International Journal of Chemistry and Pharmaceutical Sciences

Journal Home Page: www.pharmaresearchlibrary.com/ijcps

Research Article **Open Access**

Quantitative investigation of phytochemicals in Nakshtra plants and distribution analysis of ketones pertaining to LC-MS

Ajam C Shaikh, Amit Gupta and Sushama R Chaphalkar*

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

A B S T R A C T

Majority of population in developed and under developed countries totally relies on traditional forms of medicine, mainly plant based to meet the primary health care needs of the people. These medicinal plants having various chemical constituents which are bioactive and could be of the therapeutic use, known as secondary metabolites. These are the organic compounds which aid them in their normal growth and development. The systematic study regarding presence of phytochemicals may aid in the analysis, pharmacological and property predication studies. The mechanistic mode of plants producing therapeutic effects can also be better understood and investigated if the given information scrutinized well. In the given study, ethanolic extracts were analyzed for quantitative phytochemical estimation of total flavonoids and phenolics. The study inferred the presence of active phytoconstituents, flavonoids and terpenoids in particular. The extracts were further studied by LC-MS. The phytochemical identification was done on the basis of m/z ratio obtained for each individual secondary metabolite. The numeral analysis for ketones present as secondary metabolite is done along with frequency of occurrence of particular ketone in other Nakshtra plants. The representative ketones of medicinal and biological importance are also identified. The given study paves a way to the systematic analysis of the phytochemicals pertaining to ketones or any other secondary metabolite under study 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, Flavonoids and Terpenoids, Ketones

A R T I C L E I N F O

CONTENTS

Article History: Received 25 May 2016, **Accepted** 30 June 2016, **Available Online** 27 July 2016

***Corresponding Author**
Dr. Sushama R Chaphalkar
Vidya Pratishthan's School of Biotechnology
(VSBT, Research Centre affiliated to
Savitribai Phule Pune University),
Baramati, Maharashtra, India
Manuscript ID: IJCPS3065

PAPER-QR CODE

Citation: Sushama R Chaphalkar. Quantitative investigation of phytochemicals in Nakshtra plants and distribution analysis of ketones pertaining to LC-MS. *Int. J. Chem, Pharm, Sci.*, 2016, 4(7): 381-393.

Copyright© 2016 Sushama R Chaphalkar. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

1. Introduction

Medicinal plants have proven to be valuable sources for the identification of new drug candidates, as well as they are important means of studying for chemical biology and medicinal chemistry research. Historically, many natural products were used in treating various diseases because of bioactive constituents present in it. India is gifted with rich plant diversity. Therapeutic properties of these medicinal plants are widely acknowledged at global level too [1a] and it is estimate, majority of modern drugs have plant based 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. The systematic investigation, identification and characterization of drug with highest pharmaco-therapeutic activity may lead reducing the number of plants to be chopped off for medicinal purpose especially medicinal plants whose roots are used. 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. The therapeutic activity of these plants is attributed to the secondary metabolites present in it. Primary metabolites are compounds that have essential roles associated with photosynthesis, respiration, growth and development. Secondary metabolites are organic compounds which are not directly involved in the survival of plants but they produce some products which help them in their normal growth and development *e.g.* Terpenes, Alkaloids, Phenols etc. These are not essential component for survival but rather are required for the interaction of plants with their environment e.g. survival in odd and extreme conditions, fighting against diseases etc. These are structurally diverse and so can be diagnostic in chemotaxonomic studies. To date thousands of bioactive compounds have been isolated from plants and other sources. Consequently these chemical structures have been employed by chemists as good references to scan the diversity of space for drug discovery efforts. Although ignored for long, their function in plants is now attracting attention as some appear to have a key role as well as molecular markers. The presence of vast number of secondary metabolites, having intricate and complexity in the molecular structure, specific availability could be exploited them as molecular markers. In order to exploit these compounds as drug or molecular marker one need to have thorough characterization of their active ingredients which may shed light on the mode of action of plants producing therapeutic effects. By applying statistical tools to the information gathered one can have better understanding and representation of the compounds/plants under consideration.

Nakshtra plants plays pivotal role in Ayurveda, Indian tradition and modern medicine which also forms basis of our study. In this study special attention is been given to the quantitative investigation of Flavonoids, and Phenolics present in Nakshtra plants as well as identification and classification of these secondary metabolites pertaining to ketonic functionality. These carbonyl compounds are of medicinal significance when administered raw in the body. These are also used as building blocks in the synthesizing of commercially important compounds, including pharmaceuticals and polymers. Though they are well known to the mankind as secondary metabolites, they are scarcely studied and compiled. Herewith we attributed our study to tabulate data 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.

Estimation of total flavonoids2a,2b:

Quercetin was used to make the calibration curve. 10 mg quercetin was dissolved in water and diluted to 20, 40 to 100 μg/mL. The diluted standard solutions (0.5mL) were separately mixed with 1.5mL of 95% ethanol, 0.1 mL of 10% AlCl3, and 0.1 mL of potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 50 min, the absorption of the reaction mixture was measured at 415 nm using spectrophotometer. Same amount of distilled water used as a blank as was used for 10% AlCl3as substitute. Similarly, 0.5mL of ethanol extract was reacted with AlCl3for determining of flavonoid contents described above.

Estimation of total phenolics[2a,2b]:

The Folin-Ciocalteau micro method of Waterhouse was used to estimate total phenolic content (TPC). The extract (100μL) was diluted with deionized water to 4.8ml and 300μL of Folin-Ciocalteau reagent (1N) was added and shaken. After 8 min. 900 μL of 20% Na_2CO_3 solution was added with mixing. The solution was left at room temperature at 40 min before reading the absorbance at 765nm. Gallic acid was used as standard and the results were reported as mg Gallic acid equivalent per gram.

