WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 12, 1243-1254. Research Article ISSN 2277– 7105

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF FLAVONOIDS FROM *NYCTANTHES ARBORTRISTIS* AND *TYLOPHORA INDICA*

Deepika Harit* and R. A. Sharma

Department of Botany, University of Rajasthan, Jaipur- 302004.

Article Received on
20 August 2017,
Revised on 11 Sep. 2017,
Accepted on 02 Oct. 2017
DOI: 10.20959/wjpr201712-9759

***Corresponding Author**
Deepika Harit
Department of Botany,
University of Rajasthan,
Jaipur- 302004.

ABSTRACT

There are a numbers of bioactive compounds in plants, such as alkaloids, tannins, flavonoids, sterols, triterpenes, etc., noted to have the major role in nutrition, physiology and control of diseases. Flavonoids constitute one of the most characteristic classes of compounds in higher plants. The foremost important task in this paradigm is the screening of these compounds in the plants. The chromatographic study of the compounds serves to be a very useful and reliable source in the process of bioactive compounds screening in plants. According to the ethnobotanical information, it has been reported that both the plants *Nyctanthes arbortristis* and *Tylophora indica* possesses some medicinal importance. Hence in the present study, an attempt has been made to identify the flavonoid constituent in both the plants using TLC and PTLC. Further, the isolation of the same compound using the standardized solvent system viz., Benzene: Acetic Acid: Water (125:72:3). The confirmation of the isolation was done by IR Spectroscopy and GC- MS analysis.

KEYWORDS: Flavonoids, *Nyctanthes Arbortristis*, *Tylophora Indica*, TLC, IR, GC-MS.

INTRODUCTION

Chemical compounds that occur naturally in plants are responsible for the color and organoleptic properties in the plants, such as deep purple color of blueberries and smell of garlic. These chemicals are called as the secondary metabolites or phytochemicals. The term, phytochemicals, is generally used to refer to those chemicals that may have biological significance but have not been established as essential nutrients. Phytochemicals have been used as drugs since long in the past. Many of the medicinal plants have been characterized for

secondary metabolite screening and their possible use in the chemotherapy. Recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance from the plants for specific diseases (Khan et al., 2009, 2010 a, 2010 b).

Flavonoids are a large family of compounds synthesized by plants that have a common chemical structure. Flavonoids constitute one of the most characteristic classes of compounds in higher plants, where they can be easily recognized as flower pigments in angiosperms. However, their occurrence is not restricted to flowers only, but include all the parts of a plant. Flavonoids are also known to play an important role in giving resistance to the plant species, such as rotenone, which is aisoflavonoid, serves to be an effective insecticide. The possible effect of isoflavonoids on human health is also extensively investigated especially in the prevention of cancer and in particular hormone dependent cancers such as breast cancer (Cornwell et al., 2004; Dixon, 2004; Greenwald, 2004; Holzbeierlein et al., 2005). In addition, consumption of soy foods rich in isoflavones has been weakly associated with reduced colon cancer.

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). The plant remains inactive during daytime while its flowers are activated during night. The generic name '*Nyctanthes*' has been coined from two Greek words 'Nykhta' (Night) and 'anthos' (flower) (Meshram et al., 2012). 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 (Rathod et al., 2010).

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 (Bhatia et al., 2013).

MATERIALS AND METHODS

Extraction: Different plant parts of *Nyctanthes-arbor-tritis* (flowers, leaves, stem and roots) and *Tylophora indica* (leaves and stem) were air dried and powdered, separately. Each of these extracted separately with 80% methanol on water bath (Subramanian and Nagarajan, 1969) for 24 h. The methanol soluble fractions were filtered, concentrated *in vacuo* and aqueous fractions were fractioned by sequential extraction with petroleum ether (Fr1), diethyl ether (FrII) and ethyl acetate (FrIII) separately. Each step was repeated thrice for complete extraction, fraction 1 was discarded in each case because it contained fatty substance, where as fraction II and fraction III were concentrated and used for determining flavonoids.

Fraction III was further hydrolyzed by refluxing with 7% sulphuric acid ($10mLg^{-1}$ plant material for 2 h), filtered and filtrate was extracted thrice with ethyl acetate. All ethyl acetate layers were pooled separately, neutralized by distilled water with repeated washings and concentrated *in vacuo.* Both fraction II and III were taken up in small volume of ethanol (2-5mL) before chromatographic examination.

