International Journal of Chemistry and Pharmaceutical Sciences

Journal Home Page: www.pharmaresearchlibrary.com/ijcps

Research Article **Open Access**

Quantitative phytochemical credential of Nakshtra plants and distribution analysis of aldehydes pertaining to LC-MS

Ajam C Shaikh, Amit Gupta and Sushama R Chaphalkar*

Vidya of Pratishthan's School Biotechnology (VSBT, Research Centre affiliated to Savitribai Phule Pune University), Baramati, Maharashtra, India

A B S T R A C T

According to the WHO, above 80% of the world's population totally relies on traditional forms of medicine, largely plant based to meet the primary health care needs of the people. Medicinal plants contain various chemical constituents which can be used as therapeutic agents. Secondary metabolites are organic compounds which are not involved in the survival of plants but they produce some products which aid them in their normal growth and development. The systematic study regarding presence of phytochemicals may aid in the analysis which helps pharmacological and property predication studies possible. The action mechanism of plants producing therapeutic effects can also be better investigated if the given information scrutinized. In the present study, ethanolic extracts were subjected to quantitative estimation of total flavonoids, alkaloids, terpenoids and phenolics. The analysis inferred the richness of these plants in terms of active phytoconstituents, flavonoids and terpenoids in particular. Some of the plants are shown to have major quantities of all the screened phytoconstituents. The extracts were also analyzed by LC-MS. The phytochemical identification was done on the basis of m/z ratio obtained for each individual secondary metabolite. The scrupulous numeral analysis of aldehydes and ketones present as secondary metabolite is done along with frequency of occurrence of particular aldehyde / ketone in other Nakshtra plants. The classified aldehydes and ketones are having medicinal importance of which representatives have been quoted. The given study would allow the systematic analysis of the phytochemicals regarding aldehydes and ketones or any other secondary metabolite for that matter and sheds light on identification and property predication which may help in formulating a drug and study its pharmacology.

Keywords: Nakshtra Plants, Phytochemicals, LC-MS; Aldehydes, Terpenoids and Alkaloids

A R T I C L E I N F O

CONTENTS

Article History: Received 15 March 2016, **Accepted** 10 June 2016, **Available Online** 27 August 2016

***Corresponding Author**
Dr. Sushama R Chaphalkar
Vidya of Pratishthan's School Biotechnology
(VSBT, Research Centre affiliated to
Savitribai Phule Pune University),
Baramati, Maharashtra, India
Manuscript ID: IJCPS3066

PAPER-QR CODE

Citation: Sushama R Chaphalkar. Quantitative investigation of phytochemicals in Nakshtraplants and distribution analysis of ketones pertaining to LC-MS. *Int. J. Chem, Pharm, Sci.*, 2016, 4(8): 408-415.

1. Introduction

Medicinal plants form the major natural resource base of the Indian indigenous healthcare tradition as India has long tradition of Ayurvedic practices and conferred with rich plant diversity. Therapeutic properties of these medicinal plants are widely acknowledged at global level too [1a] and it is estimate, over 50% of modern clinical drugs have natural products' origin [1b].Mostly these herbal medicines are consumed either raw or as standardized plant extracts. Easy availability, efficacy and lack of side-effects make herbal medicines attractive candidate of remedy. Having said that, systematic investigation, identification and characterization has led to discovery of new, cheap drugs withhigh therapeutic potential [1c,1d] because of the unmatched availability of chemical diversity. Even though a big quantum of research work in the area of drug discovery has been undertaken in order to authenticate these herbal medicines, yet major part of it unexplored.