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) 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 with occasional stirring. Thereafter, the aqueous extract of leaves 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 electro spray ionization mode. The capillary voltage, cone voltage, fragmentor voltage were 4 kV, 45V and 170V, respectively. The gas temperature was set at 325 ºC. 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)

Mobile phase B: Acetonitrile

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

Plants are one of the major sources of large number of drugs containing different groups such as antispasmodics, emetics, anti-cancer, antimicrobials etc. Plants also play significant role in relieving various diseases in Ayurveda. Therefore, standardization, emphasis on evaluation and characterization of various plants and their active constituents against a number of diseases is highly warranted. Among the various phytochemicals the phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [3].

They are the bioactive constituents showing antiapoptosis, anticarcinogen, anti-inflammation, anti-atherosclerosis, antimicrobial as well as inhibition of angiogenesis and cell proliferation activities [4]. The phenolic compounds are also reported to show the antioxidant properties [5,6]. Phenolic compounds such as flavonoid, phenolic acids are the natural antioxidant mainly comes from plants [7].The activity may be due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [8]. They also are effective antioxidant and show strong anticancer activities [9,10,11,12], these applications compelled us to study Nakshtra plants where VSBT focused on medicinal plants such as *Prosopisspicigera* [13], *Azadirachta indica* [14], *Emblica officinalis* [15], *Jasminum auriculatum*[16], *Aeglemarmelos* [17], *Caloptropisgigantea* [18], *Calamus rotang*[19], *Ficus racemosa* [20], *Syzgiumcumini* [21], *Terminalia arjuna* [22] etc. In addition, VSBT also synthesized as well as prepared the cDNA library of *Syzygiumcumini* and *Aeglemarmelos.*

The quantitative investigation into the sample has revealed the presence of bioactive phytochemicals which are consideredas of medicinal use. Important medicinal phytochemicals such Flavonoids and Phenolicsare present in the samples [Figure 1, Table 1]. The plants are rich with phytoconstituents such as Flavonoids, and Phenolics. The comparison among the plants for total phenolics, furnished that plants such as NP-12, NP-14, NP-19, NP-20, and NP-25 contains 503.10 mg, 322.35 mg, 291.4 mg, 282.54 mg, and 277.71mg per gram of sample respectively. The appreciable quantities of total flavonoids are also present in the plants NP-3, NP-7, NP-8, NP-21, and NP-25 with 104 mg, 175 mg, 95 mg, 87.5 mg, and 71.25 mg per gram of the sample respectively.

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. Ketones are important class of compounds having therapeutic value. The drug cortisone is a ketone used to treat severe allergies, arthritis, asthma, multiple sclerosis and skin conditions. Ketones are used to create acetophenone, which is responsible for creating almond, cherry, honeysuckle, jasmine, and strawberry fragrances. The obtained data were analyzed and categorized into different groups. The general observation leads to identification of molecules containing ketonic 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 ketones present in particular plant, which revealed some intersecting facts. As compared to aldehydes, ketones are found in large numbers.

It is interesting to see structurally diverse ketones present having the assigned role being secondary metabolite depend on the type of class that belongs to. In case of ketone distribution, as expected NP-16 having most number of ketones which are 125while NP-10 is having least number of ketones present *i.e.* 10. The other plants such as NP-1, NP-17, and NP-26 are having 61,70,37 respectively [Fig.2]. If one need to consider the frequency of occurrence of a particular ketone in the plants under consideration, the ketone such as Ophiobolin A found in 25 plants, followed by Lactone of PGF-MUM in 24, 3,7-Epoxycaryophyllan-6-one in 23, Levonorgestrel acetate in 20, Dihydro-7-Desacetyldeoxygedunin in 20, Embelin in 20 and Methyl 4-[2-(2-formylvinyl)-3-hydroxy-5-oxocyclo pentyl]-butanoate in 17 plants [Table 2].

Ketones of biological and medicinal interests

Ophiobolin A, a fungal metabolite and a phytotoxin [23] was found to be a potent inhibitor of calmodulin-activated cyclic nucleotide phosphodiesterase [24]. [Fig.3] Ophioboline A exhibits a wide spectrum of bioactivity against nematodes, fungi, and bacteria [25]. It has also been reported to induce apoptotic cell death in the L1210 cell line, [26] and shows cytotoxicity against the cancer cell lines A-549, Mel-20, and P-335 with low IC_{50} values [27]. Embelin, a natural benzoquinone from plants. It is shown to exhibit anti-tumor and anti-inflammatory activity in cells.

Embelin inhibits cell growth and activates caspases to promote apoptosis in cancer cells with high expression of XIAP [28]. Additionally, it is also reported to prevent NF-κB activation by inhibiting IKK. Embelin has also extensive part in various animal models to study the role of XIAP. In the azoxymethane/dextran sulfate sodium (AOM/DSS) induced colitis-associated cancer (CAC) model, embelinis found to reduce incidence and tumor size in mice by inhibiting proliferation of tumor epithelial cells and suppressing IL6 expression and IL6-activated STAT3 *in vivo* [29]. Fluoxymesterone is an anabolic steroid having strong androgenic properties, due to which it can be used in the treatment of male hypogonadism, delayed puberty in males. The reduction or competitive inhibition of prolactin receptors can result in to the exhibit edantitumor activity of fluoxy mesterone. Pentoxifylline A, xanthine derivative is a modulator of tumor necrosis factor and of HIV replication in patients with AIDS and therefore enhanced HIV expression, and inhibition of zidovudine (AZT) activity. The obstructed arteries in the limbs may cause vascular dementia and intermittent claudication where Pentoxifylline A stood good cure to it. (+)-Tuberonic Acid has shown to have tuber-inducting properties. (+)-Tuberonic Acid is an analogue of Jasmonic Acid and a major constituent of essential oil of Jasmine so importance in fragrance industry. It is shown to have tuber-inducting properties. A class of compounds-Cucurbitacins is with wide spectrum of pharmacological activities such as anti-inflammatory and anticancer effects. There are many molecular targets for cucurbitacins have been discovered, e.g. fibrous-actin, signal transducer and activator of transcription (STAT), cyclooxygenase-2, etc [30]. Gambogic amide, proved to be an agonist for TrkA receptor that possesses robust neurotrophic activity and prevents neuronal cell death [31]. It is also used in hair treatment. Clavulone I is reported to have anti-inflammatory and anti-tumor activity [32].

