systems, protects cells against the harmful effects of reactive oxygen species such as superoxide anions, hydrogen peroxide, and hydroxyl radicals (Susmitha et al., 2013).

Among many herbs, Tylophora indica (Burm. F.) Merrill (Asclepiadaceae) is an important medicinal plant of India, reported to comprise variety of medicinal metabolites used in different ailments. The plant is found growing normally in Uttar Pradesh, Bengal, Assam, Orissa, Himalayas and sub Himalayas in India (Joshi, 2000). The plant inhabits up to an elevation of 1,260m in the sub Himalayan tract and in central and in peninsular India. It also met within Eastern, North-East and Central India, Bengal and parts of South India.

The anticancer, antioxidant, antiasthmatics, antiallergic, hepatoprotective and immunomodulatory activities of T.indica extracts has been reported by many researchers. (Mujeeb et al., 2009; Bhatia et al., 2013).

Nyctanthes arbortristis Linn. (Oleaceae) is popularly known as 'Night Jasmine' (English). Local people identify it as 'Harsinghar' (Hindi) due to the fact that it has pleasant fragrance during night by flowers which have attracted lot of researchers (Rout et al., 2007). Many bioactive compounds viz. terpenes, steroids, glycosides, flavonoids, alkaloids and aliphatic compounds have been isolated and characterized from different parts of N. arbortrisitis. The bark is rich source of glycoside and two alkaloids, one soluble in water and the

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other soluble in chloroform. Its roots are composed of alkaloids, tannins and glucosides (Suresh et al., 2011). The flowers are used in Asian continent to provoke menstruation and also used as sedatives. Flowers are also used to treat ulcers (Sasmal et al., 2007). Oral administration of decoction of flowers ward off wind in the stomach, stimulate gastric secretions and improve expectoration from the lungs (Suresh et al., 2011). The decoction is also used in treatment of gout. The inflorescence is used in scabies and skin diseases. Flower juice is used to combat hairloss and baldness (Tuntiwachwuttiku et al., 2003). Leaves are used as diuretic, laxative and diaphoretic. Leaf juice also prevents children who are infected with roundworms and threadworms. These are also used in treatment of appetite, piles, liver disorders, chronic and malarial fever and rheumatism. Leaves are also recommended in dry cough and skin infections and also used in gynecological disorders (Nawaz et al., 2009). Seeds are used in treatment of scurvy and anthelminthics. The bark is used for treatment of bronchitis and snakebite. Its roots are used as anthelminthics (Narendhirakannan and Smeera, 2010).

Materials and Methods

Collection of plant Material

Plant parts of *Tylophora indica* (leaves and stem) and *Nyctanthes arbortristis* L. (leaves , roots, flowers and stem) were collected from the fields at Jaipur and authenticated. The voucher (RUBL* No. 20304 for *Tylophora indica* L. and RUBL* No. 20864 for *Nyctanthes arbortristis* L. of experimental plant was deposited in the Herbarium of Department of Botany, University of Rajasthan, Jaipur. The plant parts were separated and washed thoroughly 2-3 times with running tap water and then air dried under shade after complete shade drying the plant material was powdered and used for phytochemical analysis.

Quantification of Primary Metabolites

Quantification of Primary Metabolites were carried out by using following methods of carbohydrates, proteins, lipids and phenols.

Carbohydrates

Total Soluble Sugars

Extraction

The dried experimental plant material (50 mg each) was homogenized in pestle and mortar with 20 mL of 80% ethanol separately and left overnight. Each sample was centrifuged at 1200 rpm for 15 minutes; the supernatants were collected separately and concentrated on a water bath using the method of (Loomis and Shull, 1973).

Starch

Extraction

The residual mass obtained after extraction of total soluble sugars of each of the test samples was suspended in 5 mL of 52% perchloric acid (McCready et al., 1950). Later, 6.5 mL of water was added to each sample and the mixture was shaken vigorously for 5 minutes.