Potency of these plants majorly due to secondary metabolites associated with it andplant synthesize a vast range of organic compounds that are traditionally classified as primary and secondary metabolites having a thin boundary between the twogroups. Primary metabolites are compoundsthat have important roles to play pertaining to photosynthesis, respiration, growth and development. Other phytochemicals, majority of which accumulate in astonishingly high concentrations, are referred to as secondary metabolites. These are organic compounds which are not directly involved in the survival of plants but they helps them in plants normal growth and development *e.g.* Terpenes, Alkaloids, Phenols etc. By definition secondary metabolites are not essential component for survival but rather are required for the interaction of plants with their environment. These are structurally diverse andso can be diagnostic in chemotaxonomic studies. Though ignored for a while, theirrole in plants is now attracting attention as some appear to have playing key functions.

In order to investigate and identify such secondary metabolites in the plant, oneneeds to have characterization of samples extracted from different parts of the plant. The information obtained after analysis makes pharmacological studies possible. The mode of action of plants producing therapeutic effects can also be better investigated if the active ingredients are characterized. Once the information is gathered, systematic representation and analysis may lead to fruitful conclusions which would put some light on the role and presence of that particular secondary metabolite. If possible the information could be treated with statistical tools in order to understand the data better as well as representation of obtained information in more effective manner.

Having pivotal place in Ayurveda, Indian tradition and modern medicine we have chosen Nakshtra plants [**Table 1**], as our dataset where emphasishas been given on identification and classification of compounds having aldehydic groups present in these plants as secondary metabolites. These carbonyl compounds not only of medicinal significance when administered raw in the body but also used as building blocks in the synthesizing of commercially important compounds, including pharmaceuticals and polymers. Even though carbonyl compounds are well known to the mankind as secondary metabolites, they are poorly studied and complied. Herewith we attributed our study to tabulatedata regarding carbonyl compounds according to presence and occurrence in different plants.

2. Experimental

Collection of Plant materials-

The leaves of Nakshtra plants were collected from the garden (Nakshtra Udyan) of Vidya Pratishthan, Baramati, Pune, Maharashtra in the month of December 2014 - August 2015.

Qualitative estimation of phytoconstituents

Phytochemical screening was carried out in order to have qualitative chemical composition information of crude extracts and to identify the major natural chemical groups such as, alkaloids, phenolic compounds, flavonoids, and terpenoids using commonly employed precipitation and coloration methods.

Quantitative estimation of phytoconstituents

For quantitative studies, one gm plant sample extracted with 25 mL 95% ethanol at 200 rpm for 24 h., filtered and used for further analysis i.e.

Determination of total alkaloids[2c]:

The plant materials were extracted with methanol for 24 h at 150 rpm. The extract was filtered and methanol was evaporated on a rotary evaporator under vacuum at a temperature of 45ºC temperature to dryness. A part of this residue was dissolved in 2N HCl and then filtered and filtrate was further washed with 10 mL chloroform (3times). The pH of this solution was adjusted to neutral with 0.1N NaOH. Then addition of 5 mL of BCG solution and 5 mL of phosphate buffer was done to this solution. The mixture was shaken and extracted with 5 mL chloroform by vigorous shaking two times. The extracts were collected in a 10 mL volumetric flask and diluted with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. For standard graph, 1 mg pure atropine was dissolved in 10 mL distilled water in order to make Atropine standard solution. Measure aliquots (0.4, 0.6, 0.8,1 and 1.2 mL) of atropine standard solution and transfer it to different separatory funnels. Then, add 5 mL of pH 4.7 phosphate buffer and 5 mL BCG solution, shake a mixture with 1,2,3,4 mL of chloroform. The extracts were collected in a 10 mL volumetric flask and then diluted with chloroform to adjust volume. The absorbance was measured at 470 nm against blank prepared as above in absence of atropine.

Determination of terpenoids[2d]:

10 g of the powdered leaves were extracted with methanol: water (4:1) at room temperature for 24 h. Filtration was done using Whatman no.1 filter paper obtained filtrate was evaporated to 1/10 volume at 40 ºC temperature. The reduced filtrate was acidified with 2M Sulphuric acid followed by chloroform extraction (three times the volume). Out of the two layers formed, the non-aqueous layer was taken and evaporated completely. The dried extract contained components like terpenoids.

