ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Isolation of Phytoconstituents and Invitro Antilithiatic activity by Titrimetic method, Antioxidant activity by DPPH scavenging assay method of alcoholic root extract of *Hedychium coronarium J.* Koenig and alcoholic leaves extract of *Ageratum conyzoides* Linn. Plant species.

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ABSTRACT

Herbal medicine is a natural remedy for all the disease. The herbal medicines do not have any side effect and it is cost effective and safest medicine from an ancient periods. Urolithiasis or Lithiasis is a consequence of complex physical processes. The major factors are supersaturation of urine with the offending salt and crystallization. This process is synonymously known as Urolithiasis or Lithiasis or Nephrolithiasis or Kidney stones. In this study Ethanolic & Aqueous extracts of roots of *Hedychium coronarium* J. Koenig and leaves extract of *Ageratum conyzoides* Linn. plant species were evaluated for their potential to dissolve experimentally prepared kidney stones like calcium oxalate by an Invitro model and Antioxidant activity was also performed by DPPH scavenging assay method. For performing *Invitro* Antilithiatic activity titrimetric method was adopted. For performing antioxidant activity DPPH scavenging assay method was adopted and for this materials used were DPPH dye, alcohol, ascorbic acid, UV visible spectrophotometer etc. Phytoconstituents were also isolated by column and thin layer chromatographic techniques from both plant species. Only Ethanolic extract of roots of *Hedychium coronarium* and leaves extract of *Ageratum conyzoides* plant species produced highest dissolution of stones when compared to standard drug cystone and at 10 mg. concentration. Also this study showed that alcoholic extract of roots of *Hedychium* plant and leaves of *Ageratum* plant species in higher concentration possess best antioxidant potential as compare to standard ascorbic acid with IC 50 value 9.3, 18.9 and 24.8 µg/ml. for ascorbic acid, alcoholic extract of roots of *Hedychium* plant species and alcoholic extract of leaves of *Ageratum* plant species respectively. For *Hedychium* plant species isolated phytoconstituents were hedychilactone like derivatives and for *Ageratum* plant species isolated phytoconstituents were precocene I and II and their structures were confirmed by IR, NMR and Mass spectroscopic datas.

Keywords: *Hedychium coronarium* J. Koenig , *Ageratum conyzoides* Linn. , Urolithiasis, Kidney stones, Calcium oxalate, antioxidants, DPPH etc.

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ISSN: 0975-8585

INTRODUCTION

Lithiasis or Urolithiasis- is the medical term used to describe stones occurring in the urinary tract. Other frequently used terms are urinary tract stone disease and nephrolithiasis. Kidney stones may contain various combinations of chemicals. The most common type of stone contains calcium in combination with either oxalate or phosphate. These chemicals are part of a person's normal diet and make up important parts of the body, such as bones and muscles. A less common type of stone is caused by infection in the urinary tract [1-2]. This type of stone is called a struvite or infection stone. Another type of stone, uric acid stones, are a bit less common, and cystine stones are rare. For unknown reasons, the number of people in the United States with kidney stones has been increasing over the past 30 years [3]. In the late 1970s, less than 4 percent of the population had stone-forming disease. By the early 1990s, the portion of the population with the disease had increased to more than 5 percent. The prevalence of kidney stones rises dramatically as men enter their 40s and continues to rise into their 70s. For women, the prevalence of kidney stones peaks in their 50s. Once a person gets more than one stone, other stones are likely to develop [4-5].

Kidney stones often do not cause any symptoms. Usually, the first symptom of a kidney stone is extreme pain, which begins suddenly when a stone moves in the urinary tract and blocks the flow of urine. Typically, a person feels a sharp, cramping pain in the back and side in the area of the kidney or in the lower abdomen. Sometimes nausea and vomiting occur [6-7].

Kidney stone formation is a complex process that results from a succession of several physico-chemical events including supersaturation, nucleation, growth, aggregation and retention within the kidneys. The incidence of Urolithiasis is more in men (recurrence rate is 70-80%) then women (47-60%). Many remedial measures have been employed during ages to treat this condition [8-9]. Most of them were from plants and prove to be useful. However, the rational behind their use is not well established except for a few plants and are reported to be effective with no side effects [10].

Antioxidants

These play an important role as health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced antioxidants like vitamin C, vitamin E, carotenes, phenolic acids etc. have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity [11-12].