Qualitative

***Thin Layer Chromatography (TLC*)**: Thin glass plates (20x20 cm) were coated with Silica gel G (250mμ thick). The freshly prepared plates were air dried at room temperature; thereafter these were kept at 100 ^{0} C for 30 minutes to activate and then cooled at room temperature. The freshly prepared and activated plates were used for analysis.

Each of the extract was co- chromatographed with authentic flavonoid as a marker (quercetin, luteolin, kaempferol). These plates were developed in an air tight chromatographic chamber saturated with solvent mixture (Benzene: Acetic Acid: Water:: 125:72:3) (Wong and Francis, 1968). The developed plates were air dried and visualized under UV light by exposure to ammonia fumes. The mouth of a 100 mL containing concentrated NH_4OH was held in contact with each spot for about 5-10 seconds and fluorescent spots corresponding to that of

standard markers were marked. The developed plates were also sprayed with 5% $FeCl_3$, 0.1% alcoholic $AlCl_3$ and kept in I_2 chamber separately. The coloured spots thus developed were noted and the Rf value of each spot was calculated. Several others solvent systems such as n-butanol, acetic acid, water (4:1:5), tertiary butanol, acetic acid, water (3:1:1) were also tested, but the solvent system containing benzene, acetic acid, water (125:72:3) gave better results.

Preparative thin layer chromatography (PTLC)

PTLC of aforementioned flavonoid extracts was carried out using silica gel G coated plates (BDH ; 500mμ in thickness) by spotting the extract as well as standard markers (luteolin, kaempferol, quercetin). These plates were developed in the solvent mixture of benzene, acetic acid and water (125:72:3), air dried and examined under UV light. Each of spots corresponding with the standard markers were marked, scraped from 200 plates and eluted with 50% methanol. The eluted fractions were filtered, air dried and again co-chromatographed along with standard markers to test their purity. The eluted fractions were subjected to crystallization separately and melting point (mp), mixed melting point (mmp) was determined. The isolates were also subjected to ultraviolet and infrared spectral studies.

Identification: The identity of the isolated flavonoids were confirmed by mp, mmp performed in capillaries (Toshniwal Melting Point Apparatus), IR (Infra-red spectrophotometer; Perkin, Elmer 337, Grating Infra-red spectrophotometer), UV (Ultraviolet and visible spectrophotometer; Carl Zeiss, Jena, DDR, VSU-2P spectrophotometer) analysis along with their respective authentic samples.

Quantification: The isolated flavonoids were estimated by spectrophotometer using established protocol (Mabry et al., 1970).

Stock solution ($1mgL^{-1}$) of kaempferol, luteolin, quercetin were prepared separately by dissolving authentic compounds in methanol. Different concentrations ranging from 20μg to 160μg of each of the compounds spotted separately on silica gel G plates. For each concentration of reference authentic standards separate plates were used and developed in the same manner as described earlier. These developed plates were air dried and visualized under UV light. The fluorescent spots were marked and collected along with the absorbance in separate test tubes. Spectroscopy methanol grade (5mL) was added to each test tube, shaken vigorously, centrifuged and supernatants were collected separately. The volume of each of the eluate was made up to 10mL by adding methanol. To each of these samples, 3mL of 0.1

M $AlCl_3$ solution was added again shaken vigorously and kept at room temperature for 20 min. Five such replicates were run in each case and their optical densities were measured using spectrophotometer at 426nm for kaempferol and luteolin and at 440nm for quercetin against blank (10ml of spectroscopic grade methanol and 3mL of 0.1 M $AlCl_3$). The standard curves were plotted between concentration and their respective average optical density of each of the compound. The regression curve so achieved followed Beer's law.

Each of the plant extract sample (ether and ethyl acetate sample) was dissolved in 5 mL of spectroscopic grade methanol and 0.1mL was applied on silica gel G coated plates along with standard markers, separately. The plates were developed as above and the spots coinciding with that of standard markers were marked on each plate under UV. Each spot was collected along with the silica gel, eluted in methanol and test samples were prepared in the same way as described above. The optical density in each case was recorded and concentration of each sample was computed using the regression curve of authentic flavonoids samples. The concentrations were calculated on mg/g dry weight basis.