Preparation of Extract for LC-MS

Freshly harvested plant leaves were washed with tap water. Thereafter, leaves were air dried and cut into small pieces and maceration was done with liquid nitrogen (-196 ºC) for one minutes to prepare fine powder. Weigh 8 g of leaves powder was macerated in 80 mL PBS (phosphate buffered saline) using mortar and pestle at room temperature for two minutes with occasional stirring. Thereafter, the aqueous extract ofleaves was filtered and filtrate collected was kept in refrigerator at 4ºC. All the extracts were subjected to LC-MS analysis.

LC-MS Analysis: All MS acquisitions were performed in the positive electrospray ionization mode. The capillary voltage, cone voltage, fragmentor voltage were 4 kV, 45V and 170V, respectively. The gas temperature was set at 325ºC. Data was acquired at scan rate of 3Hz in mass range 100-100 m/z. Further data was analyzed with Mass hunter qualitative software and METLIN database.

LC-MS specification

LC: Agilent 1260 binary LC System

Column: Agilent Zorbax SB 18 RRHT column (100×2.1 mm, 1.8µm)

Mobile phase A: Water (0.1% Formic acid)

Gradient:

S.No	Time	% A	%B
1	5.00 min	95	5
2	18.00 min	5	95
3	27.00 min	5	95
4	27.10 min	95	5
5	30.00 min	95	5

Flow rate: 0.3mL/min

Run time: 30 min

Injection Vol: 1µL

MS: 6540 ultra-high definition accurate mass QTOF LC/MS system

Parameter	Value
Gas Temp (°C)	325
Gas Flow (l/min)	10
Nebulizer (psig)	20
SheathGasTemp	320
SheathGasFlow	10
VCap	4000
Nozzle Voltage (V)	0
Fragmentor	170

Acquisition Mode MS1	
Min Range (m/z)	50
Max Range (m/z)	1700
Scan Rate (spectra/sec)	2.0

3. Results and Discussion

In India, the collection of medicinal plants from different regions *i.e.* Western Ghats, Himalayan region etc. and processing the plant products like leaf, stem, root is a part of indigenous health care system. Separation of its primary as well as secondary metabolites contributes a major part in immuno pharmacology especially for various activities like anti-inflammatory, anti-arthritic, anti-oxidant, anti-cancer, anti-allergic, immunomodulatory, adjuvant etc. These activities are important for human beings to be safe from various types of diseases.

Quantification of secondary metabolite contents in different parts of the plant is necessary. Different qualitative and quantitative tests are performed for detection of alkaloids, saponins, terpenoids, flavonoids, phenolics and glycosides. These metabolites (primary as well as secondary) isolated from various medicinal plants showed number of immune-pharmacological activities and used for various medical purposes *e.g.* anti-malarial drug artemisinin and anti-cancer drug paclitaxel.

At the same time, number of derivatives of metabolites isolated from various medicinal plants and used as efficacious compounds in human disease therapy and prevention. On the basis of these medical applications, VSBT focused on medicinal plants such as *Prosopisspicigera* [3], *Azadirachta indica* [4], *Emblica officinalis* [5], *Jasminum auriculatum* [6], *Aegle marmelos* [7], *Caloptropisgigantea* [8], *Calamus rotang* [9], *Ficus racemosa* [10], *Syzygiumcumini* [11], *Terminalia arjuna* [12]etc. In addition, VSBT also synthesized as well as prepared the cDNA library of *Syzygiumcumini* and *Aegle marmelos.*

The quantitative study has revealed the presence of phytochemicals which are considered as active medicinal chemical constituents. Important medicinal phytochemicals such as terpenoids and alkaloids are present in the samples [Fig.1, Table 1]. The result of the phytochemical analysis shows the presence of all the phytochemicals under consideration. The plants are rich interpenoids, with 4 are having alkaloids present. It is inferred from the data that among phytochmemicals screened, terpenoids are presentin major quantity followed by alkaloids.