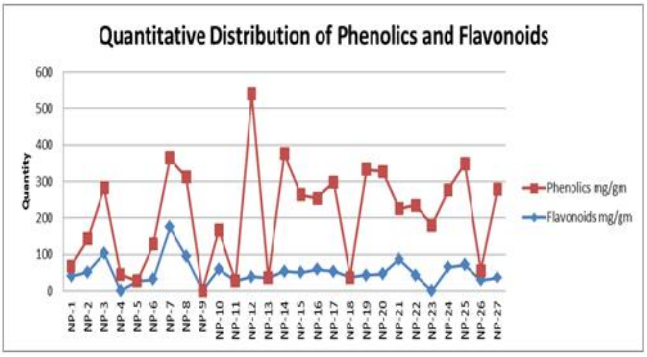

Figure 1: Graphical representation of quantitative distribution of Flavonoids and Phenolics

Figure 2: Total number of ketones present in each plant

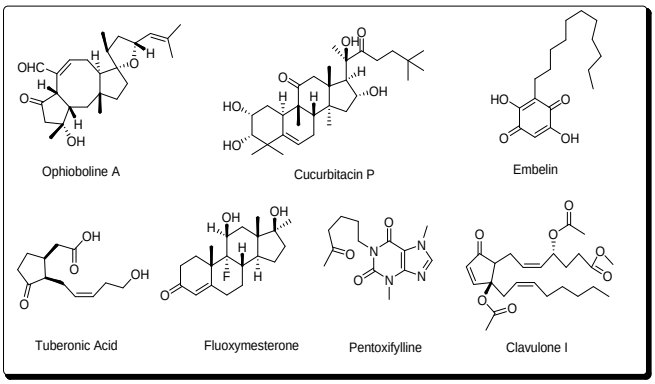

Figure 3: Representative ketones of medicinal and biological importance

4. Conclusion

The ethanolic extracts were investigated for qualitative and quantitative estimation of total flavonoids and phenolics. The study revealed the richness of these plants in terms of active phytoconstituents, flavonoids and terpenoids in particular. Comparison among the plants shows the presence of phytoconstituents in the major quantities. The extracts of Nakshtra plants were also subjected to LC-MS analysis. The phytochemical analysis and secondary metabolites identification was done on the basis of spectral information obtained. The systematic analysis and

categorization of the given phytochemicals into different classes of compounds such as quinones, flavanoides, steroids etc. pertaining to ketonic functional groups is performed. Further, the data was classified as an individual ketone based on frequency of occurrence of that particular member in all other Nakshtra plants, which yielded a systematic taxonomical documentation of the ketones. The potent molecules of biological and medicinal interest were also identified for which detailed investigation is underway. The given study helps to understand and perform systematic analysis of the phytochemicals regarding ketones or any other secondary metabolite under consideration. It also facilitates drug molecule identification, postulation of their disease curing properties as well as drug formulation and study their mode of action.

Table 1: Quantitative estimation of Flavonoids and Phenolics

Sr. No.	Common Name	Botanical Name	Code	Phytoconstituents	
				Flavonoids mg/gm	Phenolics mg/gm
1	Rui	*Calotropisgigantea*	*NP-1*	39.75	28.90
2	Kuchla	*Strychnosnuxvomica*	*NP-2*	51.5	91.46
3	Pimpal	*Ficus religiosa*	*NP-3*	104	180.07
4	Velu	*Bambusaarundinacea*	*NP-4*	-	44.80
5	Arjun	*Terminalia arjuna*	*NP-5*	26.5	-
6	Palas	*Butea frondosa*	*NP-6*	31.5	97.68
7	Jai	*Jasminum auriculatum*	*NP-7*	175	189.87
8	Amba	*Mangifera Indica*	*NP-8*	95	217.39
9	Chandan	*Santalum album*	*NP-9*	-	-
10	Khair	*Acacia catechu*	*NP-10*	60	107.82
11	Umber	*Ficusglomerata*	*NP-11*	26.75	-
12	Naagkeshar	*Mesuaferrea*	*NP-12*	39	503.10
13	Aamla	*Emblica officinalis*	*NP-13*	35	-
14	Jamun	*Eugenia jambolana*	*NP-14*	53.5	322.35
15	Payari	*Ficusinfectoria*	*NP-15*	50.6	213.09
16	Raal	*Vetiveria indica*	*NP-16*	58.25	195.67
17	Neem	*Azadirachta indica*	*NP-17*	53.5	244.92
18	Bakul	*Mimusopselengi*	*NP-18*	36.75	-
19	Shami	*Prosopisspicigera*	*NP-19*	42.5	291.4
20	Adulsa	*Justiciaadhatoda*	*NP-20*	45.75	282.54
21	Saavar	*Salmaliamalabarica*	*NP-21*	87.5	138.10
22	Fanas	*Artocarpusintegrifolia*	*NP-22*	42.6	193.10
23	Vet	*Calamusrotang*	*NP-23*	-	180.23
24	Moha	*Madhuca indica*	*NP-24*	65	212.10
25	Kadamba	*Anthocephaluscadamba*	*NP-25*	71.25	277.71
26	Bael	*Aeglemarmelos*	*NP-26*	29	26.21
27	Vad	*Ficus benghalensis*	*NP-27*	35	244.92