GC-MS analysis (Gas Chromatography and Mass Spectroscopy (GC-MS))

The extract and the standard samples were analyzed by GC-MS of Hewlett-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with using Agilent 7890A/5975C GC HP-5. Capillary column (phenyl methyl siloxane, 25 m×0.25 mm i.d) with Helium (He) was used as the carrier gas with split ratio 1:5. Oven temperature was 100°C (3 min) to 280°C at 1 to 40°C/min; detector temperature, 250 to 280°C; carrier gas, He (0.9 mL/min). Retention indices were determined by using retention times of samples that were injected under the same chromatographic conditions. The components of the standard and plant samples were identified by comparison of their mass spectra and retention time with those given in literature and by comparison with the mass spectra of the Wiley library or with the published mass spectra.

RESULTS

Flavonoids: Three spots of flavonoids were observed in different plant parts of *Nyctanthes arbortristis* and *Tylophora indica* on thin layer chromatography plates developed and sprayed with 5% $FeCl_3$. The R_f values of these spots matched with their respective authentic standards and were identified as kaempferol, quercetin, luteolin. Solvent system Benzene: Acetic Acid: Water (125:72:3) gave best results with R_f values viz., kaempferol, 0.86; luteolin, 0.56; quercetin, 0.78 (**Table-1**). When other solvents viz n-Butanol: Acetic acid: Water (4:1:5) and

conc. HCl: Acetic acid : Water (3:30:10) the R_f value of kaempferol was found to be 0.83 and 0.55, that of quercetin was found to be 0.64 and 0.41 while that of luteolin was found to be 0.83 and 0.77, respectively.

The isolated flavonoids viz., kaempferol, quercetin, luteolin were also identified and characterized by super imposable IR peaks (**Fig.1,2,3.),** mp (kaempferol, 271-273^{o}C; luteolin 327-328^{o}C; quercetin 309-311^{o}C and UV maxima (nm) in methanol (kaempferol, 253sh, 266, 394sh, 322sh, 368; quercetin 255, 269sh, 301sh 374;luteolin 242sh, 253, 267, 291sh, 349.

Quantitative analysis

Nyctanthes arbortristis: Among the plant parts total flavonoid content (free & bound) was maximum in flowers (8.75 mg/gdw) and minimum (2.4mg/gdw) in roots. Flavonoid content in its free form was more as compared to the bound form in plant parts. Individually, all the isolated flavonoids were more in flowers with the highest level of quercetin (2.41mg/gdw) followed by kaempferol (1.62 mg/gdw) in root it was minimum **(Table 2).**

Tylophora indica: Here maximum content was observed in leaves (9.2mg/gdw) with maximum level of quercetin (2.89mg/gdw). Among these two plants it was found to be better than ***N. arbortristis*** **(Table 3).**

GC-MS analysis: When flavonoid frcation of leaves of *Nyctanthes arbortristis* were analysed by GC MS about 41 compounds were observed. Maximum retention time (34.44) was observed in Butanoic acid, 2methyl, 2methoxy 4(2propenyl) phenyl ester and **(Fig and Table 4**).

Table. 1: Chromatographic behavior and physicochemical characteristics of isolated flavonoids.

Isolated compounds	R_f value			Physical appearance			Color after spray				Melting point (°C)	IR Spectral Peaks ν (KBr) cm^{-1}
	S_1	S_2	S_3	Day light	UV ammonia	Iodine Vapor	R_1		R_2			
							Visible	UV	Visible	UV		
Kaempferol	0.86	0.83	0.55	GN-YW	BT-YW	YW-BN	BN	BK	YW	YW-GN	276-278	(O-H) (3410cm-1(270, 295, 344 , 1690
Luteolin	0.56	0.83	0.77	GN-YW	YW	YW-BN	TN	BK	DL-YW	YW-GN	326-329	3421, 2965, 1736 (lactone), 1510 (furan), 1461, 1388, 1360, 1274, 1242, 1187, 1136, 1028, 903, 850 cm^{-1}.
Quercetin	0.78	0.64	0.41	GN-YW	YW	YW-BN	BT-GY	BK	DL-YW	YW-GN	315-320	3423, 1739, 1655 (O-H), 1608, 1508, 1305, 1203 (C=C), 1088.