The total alkaloid content of the plants is found to be in microgram scale *e.g.* plants NP-6, NP-15, NP-16, and NP-19 having quantities 1.6 μg, 7.6 μg, 6.8 μg, and 9.5 μg per gram, respectively, whereas the total terpenoids content in the plant NP-10, NP-15, NP-17, NP-21and NP-26 found to be 131 mg, 94 mg, 81 mg, 143 mg, and 55 mg per gram of the sample. The present data shows the richness of the plants screened in terms of the active phytoconstituents which ultimately contributes to the efficacy of that plant/compound as a drug. Aldehydes are important classes

of molecules having therapeutic value. Both ketones and aldehydes are found in a number of perfumes. Compared to ketones, aldehydes are a more popular source for perfumes fragrances. As well as aldehydes are an important part of some sugars and are contained in many substances used in baking, such as cinnamon, vanilla, and more. They also play a crucial role in the caramelization of sugars. It is been reported to have disinfectant and antiseptic property too.

The obtained data were analyzed and categorized into different groups. The general observation leads to identification of molecules containing aldehydic functional groups, those having structural similarity to prostaglandins, steroids, flavonoids, quinones etc. which are of medicinal importance. One of the classifications based on the number of aldehyde present in particular plant, which may lead to revelation of important information about necessity of the certain aldehyde in the plant as a secondary metabolite and its mode of action.

The occurrence of aldehydes in the plantis found in low numbers as compared to other molecules. Whereas pertaining to aldehyde, drastic variation is seen when distribution of number of aldehydes in different plants taken into account, such as in NP-16 contains 24 aldehydes present, while NP-2, NP-12 and NP-19 contains as low as 2 aldehydes [Figure 2]. Some of the plants such as NP-5, NP-17 and NP-26 have moderate number of aldehydes ranging from 10-15.

The second tabulation based on the occurrence of a particular aldehyde in a chosen plant along with frequency of occurrence of the same aldehyde in other [Table 2]. It is been seen that few of them are present in almost all of plants under consideration, such as Ophiobolin A which shows presence in 24 plants. The other aldehydes Methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxocyclopentyl] octanoate in 16, Methyl 4-[2-(2-formyl-vinyl)-3-hydroxy-5-oxocyclo pentyl]-butanoate in 13, acyclic unsaturated aldehyde 3-heptenal in 11, 4, 6, 11-Hexadecatrienal in 5, 2-Dodecenal in 5 plants. Few of the plants observed with presence of a single aldehyde of variable structures.

Aldehyde of biological and medicinal interests

Ophiobolin A, a fungal metabolite and a phytotoxin [13] was found to be a potent inhibitor of calmodulin-activated cyclic nucleotide phosphodiesterase [14]. [Figure.3] Ophioboline A exhibits a wide spectrum of bioactivity a ainst nematodes, fungi, and bacteria [15]. It has also been reported to induce apoptotic cell death in the L1210 cell line,[16] and shows cytotoxicity against the cancer cell lines A-549, Mel-20, and P-335 with low IC_{50} values[17]. Convallatoxin is found as dual inducer of autophagy and apoptosis, inhibits angiogenesis in vitro and in vivo as well, it is also proved to be new P-glycoprotein substrate [18].Calotropin possesses cytotoxicity against several cancer cells, e.g. human chronic myeloid leukemia K562 cells, with unclear mode of action. Calotropin inhibited the growth of K562 cells in a time- and dose-dependent manner by G_2/M phase arrest [19].