Quantitative Estimation

1mL of aliquot of each sample was used for the estimation of carbohydrates using the phenol sulphuric acid (Dubois et al.,1951)

Proteins

Extraction

The test sample (50mg each) were separately homogenized in 10 mL of cold 10% trichloroacetic acid (TCA) for 30 min and kept at 4^0C for 24 hours. These mixtures were centrifuged separately and supernatants were discarded. Each of the residues was again suspended in 10 mL of 5% TCA and heated at 80^0 on a water bath for 30 minutes. The samples were cooled, centrifuged and supernatants of each were discarded. The residue was then washed with distilled water, dissolved in 10 ml of 1N NaOH, and left overnight at room temperature (Osborne, 1962).

Quantitative Estimation

Each of the above samples (1mL) was taken and the total protein content was estimated using the spectrophotometer (Lowry et al., 1951).

Lipids

Extraction and Quantification

The test sample were dried, powdered and 100mg was macerated with 10 mL distilled water, transferred to a conical flask containing 30 mL of chloroform and methanol (2/1:v/v) (Jayaraman,1981). The mixture was thoroughly mixed and left overnight at room temperature in dark for complete extraction. Later, 20 mL of chloroform mixed with 2 mL of water were added and centrifuged. Two layers were separated, the lower layer of chloroform, which contained all the lipids, was carefully collected in the pre-weighed glass vials and the colored aqueous layer of methanol which contained all the water soluble substances and thick interface layer were discarded in each test sample. The chloroform layers dried *in vacuo* and weighed. Each treatment was repeated thrice and their mean values were calculated.

Phenols

Extraction

The deproteinized test materials (200mg each) were macerated with 10 mL of 80% ethanol for 2 hours, and left overnight at room temperature. The mixtures were centrifuged and the supernatants were collected separately and maintained up to 40 mL by adding 80% ethanol .

Quantitative Estimation

Total phenol content in each sample was estimated by using established protocol (Bray and Thorpe, 1954).

FRAP Assay (Ferric reducing ability of Plasma)

The FRAP assay depends upon the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant at low pH (Varga et al., 1998).

Reagents:

- Acetate buffer, 300mM/L pH 3.6 (3.1 g sodium acetate x H_2O and 16 mL conc. Acetic acid per 1 mL of buffer solution).
- 10mM 2, 4, 6-tripyridyl –s- triazine (TPTZ) in 40 mM 1 HCl.
- 20mM $FeCl_3$ x 6 H_2O in distilled water

Catalase (CAT) assay

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

Reagents and Test sample solutions

- Phosphate buffer (0.1M, pH- 6.8)
- Phosphate buffer (50M, pH- 7)
- H_2O_2 (24 mM)
- Na_2EDTA (0.1mM)
- PVP (1%)

Procedure

Plant sample (200mg) was homogenized with 5mL of phosphate buffer with Na_2EDTA and refrigerated centrifuged at 10000rpm for 20 minutes at 4°C. The clear supernatant was taken as the enzyme extract. The activity was assayed after the method of (Aebi, 1984) with the following modifications. 2.0mL of phosphate buffer, 0.8mL of H_2O_2 was added and

finally 0.2 mL enzyme extracts then immediately absorbance taken at 240nm.

Peroxidase Assay (POXA)

The method of assay measures the oxidation of pyrogallol to purpurogallin by peroxidase when catalyzed by peroxidase at 420 nm and at 20° C.

Pyrogallol+ H_2O_2 $\xrightarrow{\text{Peroxidas}}$ Purpurogallin

Reagents and Test sample solutions:

- Phosphate buffer (0.1M, pH- 6.8)
- Phosphate buffer (125µmol, pH- 6.8)
- Pyrogallol (50µmol)
- H_2O_2 (30%)

Procedure

Plant sample (200mg) was homogenized with 10mL of phosphate buffer and refrigerated centrifuged at 10000 rpm for 20 minutes. The clear supernatant was taken as the enzyme extract.