Abbreviations:

S_1 – Benzene : acetic acid : water (125 : 72 : 3), S_2 – n-Butanol : acetic acid : water (4 : 1 : 5), S_3 – Conc. Hydrochloric acid : acetic acid : water (3 : 30 : 10), R_1 – 5% $FeCl_3$ solution, R_2 – 5% alc. $AlCl_3$ solution, YW – Yellow, BK – Black, BN – Brown, BT – Bright, DL – Dull, GN – Green, GY – Gray.

Table. 2: Flavonoids content (mg/gdw) in different plant parts of *Nyctanthes arbortristis.*

S. No.	Plats Parts	Free flavonoids (mg/gdw)				Bound flavonoids (mg/gdw)				Total flavonoids (free+bound) (mg/gdw)
		K	Q	L	T	K	Q	L	T	
1.	Flowers	1.62	2.41	0.22	4.45	1.59	1.63	1.08	4.30	8.75
2.	Stem	0.58	0.98	0.44	2.00	0.51	0.88	0.46	1.85	3.85
3.	Leaves	0.88	1.26	0.86	3.30	0.76	1.97	0.47	3.2	6.5
4	Root	0.18	0.87	0.20	1.25	0.22	0.78	0.15	1.15	2.4

Table. 3: Flavonoids content (mg/gdw) in different plant parts of *Tylophora indica.*

S. No.	Plats Parts	Free flavonoids (mg/gdw)				Bound flavonoids (mg/gdw)				Total flavonoids (free+bound) (mg/gdw)
		K	Q	L	T	K	Q	L	T	
2.	Stem	1.10	2.34	1.01	4.45	0.85	2.78	0.52	4.15	**8.6**
3.	Leaves	1.45	2.66	0.55	4.65	1.59	2.89	0.07	4.55	**9.2**

Table. 4. GC MS analysis of flavonoids from leaves of *Nyctanthes arbortristis.*

RT	Compound Name	Area	Area %
4.35	2Methyl1(2methyl[1,3]dioxolan2yl) but3yn2ol	1920264	0.23
4.50	2,6Piperazinedione, 4benzoyl, 2oxime	823538	0.10
6.14	3,5Dimethyl2octanone	1103290	0.13
7.09	Benzoic acid	59188892	6.95
7.37	SBenzyl benzenesulfonothioate	20556928	2.41
9.09	Hydroquinone	425432708	49.94
9.24	3Selenetanol, 3(4methoxyphenyl)	2710856	0.32
10.12	Fosfosal	394385	0.05
11.04	4Methylcoumarine7cinnamate	796274	0.09
11.60	2Iodocinnamic acid	13768394	1.62
12.59	Ethyl 2hydroxybenzyl sulfone	9263850	1.09

12.91	(3Nitrophenyl) methanol, nbutyl ether	15604101	1.83
13.04	2Propenoic acid, 3phenyl	66466801	7.80
13.15	2Iodocinnamic acid	721524	0.08
14.12	áDGlucopyranose, 1,6anhydro	5621479	0.66
15.28	SBenzyl benzenesulfonothioate	844769	0.10
15.54	Hexadecane	95239560	11.18
15.63	1,3Oxazolidine, 4methylcis5phenyl2[4(dimethylamino)phenyl	899952	0.11
16.53	Ditertbutyl carbonotrithioate	1377730	0.16
16.63	4Methylcoumarine7cinnamate	3826124	0.45
17.53	Z4Dodecenol	3495931	0.41
19.14	Tricyclo[4.1.0.0(2,4)]heptane, 5(phenylthio),	4084553	0.48
19.56	Cyclohexane, 1bromo2methoxy, trans	703262	0.08
20.04	Montanol	4536980	0.53
22.59	Tetradecanoic acid, 10,13dimethyl, methyl ester	1723407	0.20
22.97	DglyceroDmannoHeptitol	962726	0.11
23.18	DglyceroDmannoHeptitol	2633848	0.31
23.42	Dibutyl phthalate	6457306	0.76
24.19	1,2,4,5Tetroxane, 3,3,6,6tetraphenyl	741184	0.09
24.70	Cyclohexane, 1(1,5dimethylhexyl) 4(4methylpentyl**)**	4020680	0.47
24.90	5Bromo1hexene	1606850	0.19
26.02	Phytol	8275270	0.97
26.98	Acetic acid, mercapto, cyclohexyl ester	1327727	0.16
28.99	3,7,11Trimethyl3hydroxy6,10dodecadien1yl acetate	24994585	2.93
29.16	3,7,11Trimethyl3hydroxy6,10dodecadien1yl acetate	7518448	0.88
29.31	Docosa8,14diyncis1,22diol, bis(trimethylsilyl) ether	1489346	0.17
31.22	2Methyl3(3methylbut2enyl) 2(4methylpent3enyl) oxetane	18510673	2.17
32.25	3Cyclopropylphenyl trifluoromethanesulfonate	1189488	0.14
32.35	Benzoic acid, 2benzoyl1(phenylmethyl)hydrazide	1391295	
34.26	Trimethyl[4(1,1,3,3,tetramethylbutyl)phenoxy]silane	1469886	0.16
34.44	Butanoic acid, 2methyl, 2methoxy4(2propenyl) phenyl ester	9965974	1.17