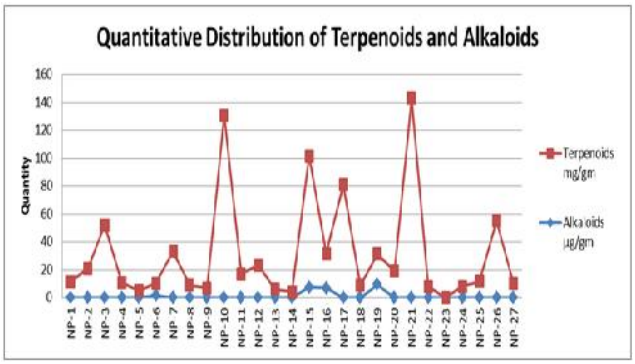

Figure 1: Graphical representation of quantitative distribution of Flavonoids, Alkaloids, Terpenoids, and Phenolics

Figure 2: Total number of aldehydes present in each plant

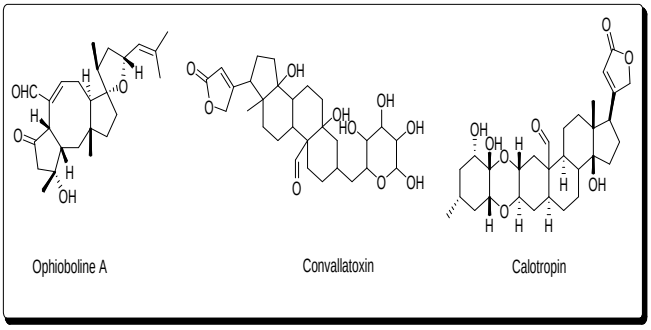

Figure 3: Representative aldehydes of medicinal importance

4. Conclusion

In the present study, ethanolic extracts were subjected to qualitative and quantitative estimation of total flavonoids, alkaloids, terpenoids and phenolics. The quantitative analysis inferred the richness of these plants in terms of active phytoconstituents, alkaloids and terpenoids in particular. Some of the plants are shown to have major quantities of all the screened phytoconstituents. The extracts of Nakshtra plants were also subjected to LC-MS analysis. The phytochemical investigation is done on the basis of spectral information obtained and respective secondary metabolites were identified. The systematic analysis and categorization of the given phytochemicals into different classes of compounds such as quinones, flavanoides, steroids etc. pertaining to aldehydic functional

group is performed. Further tabulation regarding classification of an individual aldehyde based on frequency of occurrence of that particular member in all other Nakshtra plants is arranged, which yielded a systematic taxonomical documentation of the said group. The potential bioactive candidates for biological and medicinal interest were also identified for which scruples investigation is underway. The given study paves a way towards the systematic analysis of the phytochemicals regarding aldehydes or any other secondary metabolite for that matter and facilitates identification and property postulations as well as drug formulation and study their pharmacology.

Table 1: Quantitative estimation of Flavonoids, Alkaloids, Terpenoids and Phenolics

S. No	Common Name	Botanical Name	Code	Phytoconstituents	
				Alkaloids *µ*g/gm	Terpenoids mg/gm
1	Rui	*Calotropisgigantea*	*NP-1*	-	11.3
2	Kuchla	*Strychnos nuxvomica*	*NP-2*	-	21
3	Pimpal	*Ficus religiosa*	*NP-3*	-	52
4	Velu	*Bambusa arundinacea*	*NP-4*	-	11
5	Arjun	*Terminalia arjuna*	*NP-5*	-	5
6	Palas	*Butea frondosa*	*NP-6*	1.6	8.6
7	Jai	*Jasminum auriculatum*	*NP-7*	-	33
8	Amba	*Mangifera Indica*	*NP-8*	-	9
9	Chandan	*Santalum album*	*NP-9*	-	7
10	Khair	*Acacia catechu*	*NP-10*	-	131
11	Umber	*Ficus glomerata*	*NP-11*	-	17
12	Naagkeshar	*Mesua ferrea*	*NP-12*	-	23.3
13	Aamla	*Emblica officinalis*	*NP-13*	-	6
14	Jamun	*Eugenia jambolana*	*NP-14*	-	4
15	Payari	*Ficus infectoria*	*NP-15*	7.5	94
16	Raal	*Vetiveria indica*	*NP-16*	6.8	25
17	Neem	*Azadirachta indica*	*NP-17*	-	81
18	Bakul	*Mimusops elengi*	*NP-18*	-	9
19	Shami	*Prosopis spicigera*	*NP-19*	9.5	22
20	Adulsa	*Justicia adhatoda*	*NP-20*	-	19
21	Saavar	*Salmalia malabarica*	*NP-21*	-	143
22	Fanas	*Artocarpus integrifolia*	*NP-22*	-	8
23	Vet	*Calamus rotang*	*NP-23*	-	-
24	Moha	*Madhuca indica*	*NP-24*	-	8
25	Kadamba	*Anthocephalus cadamba*	*NP-25*	-	11.6
26	Bael	*Aegle marmelos*	*NP-26*	-	55
27	Vad	*Ficus benghalensis*	*NP-27*	-	10