Table 2: Distribution of Ketones in Nakshtra plants

SN	Molecule	Sample																											
		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	**T**
1	Embelin	x	x	x	x	x	x	x	x	x	x	x	x					x			x	x	x		x	x	x	x	**20**
2	Dihydrojamonic acid methyl ester	x	x	x	x	x																							**5**
3	Cucurbitacin H	x																											**1**
4	Oleandomycin	x																											**1**
5	Rifamycin B	x																											**1**

6	Triptonide	x		x						x				x							x				x		x	x	**8**
7	Quercitrin	x				x																							**2**
8	Dihydroquercetin	x				x					x																		**3**
9	Gestrinone	x																											**1**
10	Diethylpropion(metabolite X-glucuronide)	x														x						x				x		**4**
11	Gestrinone	x																											**1**
12	3beta-Acetoxy-23-bromo-isoallospirost-9 (11)-ene-12-one	x				x		x	x																				**4**
13	6-Desmethylgriseofulvin glucuronide	x																											**1**
14	Cortisol 21-acetate	x																											**1**
15	3,7,12-Trioxochol-4-en-24-oic Acid	x																											**1**
16	5-Oxo-7-decynoic acid	x																											**1**
17	Deoxysappanone B 7,3'-dimethyl ether acetate	x					x									x													**3**
18	Epirubicinolglucuronide	x																											**1**
19	11-Ketorockogenin acetate	x										x													x				**3**
20	Deoxysappanone B trimethyl ether	x																											**1**
21	Ciprofloxacin	x																											**1**
22	Captopril disulfide	x			x																								**2**
23	Naringenin-7-O-Glucoside	x																											**1**
24	Medroxyprogesteroneglucuronide	x																											**1**
25	Methyl jasmonate	x				x										x	x	x	x	x	x	x	x	x	x	x	x		**1 4**
26	Pregnenolone sulfate	x																											**1**
27	Hydroxyprogesterone acetate	x														x													**2**
28	9a-Fluoro-B-hydroxyandrosterone	x			x																								**2**
29	10-Deoxymethymycin	x																											**1**
30	Warfarin	x																											**1**
31	2,3-Dihydroxy-4-methoxy-4'-ethoxybenzophenone	x																											**1**
32	Pregnenolone	x																											**1**
33	Isorhamnetin	x																											**1**
34	4S-Hydroxy-8-oxo-(5E,9Z,13Z,16Z,19Z)-neuroprostapentaenoic acid-cyclo[7S,11S]	x																											**1**
35	3,7,12-Trioxochol-4-en-24-oic Acid	x																											**1**
36	1-Deacetoxy-1-oxo-3,7-dideacetylkhivorin	x															x												**2**
37	17a-Hydroxypregnolone	x																											**1**
38	Epirubicinglucuronide	x																											**1**
39	Pregnenolone sulfate	x																											**1**
40	Phenylmethyl methyl ketone	x		x			x									x										x		**5**
41	Carvone	x																											**1**
42	cis-Jasmone	x														x													**2**
43	Nandrolone	x			x																								**2**
44	(+)-Bornane-2,5-dione	x																											**1**
45	6,5-Diketo-13,14-dihydro-PGF1 alpha	x																											**1**
46	Urocortisone	x																											**1**
47	12-Oxo-9-octadecynoic acid		x	x	x		x			x	x	x																	**7**
48	20,alpha-Dihydroperdnisolone	x																											**1**
49	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	x	**25**
50	3-Keto palmitic acid	x								x																			**2**

51	6-Keto-PGF1a	x														x												**2**
52	9a-Fluorotetrahydrocortisol	x				x						x		x				x	x	x				x					**8**
53	11-Keto pentadecanoic acid	x																											**1**
54	3beta,7beta-Dihydroxy-12-oxo-5beta-cholan-24-oic Acid	x		x																									**2**
55	Bicyclo-PGE2	x					x																						**2**
56	15-Deoxy-delta-12,14-PGD2	x																											**1**
57	3beta,7beta-Dihydroxy-12-oxo -5beta-cholan-24-oic Acid	x																											**1**
58	PGA3	x			x	x	x		x	x	x	x				x													**9**
59	1a,1b-Dihomo-PGE1	x	x	x	x		x									x	x												**7**
60	Triamcinolone acetonideglucuronide	x	x		x																								**3**
61	Lactone of PGF-MUM	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**
62	2,3-Diketo-13,14-dihydro-PGF1a	x				x					x		x																**4**
63	12-oxo-14,18-Dihydroxy-9Z,13E,15Z-octadecatrienoic acid		x																								x		**2**
64	10-Keto tridecanoic acid		x			x										x													**3**
65	Koparin 2'-methyl ether		x	x			x									x													**4**
66	Levonorgestrel acetate		x			x	x		x	x	x	x	x				x	x	x	x	x	x	x	x	x	x	x	x	**20**
67	(9R,13R)-1a,1b-Dihomo-jasmonic acid		x				x														x					x	x	x	**6**
68	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	x	x	x	x	**17**
69	6b,11b,16a,17a,21-Pentahydroxypregna-1,4-diene-3,20-dione 16,17-acetonide		x										x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	**16**
70	Tetrahydrodeoxycorticosterone		x																										**1**
71	13-Keto-9Z,11E,15Z-octadecatrienoic acid		x	x	x																								**3**
72	Isotectorgenin, 7-methyl ether		x				x	x								x													**4**
73	3-Oxo-tridecanoic acid			x		x	x	x	x	x	x	x	x			x		x			x	x		x		x		x	**16**
74	Pyrogallin			x																									**1**
75	Fluoxymesterone			x		x								x	x	x	x	x										x	**8**
76	11-Deoxy-16,16-dimethyl-PGE2			x																									**1**
77	11beta-PGE2			x																									**1**
78	Khayanthone			x	x													x	x									x	x	**6**
79	3,7-Epoxycaryophyllan-6-one			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x		x	x	x	x	**23**
80	Dihydrokaempferol				x																								**1**
81	Pentoxifylline				x																								**1**
82	2-Oxo capric acid				x											x													**2**
83	4-Oxo capric acid				x																								**1**
84	9-Hydroxy-10-oxo-12-octadecenoic acid				x											x				x							x		**4**
85	Xanthurenic acid					x										x													**2**
86	13-Ethyl-16,17-dihydroxy-18,19-Dinorpregna-4,9,11-trien-20-yn-3-one					x										x													**2**
87	3-Prenyl-4-hydroxyacetophenone					x										x													**2**
88	Mitoxantrone					x																							**1**
89	Santonin					x																							**1**
90	Idebenone Metabolite (QS-10)					x																							**1**
91	Mitomycin					x																							**1**
92	Tuberonic acid					x	x				x		x			x	x	x		x		x	x	x		x	x	x	**14**
93	3-Butyrl propionic acid					x																							**1**
94	9-Oxo-2Z-decenoic acid					x																							**1**