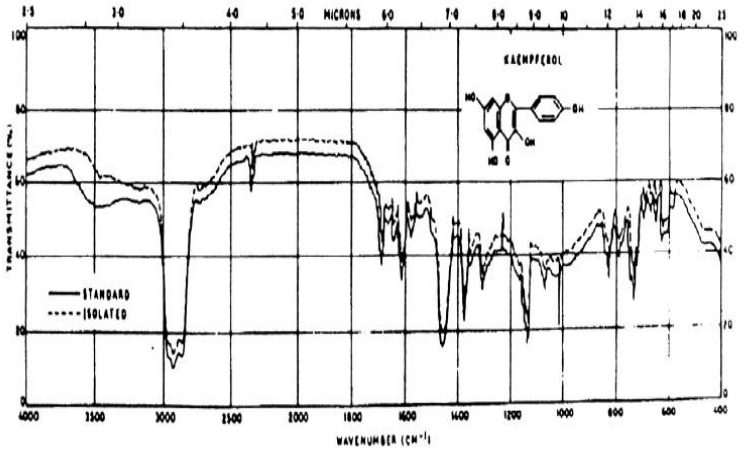

Fig. 1 IR Spectra of Kaempferol.

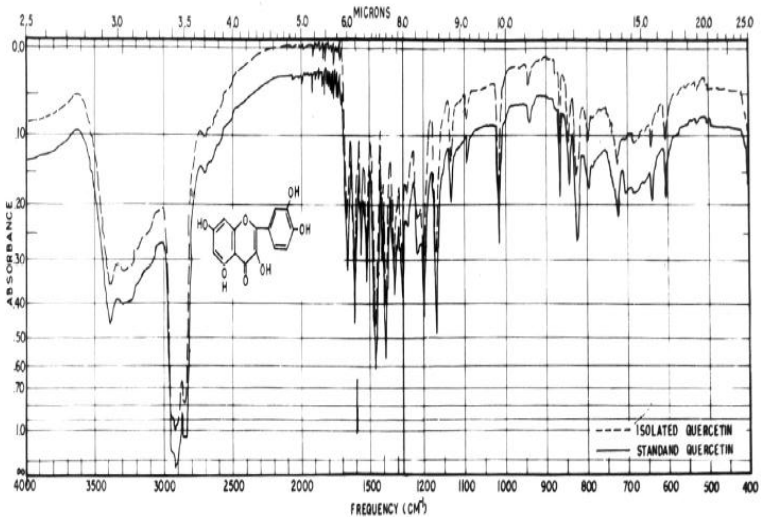

Fig. 2: IR Spectra of Quercetin.

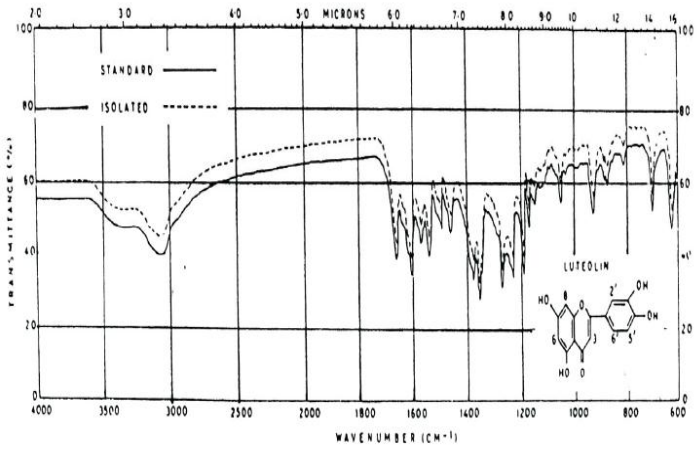

Fig. 3: IR spectra of Luteolin.