Table 2: Distribution of aldehydes in Nakshatra plants

SN	Molecule	Sample																											T
		NP-1	NP-2	NP-3	NP-4	NP-5	NP-6	NP-7	NP-8	NP-9	NP-10	NP-11	NP-12	NP-13	NP-14	NP-15	NP-16	NP-17	NP-18	NP-19	NP-20	NP-21	NP-22	NP-23	NP-24	NP-25	NP-26	NP-27	
1	Ergoline-1-carboxaldehyde, 8-(hydroxymethyl)-10-methoxy-6-methyl-, (8b)-	x																											**1**
2	Ipecac (Protoemetine)	x																											**1**
3	5,8-Tetradecadienal	x															x												**2**
4	2,4-Octadienal	x			x	x	x																						**4**
5	Convallatoxin	x																x											**2**
6	Indoleacetaldehyde	x																											**1**
7	2,4,7-Tridecatrienal	x		x		x											x	x							x		x		**7**
8	Calotropin	x																											**1**
9	2-Methyl-5-isopropylhexa-2Z,5-dienal	x																											**1**
10	2,6-Nonadienal	x		x		x	x																						**4**
11	2-Nonenal	x				x											x										x		**4**
12	2,5-Undecadienal	x				x	x										x										x		**5**
13	4,10-Undecadiynal	x															x												**2**
14	12-Oxo-9(Z)-dodecenoic acid	x															x			x	x	x							**5**
15	4-Octenal	x							x	x	x	x		x	x	x	x												**9**
16	Ophiobolin A	x	x	x	x	x	x		x	x	x		x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	**24**
17	12-Oxo-9-octadecynoic acid	x																											**1**
18	13-Keto-9Z,11E,15Z-octadecatrienoic acid	x																											**1**
19	Methyl 4-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-butanoate		x	x		x	x					x			x				x			x		x	x	x	x	x	**13**
20	3-Heptenal				x	x		x	x	x											x	x		x	x	x	x		**11**
21	9,12-Octadecadienal				x												x												**2**
22	Digitoxose					x																							**1**
23	2,4,6-Octatrienal					x																							**1**
24	2,4,6,8-Decatetraenal					x											x												**2**
25	Gibberellin A36					x				x																			**2**
26	12-Oxo-10Z-dodecenoic acid					x	x				x																		**3**