95	1-Cyclohexene-1-acrylic acid, 2,6,6-trimethyl-3-oxo-					x										x											**2**
96	2-Methoxyestrone 3-glucuronide					x																					**1**
97	Myricetin					x																					**1**
98	Dihydromyricetin					x										x											**2**
99	Abscisic acid (cis,trans)					x										x	x										**3**
100	17-alpha, 21-dihydroxy-11,20-dioxo-5-beta-pregnan-3-alpha-yl-beta-d-glucuronide					x																					**1**
101	DMG-MINO					x																					**1**
102	3-Hydroxy-5,9,17-trioxo-4,5:9,10-disecoandrosta-1(10),2-dien-4-oate					x										x											**2**
103	Bromovulone I					x																					**1**
104	2,5-Undecadienal					x																					**1**
105	Triamcinolone acetonide sulfate					x										x											**2**
106	25-O-Desacetylrifampin					x																					**1**
107	Gibberellin A8-catabolite					x				x																	**2**
108	(5b,7a,12a)-2-(3-methoxyphenyl)-2-oxoethyl ester-7,12-dihydroxy-cholan-24-oic acid					x																					**1**
109	Testosterone decanoate					x																					**1**
110	Dihydro-7-desacetyldeoxygedunin					x	x		x		x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	**20**
111	Cucurbitacin P					x																			x			**2**
112	Oleandolide					x										x		x		x		x				x	**6**
113	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	**17**
114	15-Deoxy-ä-12,14-PGJ2					x	x		x	x						x												**5**
115	3,12-Dioxochola-1,4,9(11)-trien-24-oic Acid					x															x	x	x			x		**5**
116	Parthenin															x	x	x			x					x	x	**6**
117	Sappanone A 7-methyl ether						x						x															**2**
118	Jasmonic acid						x																					**1**
119	Hematein						x																					**1**
120	Dihydrojasmonic acid, methyl ester						x		x	x	x	x	x	x		x	x		x	x		x	x		x		x	x	**16**
121	Flumequine						x																					**1**
122	3-Oxo-eicosanoic acid						x																					**1**
123	Nobiletin						x	x								x												**3**
124	(1S,2S)-3-oxo-2-pentyl-cyclopentanebutanoic acid						x											x										**2**
125	Gambogic acid amide						x	x	x	x	x	x	x	x		x				x					x	x			**12**
126	Alprostadil(15-keto-13,14-dihydro-PGE)							x																				**1**
127	Lobaric acid							x																				**1**
128	beta-Toxicarol							x																				**1**
129	Phloridzin							x								x												**2**
130	Ketoprofenglucuronide							x																				**1**
131	Anhydrotetracycline							x																				**1**
132	3-Oxo-4-pentenoic acid							x								x												**2**
133	2',4'-Dhydroxychalcone 4'-glucoside							x	x	x																		**3**
134	Castasterone								x		x	x	x	x	x	x												**7**
135	Dihydrocaryophyllen-5-one									x			x			x										x	x	**5**
136	Mebendazole metabolite (2-Amino-5-benzoylbenzimidazole)									x	x																	**2**
137	PGD3									x																		**1**