The activity was assayed after the method of (Chance and Maehly, 1955) with the following modifications. 2.4mL of phosphate buffer, 0.3mL of pyrogallol and 0.2mL of H_2O_2 was added. The amount of purpurogallin formed was determined by taking the absorbency at 420nm immediately after adding 0.1mL enzyme extract.

Results and Discussion

Quantification of Primary metabolites

Table 1.Primary metabolites (in mg/gdw) *Tylophora indica* L.

Phytochemical Name		*Tylophora indica* L.	
		Leaf	Stem
Proteins		292±2.79	280±2.31
Lipids		6±0.04	2±0.01
Phenols		3.0±0.02	1.5±0.03
Carbohydrates	TSS	2.48±0.02	1.38±0.05
	Starch	2.84±0.04	2.50±0.06

Values are the mean ± SEM (n = 3 rats in each group).
*P <0.05; **P < 0.001 compared with the control ; P < 0. 001

Table 2.Primary metabolites (in mg/gdw) from *Nyctanthes arbortristis* L.

Phytochemical Name	Leaf		Stem	Flower	Root
Proteins	90±3.29		84±2.87	30±1.2	150±4.1
Lipids	26±0.09		4±0.02	2±0.01	22±0.18
Phenols	2.5±0.02		1.5±0.008	5.5±0.03	3.2±0.02
Carbohydrates	TSS	3.5±0.009	3.3±0.007	4.0±0.009	6.8±0.01
	Starch	1.5±0.04	1.4±0.03	5.5±0.05	6.5±0.06

Values are the mean ± SEM (n = 3 rats in each group).
*P< 0.05; **P < 0.001 compared with the control ; P< 0. 001

In the present study, quantification of primary metabolites in leaves and stem of *Tylophora indica* L. and various plant parts of *Nyctanthes arbortristis* L. have been under taken, the results are present in Table 1 and Table 2 respectively.

Determination of antioxidant activity of *Tylophora indica* L.

The antioxidant activity of selected plants are present in Table 3 and Table4 respectively.

Table 3: Antioxidant activity of *Tylophora indica* L.

Antioxidant assay (in (mM/g/fresh wt)	.	
	Leaf	Stem
FRAP	0.682±0.009	0.129±0.002
Catalase	2.92±0.08	2.44±0.03
Peroxidase	0.907±0.001	0.778±0.001
Lipid Peroxidation Assay	7.795±0.11	1.705±0.01

Values are the mean ± SEM (n = 3 rats in each group).
*P<0.05; **P < 0.001 compared with the control ; P < 0. 001

Table 4. Antioxidant activity of *Nyctanthes arbortristis* L.

Antioxidant assay (in (mM/g/fresh wt)	Leaf	Stem	Flowers	Roots
FRAP	0.954±0.009	0.550±0.005	2.140±0.04	0.829±0.008
Catalase	2.76±0.08	1.40±0.02	2.56±0.04	0.80±0.009
Peroxidase	1.072±0.01	0.842±0.008	0.110±0.001	4.330±0.04
Lipid Peroxidation Assay	7.28±0.11	6.70±0.08	2.41±0.04	3.59±0.0.05

Values are the mean ± SEM (n = 3 rats in each group).
*P <0.05; **P <0.001 compared with the control ; P < 0. 001

The maximum amount of primary metabolite is protein found in leaves of *Tylophora indica* L. and in roots of *Nyctanthes arbortristis* L., while the minimum is TSS in stem of *Tylophora indica* L. and starch in stem of *Nyctanthes arbortristis* L.

Antioxidant activity was carried out using different methods. Stem of *Tylophora indica* was found to be better antioxidant when observed by all antioxidant assay while variation in potent antioxidant activity was observed in *Nyctanthes arbortristis* L when assayed by different methods. The present results showed potential of this medicinal plant which can be used as herbal drug as therapeutic ventures in future aspects.

Conclusion

In the present study, both selected plants L. contain many primary metabolites like protein, phenol, carbohydrates etc. and they are rich source of antioxidants which may play a role in preventing human diseases in which free radicals are involved, such as cancer, ageing and cardiovascular diseases.

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