27	Methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate					x				x			x	x	x	x		x	x		x	x	x	x	x	x	x	x	**16**
28	4,4-Dimethyl-14a-formyl-5a-cholesta-8,24-dien-3b-ol						x																							**1**
29	Mebendazole metabolite (2-Amino-5-benzoylbenzimidazole)								x																					**1**
30	p-Hydroxy cinnamaldehyde									x							x										x		**3**
31	(R)-(+)-Citronella									x																			**1**
32	2-Decene-4,6,8-triyn-1-al											x																	**1**
33	Cinnamaldehyde											x																	**1**
34	Acetaminobenzaldehyde																x												**1**
35	2,4,6,8,10-Dodecapentaenal																x	x											**2**
36	2-Tridecene-4,7-diynal																x												**1**
37	2-Undecenal																x		x										**2**
38	Geranial																x												**1**
39	Sinapyl aldehyde																x												**1**
40	2-Tridecenal																x												**1**
41	Haematommic acid																x							x				x	**3**
42	10-Hydroxy-16-oxo-hexadecanoic acid																x		x										**2**
43	Clavirin I																x	x											**2**
44	3-Hexenal																x									x	x		**3**
45	Octanal																x	x	x			x				x			**5**
46	3-Formyl-25-desacetylrifampin																x												**1**
47	Gossypol																	x											**1**
48	Peruvoside																	x											**1**
49	(11Z)-8,18-Ethanoretinal																	x											**1**
50	4-Methylpentanal																				x				x	x			**3**
51	3-Octenal																				x	x	x				x		**4**
52	Glycolaldehyde																				x								**1**
53	4,6,11-Hexadecatrienal																				x				x	x	x	x	**5**
54	2-Dodecenal																					x	x	x			x	x	**5**
55	6-Octenal																							x	x	x			**3**
56	Capraldehyde																										x		**1**
57	2-Heptenedial																										x		**1**
58	Myristaldehyde																										x		**1**
	T	**18**	**2**	**4**	**4**	**14**	**7**	**1**	**4**	**7**	**3**	**4**	**2**	**3**	**4**	**3**	**24**	**10**	**6**	**2**	**8**	**8**	**4**	**7**	**7**	**9**	**15**	**6**	

x – Present, **T**– Total

5. References

[1] a) Anwar F, Ali M, Hussain AI, Shahid M. Antioxidant and antimicrobial activities of essential oil and extracts of fennel (Foeniculum vulgare Mill.) seeds from Pakistan. Flavour and Fragrance Journal 2009; 24:170–176. b) Stuffness M, Douros J. Current status of the NCI plant and animal product program. Natural Products 1982; 45:1–14. c) Huie CW. Review: Modern sample preparationtechniques for the extraction and analysis ofmedicinal plants. Journal of Analytical andBioanalytical Chemistry, 2002; 373:23-30.d) Ukwuani AN, Abubakar MG, Hassan SW, Agaie BM. Antinociceptive activity of hydromethanolic extract of some medicinalplants in mice. International Journal of Pharmacy. Photon. 2013: 104: 120-125.

[2] a) Chang C-C, Yang M-H, Wen H-M,Chern J-C. Estimation of total flavonoids content in Propolisby two complementary colorimetric methods, Journal of Food and Drug Analysis 2002. 10:178-182. b) Wangcharoen W. Morasuk W. Antioxidant capacity and phenolic content of some Thai culinary plants. Maejo International Journal of Science and Technology 2007; 01:100–106.c) Shamsa F, Monsef H, Ghamooshi R, Verdian-rizi M. Spectrophotometric determination of total alkaloids in some Iranianplants, Thai Journal of Pharmaceutical Sciences 2008; 32:17-20. d) Majaw S,Moirangthem J. Qualitative and Quantitative Analysis of *Clerodendroncolebroo kianum* Walp. Leaves and *Zingiber cassumunar* Roxb. Rhizomes. Ethnobotanical Leaflets 2009; 13:578-89

[3] Gupta A, Chaphalkar SR. Anti inflammatory activity of aqueous extract of leavesof *Prosopisspicigera*. International J. of Research in Pharmacy & Life Sciences 2015; 3: 829–834.

[4] Gupta A, Jagtap RB, Chaphalkar SR. Anti-viral activity of *Azadirachtaindica* leaves against Newcastle disease virus: Astudy by *In-vitro* and *in vivo* immunological approach. International Journal of Current Trendsin Pharmaceutical research. 2014, 2: 494 –501.