138	12-Oxo-ETE									x																			**1**
139	Ethosuximide M5										x																			**1**
140	3alpha,6alpha,12alpha-Trihydroxy-7-oxo-5beta-cholan-24-oic Acid										x																			**1**
141	Testosterone undecanoate										x																			**1**
142	Punaglandin 3										x																			**1**
143	3alpha-Hydroxy-1,7-dioxo-5beta-cholan-24-oic Acid												x																	**1**
144	3alpha,12alpha-Dihydroxy-15-oxo-5beta-chol-8(14)-en-24-oic Acid												x																	**1**
145	Hydrocortisone butyrate propionate												x																	**1**
146	Cucurbitacin C												x																	**1**
147	Testosterone sulfate												x																	**1**
148	17,18-dehydro-clavulone I												x																	**1**
149	Rotenonic acid, methyl ether												x																	**1**
150	Betamethasone valerate												x																	**1**
151	Cucurbitacin L												x																	**1**
152	Cucurbitacin F												x																	**1**
153	Cedrelone												x																	**1**
154	7-Deacetoxy-7-oxodeoxygedunin												x					x												**2**
155	6beta-Hydroxyprednisolone													x																**1**
156	Tridesacetoxykhivorin													x	x	x	x	x												**5**
157	Cucurbitacin A															x		x												**2**
158	Nalidixic acid																x													**1**
159	Gibberellin A51-catabolite																x													**1**
160	Desacetylcolchicine																x													**1**
161	12-Oxo-9(Z)-dodecenoic acid																x													**1**
162	Acetoaceticaci																x													**1**
163	Tangeritin																x													**1**
164	Oxycodone																x													**1**
165	Hydroxypyruvic acid																x													**1**
166	Artemisinin																x													**1**
167	Idarubicin																x													**1**
168	Cosmosiin																x													**1**
169	Sarafloxacin																x													**1**
170	deacetoxy(7)-7-oxokhivorinic acid																x													**1**
171	Naringin																x													**1**
172	Levobunololglucuronide																x													**1**
173	6-Demethylsterigmatocystin																x	x												**2**
174	Diethyl Oxalpropionate																x													**1**
175	Leucodin																x													**1**
176	N-Didesmethylmifepristone (RU 42848)																x													**1**
177	Pelargonyl acetic acid																x													**1**
178	16a-Hydroxyestrone																x													**1**
179	6-Ketoestriol																x													**1**
180	Epirubicin																x													**1**
181	Chrysophanol 8-O-beta-D-glucoside																x													**1**
182	Sodium chlorovulone II																x													**1**
183	Formestane-4-O-glucuronide																x													**1**
184	Sterigmatocystin																x													**1**
185	Mitoxantrone																x													**1**
186	16a-Hydroxyprednisolone																x													**1**
187	Idarubicinolaglycone																x													**1**
188	Dihydrolevobunololglucuronide																x													**1**
189	(-)-Isopiperitenone																x													**1**

190	3-Carboxy-2,4,4-trimethyl-2-cyclohexenone															x											**1**
191	Carvone oxide															x											**1**
192	Ketoleucine															x											**1**
193	3-Formyl-25-desacetylrifampin															x											**1**
194	Daunorubicin															x											**1**
195	(-)-Usnic acid															x											**1**
196	Idarubicinol															x											**1**
197	Norfloxacinglucuronide															x											**1**
198	Fluocinonide															x											**1**
199	Gemifloxacin															x											**1**
200	2-Butanone, 4-(6-hydroxy-2-naphthalenyl)-															x	x					x		x			**4**
201	Ubiquinone															x	x	x				x		x	x	x	**8**
202	b-D-Glucopyranosiduronic acid, 6-(3-oxobutyl)-2-naphthalenyl															x											**1**
203	Dihydrorobinetin															x											**1**
204	Epothilone B															x											**1**
205	Ofloxacin-N-oxide															x											**1**
206	Flumethasone															x											**1**
207	Ondansetron															x											**1**
208	Idebenone Metabolite (QS-8)															x											**1**
209	16-Hydroxy-4-carboxyretinoic acid															x											**1**
210	Estronehemisuccinate															x											**1**
211	7C-aglycone															x											**1**
212	15-Oxo-11Z,13E-eicosadienoic acid															x											**1**
213	Loxoprofen Metabolite (b-D-Glucopyranuronic acid, 1-[a-methyl-4-[(2-oxocyclopentyl)methyl]benzeneace															x											**1**
214	Avobenzone															x											**1**
215	Diosmin															x											**1**
216	3-oxo-nonadecanoic acid															x											**1**
217	Kobusone															x	x			x	x	x	x	x	x	x	**10**
218	Prednisone															x											**1**
219	Helenine															x											**1**
220	11-Deoxy-PGE1															x											**1**
221	4-Keto pentadecanoic acid															x	x				x	x	x	x	x		**7**
222	Icariin															x											**1**
223	5-O-Methylvisamminol															x											**1**
224	Irigenin, dibenzyl ether															x											**1**
225	16-Oxo-heptadecanoic acid															x											**1**
226	Clavirin I															x	x										**2**
227	4-Heptanone															x				x	x	x	x		x	x	x	**8**
228	Hesperetin															x											**1**
229	6-Hydroxydesmethylondanse-tron															x											**1**
230	Angolensin															x											**1**
231	Phorbol															x											**1**
232	2,3-Dinor-6-keto-PGF1a															x		x					x		x	x		**5**
233	15-Deoxy-delta-12,14-PGJ2															x				x		x		x				**4**
234	Vulpinic acid															x											**1**
235	10-Deoxymethynolide															x	x		x				x					**4**
236	Isoeugenitol															x			x	x		x	x			x		**6**
237	4-Keto lauric acid															x		x	x	x	x	x			x	x	x	**9**
238	3,4-Dihydroxy-9,10-secoandrosta-															x										x		**2**