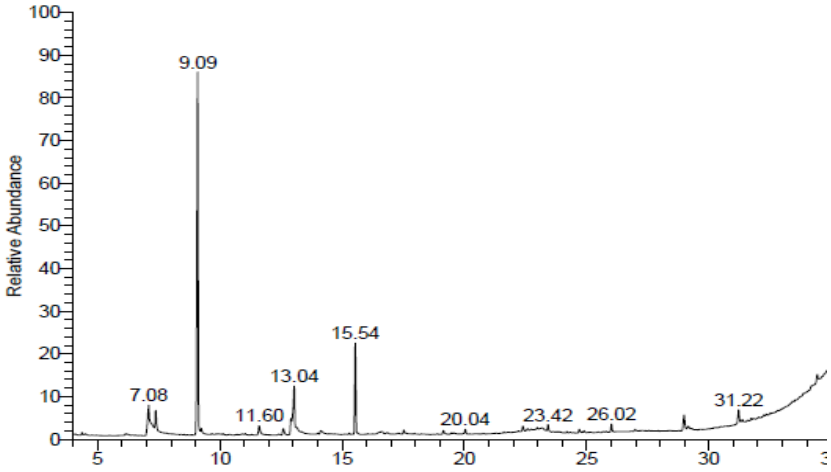

Fig. 4: GC-MS of flavonoid fraction of leaves *Nyctanthes arbortristis.*

DISCUSSION

Plant cell cultures mays serve as an alternative industrial source of phytochemicals. The study of compartmentation mechanism together with metabolic studies, improvement of culture media, and selection of cell lines is particularly relevant here in order to increase the production of phytochemicals. It has been emphasized that secondary metabolites have not been systemically assayed in the culture medium (Petiard and Courtois, 1983). Careful checks on the kinetics of cell death compared to the kinetics of metabolite excretion has shown that in most cases the excretion of phytochemicals is a real physiological process contributing to the overall dynamics of these metabolites. Some are restricted to specific organs e.g. the roots or seeds, others to specific tissues such as epidermis. While we have rather extensive knowledge of the biosynthetic pathways of secondary compounds, information on precise sites of accumulation is still scanty.

Flavonoids are a series of related water soluble phenolic glycosides derived from aromatic amino acids, occur almost universally in higher plants. They impart colour to flowers and fruits, which play a role in attraction of pollinating insects. Flavonoids have also been reported to have pathological significance in plants by providing resistance to the plants against pests and insects (Cruickshank and Perrin, 1964) besides physiological importance for animals.

The flavonoids usually present in their free form and at reactive sites in their bound form as glycosides. Therefore, the difference in content between free and bound forms shows their involvement at resting and active stages, thus giving higher or lower recovery of free and/or bound flavonoids (Harborne et al.,1975).

Phytometabolites that appear first in different morphogenetic callus are phenolics and flavonoids. Cells producing them generally do not need specialized structures i.e. common parenchyma cells and are less complex (Constable, 1988). Media components and composition are also important factors which results in product formation and accumulation. Amongst all the media components hormones have received greatest attention. There is sufficient evidence to indicate that type and concentration of auxins and cytokinins, as well as their relative ratios in the culture medium, control the biosynthesis of secondary metabolites.

CONCLUSION

The cell differentiation follows specific biochemical and morphological principles. The synthesis and accumulation of secondary metabolites can be endogenously controlled by various exogenous factors. In some plants, initiation of morphological differentiation represents triggering signal and different metabolites require degrees of tissue differentiation. Therefore, it is necessary to maintain differentiated and organized tissue for purpose of extraction of required metabolites.