[5] Gupta A, Chaphalkar SR. Flow cytometric analysis of immunoadjuvant activity of *Emblica officinalis* on human whole blood. World Journal of Pharmaceutical Research 2015; 4:1063– 1071.

[6] Gupta A, Chaphalkar SR. Use of flow cytometry to measure the immune stimulatory activity of aqueous extract of *Jasminum auriculatum.* International Journal of Current Advanced Research 2015; 4:87–91.

[7] Gupta A, Jagtap RB, Chaphalkar SR. Flow cytometric evaluation of antiviral activity of *Aeglemarmelos* against New castle disease virus. International Journal of Research in Pharmacy and Life Sciences 2015; 3: 283 –287.

[8] Gupta A, Chaphalkar SR. Immunosuppressive activity of crude saponins from the leaves of *Caloptropis gigantean, Calamusrotang* and *Artocarpusintegrifolia*. International Journal of Pharma Sciences and Research 2015; 5: 1–5.

[9] Gupta A, Chaphalkar SR. Rapiddetection of immunosuppressive activity ofaqueous extract of *Calamusrotang* using flow cytometry. Journal of Medicinal Chemistry and Drug Discovery 2015; 2:01–08.

[10] Gupta A, Chaphalkar SR. Flow cytometric immune pharmacological studies of aqueous extract of *Ficusracemosa*as animmuno modulator. Journal of International Research in Medical and Pharmaceutical Sciences2016; 6:108–114.

[11] Gupta A, Chaphalkar SR. Flowcytometry based assay of formulation from*Syzygiumcumini*in human whole blood andglycosylated red blood cells. Journal of Pharma Research 2014; 3:265–270.

[12] Gupta A**,** Khamkar PR, Chaphalkar SR. Inhibition of nitric oxide productionand proinflammatory cytokines by aqueous extract of *Terminalia arjuna*in human peripheral blood mononuclear cells. International Journal of Pharmaceutical and Biological Science Archive2014; 2: 29–33.

[13] Leung PC, Taylorg WA, Wang JH, Tipton CL, Role of Calmodulin Inhibition in the Mode of Action of Ophiobolin A. The Journal ofBiological Chemistry 1984; 259:2742–2747.

[14] Au TK, Chick WS, Leung PC. The biology of ophiobolins. Life Science, 2000; 67:733 –742.

[15] Leung PC, Taylor WA, Wang JH, Tipton CL. A natural product inhibitor of calmodulin. Journal of Biological Chemistry1984; 259:2742– 2747.

[16] Shen X, Krasnoff SB, Lu S-W, Dunbar CD, O'Neal J, Turgeon BG, Yoder OC, Gibson DM, Hamann MT. Characterization of 6-epi-3-anhydroophiobolin B from *Cochliobolus heterostrophus*. *Journal of Natural Products* 1999; 62:895–897.

[17] a) Tsuna k, Noguchi N, Nakada M. Angewandte Chemie International Edition 2011; 50: 9452 – 9455, b)Gozalpour E, Greupink R, Bilos A, Verweij V, van den Heuvel JJMW, Masereeuw M, Russel FGM, Koenderink JB. Convallatoxin: A new P-glycoprotein substrate. European Journal of Pharmacology2014; 744:18–27.

[18] Wang S-C, Lu M-C, Chen H-L, Tseng H-I, Ke Y-Y, Wu Y-C, Yang P-Y. Cytotoxicity of calotropin is through caspase activation and down regulation of anti-apoptotic proteins in K562 cells. Cell Biology International 2009; 33:1230–1236.

[19] Nikolovska-Coleska Z, Xu L, Hu Z, Tomita Y, Li P, Roller P. P, Discovery of embelin as a cell-permeable, small-molecular weight inhibitor of XIAP through structure-based computational screening of a traditional herbal medicine three-dimensional structure database. Journal of Medicinal Chemistry 2004; 47:2430-2440.