	1,3,5(10)-triene-9,17-dione																											
239	2-(Diethylamino)-4'-hydroxy-Propiophenone															x												**1**
240	4-Demethoxydaunomycinone (Idarubicinaglycone)															x												**1**
241	16,16-Dimethyl-PGE1															x				x								**2**
242	Pentan-3-one															x												**1**
243	Swietenine															x												**1**
244	Rifaximin															x		x										**2**
245	2,3-dinor-PGE1																x											**1**
246	Triamcinolone diacetate																x											**1**
247	Bromopyruvic acid																x											**1**
248	Methoxyvone																x											**1**
248	Dehydrorotenone																x											**1**
249	2,4,6-Heptatrienoic acid, 5-methyl-7-(2,6,6-trimethyl-3-oxo-1-cyclohexen-1-yl)-, (2E,4E,6E)-																x											**1**
250	4-Ketoretinoic acid glucuronide																x											**1**
251	3b,17a,21-Trihydroxypregnenone																x											**1**
252	Oxymetholone																x											**1**
253	dhk-PGF1alpha																x											**1**
254	Carapin-8 (9)-ene																x											**1**
255	Swietenolide-3-acetate																x				x							**2**
256	6-Hydroxyangolensic acid methyl ester																x				x							**2**
257	Gibberellin A29-catabolite																x											**1**
258	Fluticasone propionate																x											**1**
259	17,18-Dehydro-clavulone I																x											**1**
260	8beta-Hydroxycarapin, 3,8-hemiacetal																x											**1**
261	Neohesperidindihydrochalcone																x											**1**
262	Fissinolide																x											**1**
263	Methylprednisolone succinate																x											**1**
264	7alpha-(Thiomethyl)spironolactone sulfone																x											**1**
265	Dihydroxy (3alpha,12alpha)pregnan-20-one																x				x							**2**
266	Prieurianin																x											**1**
267	Dexamethasone																x											**1**
268	1,7-Dideacetoxy-1,7-dioxo-3-deacetylkhivorin																x											**1**
269	Khayasin c																x										x	**2**
270	Mundulone																x											**1**
271	6-Hydroxydexamethasone																x				x							**2**
272	Dihydromundulone																x											**1**
273	Angolensic acid, methyl ester																x				x							**2**
274	Budesonide																x											**1**
275	3,7,12-Trioxochola-1,4-dien-24-oic Acid																x											**1**
276	8-Hydroxydesmethylondansetron																x											**1**
277	1,3-Dideacetyl-7-deacetoxy-7-oxokhivorin																x											**1**
278	Flurandrenolide																x											**1**
279	3,16-Dideoxymexicanolide-3beta-diol																x				x							**2**
280	9a-Fluoroallotetrahydrocortisol																x											**1**
281	Dihydrogambogic acid																x											**1**
282	Avocadynone acetate																x									x		**2**

283	Gedunin																	x				x						**2**
284	6beta-Hydroxytriamcinolone acetonide																	x										**1**
285	Aflatoxin B1																	x										**1**
286	8-Hydroxyondansetron glucuronide																	x	x									**2**
287	N-(3-oxododecanoyl) homoserine lactone																		x		x			x	x			**4**
288	11b-Hydroxyaetiocholanolone																		x								x	x	**3**
289	Tetranor-PGEM																		x										**1**
290	4-Keto myristic acid																		x	x		x		x	x		x	x	**7**
291	PGJ3																		x										**1**
292	Idebenone																		x							x	x		**3**
293	Deoxysappanone b 7,3'-dimethyl ether																			x										**1**
294	12alpha-Hydroxy-3-oxochola-1,4,6-trien-24-oic Acid																				x	x	x					x		**4**
295	4-Oxo-9Z,11E,13E-octadecatrienoic acid																				x									**1**
296	Cucurbitacin J																				x									**1**
297	4-Oxomytiloxanthin																				x					x	x		x	**4**
298	Zearalenone																					x								**1**
299	17a-Hydroxyprogesterone																					x								**1**
300	Mexicanolide																					x								**1**
301	Crustecdysone																					x				x			x	**3**
302	15-keto-PGE2																					x	x							**2**
303	m-Hydroxyphenylpyruvic acid																						x							**1**
304	Clavulone I																						x			x				**2**
305	6-Hydroxyondansetron																						x							**1**
306	10-Oxo-docosanoic acid																						x					x		**2**
307	Methyl 10,13-dihydroxy-9-oxo-11-octadecenoate																							x						**1**
308	Androstanedione																							x						**1**
309	Allotetrahydrocortisone																										x			**1**
310	PGK2																										x	x		**2**
311	Clobetasol propionate																										x			**1**
312	N-Despropylpropafenone																											x		**1**
313	Morphinone																											x		**1**
314	2,3-Dinor-6,15-diketo-13,14-dihydro-20-carboxyl-PGF1a																												x	**1**
315	11-Ketorockogenin acetate																												x	**1**
T		**61**	**17**	**19**	**19**	**48**	**28**	**15**	**14**	**20**	**19**	**12**	**30**	**14**	**10**	**17**	**125**	**70**	**25**	**16**	**25**	**33**	**29**	**22**	**24**	**26**	**37**	**32**	

x – Present, **T** – Total

5. References

[1] Anwar F, Ali M, Hussain AI, Shahid M: Antioxidant and antimicrobial activities of essential oil and extracts of fennel (Foeniculumvulgare Mill.) seeds from Pakistan. FlavourFragr 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):1–14.

[2] Singh, R., Singh, S.K., Arora, S. 2007. Evaluation of antioxidant potential of ethyl acetate extract/fractions of Acacia auriculiformis A. Cunn. Fod Chem. Toxicol., 45: 1216-1223.

[3] a) Chia-Chi Chang, Jiing-Chaun Chem. &*et al*, 2002. Estimation of total flavonoids content in *Propolis* by two complementary colorimetric methods, J. of Food & Drug Ana. 10:178-182. b) Wiwat Wangcharoen & Wallaya Morasuk, 2007.

[4] Antioxidant capacity and phenolic content of some Thai culinary plants, Mj. Int. J. Sci. tech, 2007, 01(02), 100-106.

[5] Han, X., Shen, T., Lou, H. 2007. Dietry polyphenols and their biological significance. Int. J. Mol. Sci., 950-988.

[6] Brown, J.E., Rice-Evans, C.A. 1998. Luteolin rich artichoke extract protects low density lipoprotein from oxidation in vitro. Free Radical Res., 29: 247-255.

[7] Krings, U., Berger, R.G. 2001. Antioxidant activity of roasted foods. Food Chem., 72: 223-229.