REFERENCES

1. Bhatia A, Anand M, Singla R, Sharma A. In-vitro evaluation of antioxidant activity and total phenolic content of Tylophora indica (Burm f.) Merill. J Nat Prod Plant Resour, 2013; 3(1): 1-7.
2. Constable F. Cell culture in phytochemistry. In: Cell Structure and Somatic Cell Genetics of Plants, Cell Culture in Phytochemistry. Vol. 4.Constable F and Vasil IK (Eds.). Academic Press Inc. San Diego, USA., 1988; 3-13.
3. Cornwell, T, W Cohick and I Raskin. "Dietary phytoestrogens and health." Phytochemistry, no. 2004; 65: 995-1016.
4. Cruickshank IAM and Perrin DR In: Biochemistry of Phenolic Compounds, Harborne JB (Ed.). Academic Press. New York, USA. 1964; 511.
5. Dixon, R A. "Phytoestrogens." Annu. Rev. Plant Biol., 2004; 55: 225-261.
6. Greenwald, P. "Clinical trials in cancer prevention: Current results and perspectives for the future." J. Nutr. 2004; 134(12): 3507-3512.
7. Harborne JB Mabry TJ and Mabry H. (Eds.). 1975. The Flavonoids. Chapman & Hall. London, UK.
8. Holzbeierlein, J M, McIntosh and J B Thrasher. "The role of soy phytoestrogens in prostate cancer."Curr. Opin. Urol., 2015; 15: 17-22.
9. Joshi, S. G. Medicinal plants. New Delhi: Oxford and IBH Publishing. 2000.

10. Khan, M R, W Rizvi, G N Khan, R A Khan, and S Sheen. "Carbon tetrachloride induced nephrotoxicity in rats: Protective role of Digeramuricata." J. Ethnopharmacol, 2009; 122: 91-99.
11. Khan, R A, M R Khan, and S Sahreen. "Evaluation of Launaeaprocumbens use in renal disorders: A rat model." J. Ethnopharmacol, 2010a; 128: 452-461.
12. Khan, R A, M R Khan, S Sahreen, and J Bukhari. "Prevention of CCl4 induced nephrotoxicity with Sonchusasper in rat." Food Chem. Toxicol, 2010b; 23: 1304-1321.
13. Mabry TJ, Markham KR and Thomas MB. 1970. The Systematic Identification of Flavonoids. Springer Verlag, Berlin, New York, USA.
14. Meshram MM, Rangari SB, Kshirsagar SB, Gajbiye S, Trivedi MR, Sahane RS. Nyctanthes arbor tristis- A herbal panacea. International Journal of Pharmaceutical Sciences and Research, 2012; 3(8): 2432-2440.
15. Nawaz AHMM, Hossain M, Karim M, Khan M, Jahan R, Rahmatullah M.. An ethnobotanicals survey of Jessore district in Khulna Division, Bangladesh. American-Eurasian Journal of Sustainable Agriculture, 2009; 3: 238-243.
16. Petiard V and Courtois D (1983) Recent advances in research for novel alkaloids in Apocynaceae tissue cultures. Physiol. Veg., 21: 217-227.
17. Rathod N, Raghuveer I, Chitme HR, Chandra R. Free Radical scavenging activity of Nyctanthes arbortristis in streptozotocin-induced diabetic rats. Indian Journal of Pharmaceutical Educational Research, 2010; 4: 288-294.
18. Rout GR, Mahato A, Senapati SK. In vitro clonal propagation of Nyctanthes arbortristis Linn.-a medicinal tree. Horticulture Science (Prague), 2007; 34: 84-89.
19. Sahreen, S, M R Khan, and R A Khan. "Evaluaion of antioxidant activities of various solvent extracts of Carissa opaca fruits." Food Chem, 2010; 122: 1205-1211.
20. Sasmal D, Das S, Basu SP. Phytoconstituents and therapeutic potential of Nyctanthes arbortristis Linn. Pharmacognosy Rev., 2007; 1(2): 344-349.
21. Subramanian SS and Nagarajan S. 1969. Flavonoids of seeds of Crotalaria retusaand C. striata.Curr. Sci., 1969; 38: 365.
22. Suresh V, Jaikumar S, Arunachalam G. Antidiabetic activity of ethanolic extract of stem bark of Nyctanthes arbortristis Linn. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2010; 1: 311-317.
23. Tuntiwachwuttiku P, Rayanil K, Taylor WC. Chemical constituents from the flowers of Nyctanthes arbortristis. Science Asia, 2003; 29: 21- 30.
24. Wong E and Francis CM. Flavonoids in genotypes of Trifolium subterraneum: I. The normal flavonoids pattern of the geraldton variety. Phytochem. 1968; 7: 2123-2129.