[8] Ali, S.S., Kasoju, N., Luthra, A., Singh, A., Sharanabasava, H., Sahuand, A., Bora, U. 2008. Indian medicinal herbs as source of antioxidants. Food Res. Int., 41: 1-15.

[9] Marjorie, C. 1996. Plant products as antimicrobial agents. ClincalMicrobiol. Rev., 12: 564-582.

[10] Salah, N., Miller, N.J., Pagange, G., Tijburg, L., Bolwell, G.P, Rice, E., Evans, C. 1995. Polyphenolic flavonoids as scavenger of aqueous phase radicals as chai breaking antioxidant. Arc. Biochem. Broph., 2: 339-346.

[11] Del-Rio, A., Obdululio, B.G., Casfillo, J., Main, F.G., Ortuno, A. 1997. Uses and properties of citrus flavonoids. J. Agric. Food Chem., 45: 4505-4515.

[12] Okwu, D.E. 2004. Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. J.Sustain. Agric. Environ., 6(1): 30-37.

[13] Yadav, R.N.S.,Agarwala, M. 2011. Phytochemical analysis of some medicinal plants, J.Phyt., 3: 10-14

[14] Amit Guptaand Sushama R Chaphalkar. Anti-inflammatory activity of aqueous extract of leaves of *Prosopisspicigera*. International Journal of Research in pharmacy and life sciences 2015, 3(1): 829 – 834.

[15] Amit Gupta,Ramesh B Jagtap and Sushama R Chaphalkar. Anti-viral activity of *Azadirachta indica* leavesagainst Newcastle disease virus: A study by *in vitro* and *in vivo* immunological approach. International Journal of Current trends in Pharmaceutical research 2014; 2(6): 494 – 501.

[16] Amit Gupta and Sushama R Chaphalkar. Flow cytometric analysis of immunoadjuvant activity of *Emblica officinalis* on human whole blood. World Journal of Pharmaceutical research 2015; 4 (2): 1063 - 1071.

[17] Amit Gupta and Sushama R Chaphalkar. Use of flow cytometry to measure the immuno stimulatory activity of aqueous extract of *Jasminum auriculatum.* International Journal of Current Advanced research 2015; 4(5): 87 - 91.

[18] Amit Gupta, Ramesh B Jagtap and Sushama R Chaphalkar. Flow cytometric evaluation of anti-viral activity of *Aeglemarmelos* against Newcastle disease virus. International Journal of Research in pharmacy and Life Sciences 2015; 3 (2): 283 – 287.

[19] Amit Guptaand Sushama R Chaphalkar. Immunosuppressive activity of crude saponins from the leaves of *Caloptropis gigantean*, *Calamusrotang* and *Artocarpusintegrifolia*. International Journal of Pharma sciences and research. 2015; 5(7): 1 – 5.

[20] Amit Guptaand Sushama R Chaphalkar. Rapid detection of immunosuppressive activity of aqueous extract of *Calamusrotang* using flow cytometry. Journal of Medicinal chemistry and drug discovery 2015; 2: 01- 08.

[21] Amit Guptaand Sushama R Chaphalkar. Flow cytometric immunopharmacological studies of aqueous extract of *Ficusracemosa* as an immunomodulator. Journal of International research in Medical and Pharmaceutical Sciences 2015; article in press

[22] Amit Gupta and Sushama R Chaphalkar. Flow cytometry based assay of formulation from *Syzygiumcumini*in human whole blood and glycosylated red blood cells. Journal of Pharma research 2014, 3 (12): 265 - 270.

[23] Amit Gupta**,** Pallavi R Khamkar and Sushama R Chaphalkar. Inhibition of nitric oxide production and proinflammatory cytokines by aqueous extract of *Terminaliaarjuna* in human peripheral blood mononuclear cells. International Journal of pharmaceutical and biological science archive 2014, 2 (8): 29-33.

[24] Pak C. Leung, William A. Taylorg, Jerry H. Wangg, and Carl L. Tipton, The Journal of Biological Chemistry, 1984,259(5): 2742-2747.

[25] Au T. K., Chick W. S. H., Leung P. C., Life Science, 2000, 67: 733 –742.

[26] Leung P. C., Taylor W. A., Wang J. H., Tipton C. L., Journal of Biological Chemistry1984, 259:2742 – 2747.

[27] Shen X., Krasnoff S. B., Lu S.-W., Dunbar C. D., Neal J. O_, Turgeon B. G., Yoder O. C., Gibson D. M., Hamann M. T., Journal of Natural Products1999, 62: 895 –897.

[28] Ahn K. S, Sethi G, Aggarwal B. B. Embelin, an inhibitor of X chromosome-linked inhibitor-of-apoptosis protein, blocks nuclear factor-kappaB (NF-kappaB) signaling pathway leading to suppression of NF-kappaB-regulated antiapoptotic and metastatic gene products. Molecular Pharmacology 2007, 71: 209-219.

[29] Dai Y, Jiao H, Teng G, Wang W, Zhang R, Wang Y, et al. Embelin reduces colitis-associated tumorigenesis through limiting IL-6/STAT3 signaling. Molecular Cancer Therpy 2014, 13: 1206-1216.

[30] Abdullah A. Alghasham, Cucurbitacins –A Promising Target for Cancer Therapy International Journal of Health Sciences, 2013, 7(1):67-79.

[31] Jang SW, Okada M, Sayeed I, Xiao G, Stein D, Jin P, Ye K. Proceedings of Natural Academy of Sciences U S A. 2007 ;104(41):16329-34

[32] Kikuchi, H.; Tsukitani, Y.; Iguchi, K.; Yamada, Y. TetrahedronLetters 1982, 23:5171.