Hedychium coronarium J. Koenig plant (Zingiberaceae) - is a small herbaceous plant. It is hard, perennial, erect, branched, annual weed up to 3-6 feet height. The leaves are simple arranged in alternate manner with undulate margin. . The flowers are white in colour and have pleasant fragrance; summer flowering; fall flowering. The trunk is green in colour, very thick. It is widely distributed over the tropical and subtropical region of the Asia and Africa. It is an annual branching herb which grows well on wastelands and in tropical region after the rainy season. It contain many bioactive compounds including Saponins, Glycosides, flavonoids, fats and volatile oil [13].

Ageratum conyzoides Linn. (Asteraceae) - is an annual herbaceous plant with a long history of traditional medicinal uses in several countries of the world and also reputed to possess varied medicinal properties. *Ageratum* contain many bioactive compounds including flavonoids, alkaloids, coumarins, essential oils, chromenes, benzofurans, terpenoids and tannins. Whole plant have an antibacterial action against *Staphylococus aureus, Bacillus subtilis, Eschericichia coli* etc. In animal studies, it demonstrated a muscle relaxing and pain relieving effect, confirming its popular use for rheumatism [14]

These plant species have been reported to possess antilithiatic, antioxidant properties. So, this study has been undertaken to evaluate *Hedychium coronarium* J. Koenig and *Ageratum conyzoides* Linn. plant species for their possible potential to dissolve experimental urinary stones/Calcium oxalate using a modified invitro model

by titrimetric method and antioxidant action by UV spectroscopically using DPPH scavenging assay method. Phytochemical constituents were also isolated by chromatographic techniques from both plant species.

MATERIALS AND METHOD

Plant Material

The matured crude drug of *Ageratum conyzoides* Linn. (Asteraceae) was colleted carefully from local areas of Dehradun ,Uttarakhand, India. Another crude drug *Hedychium coronarium* J. Koenig under the name of Kapurkachri was collected from medicinal plants garden of Shantikunj-Haridwar (Uttarakhand) India. Then, these crude drugs were authenticated from Botanical Survey of India (BSI), Dehradun under Accession no.- 114004 & 114682 respectively. A voucher specimen herbarium of these plant species were submitted to the Botanical Survey of India, Dehradun.

Extraction

Soon after authentification, crude drugs were dried. *Ageratum conyzoides* (Asteraceae) leaves & *Hedychium coronarium* (Zingiberaceae) underground part roots were dried under shade and at room temperature until they were free from moisture and coarsely powdered and then pulverized. Then about, about 100 gms of powdered roots & rhizomes of Hedychium and leaves of Ageratum plant species were extracted with increasing order of polarity solvents series starting from Pet. ether, Chloroform, Ethanol via Soxhlet apparatus by successive hot continuous percolation method. Simultaneously, same amount of the roots and leaves of these plant species was also macerated separately in Chloroform water for preparing aqueous extracts for 72 hrs. At last, all extracts were concentrated in a rotary flash evaporator and the residue were dried in a desiccator over sodium sulphite. Then, Pharmacognostic evaluation was performed on all these extracts. Phytochemical screening tests were performed on these extracts.

EVALUATION FOR ANTILITHIATIC ACTIVITY (INVITRO)

Aqueous and ethanolic extracts of roots and leaves of *Ageratum* and roots of *Hedychium* plant species were evaluated for antilithiatic potential by titrimetric method using an invitro model. Formulation cystone was used as a reference standard.

Preparation of experimental kidney stones (Calcium oxalate stones by homogenous precipitation method):

Equimolar solution of Calcium chloride dihydrate in distilled water and Sodium oxalate in 10 ml of (2N sulphuric acid) were allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipate was calcium oxalate. precipitate was freed from traces of sulphuric acid by Ammonia solution, washed with distilled water and dried at 60˚ C for 4 hours.

Preparation of semi-permeable membrane from farm eggs :

The semi- permeable membrane of eggs lies between the outer calcified sheel and the inner contents like albumin & yolk. Shell was removed chemically by placing the eggs in 2M HCl for an overnight, which caused complete decalcification. Further, washed with distilled water, and carefully with a sharp pointer ahole is made on the top and the contents squeezed out completely from decalcified egg. Washed thoroughly with water and stored in refrigerator at a pH of 7-7.4

Estimation of Calcium oxalate by Titrimetry:

Exactly 1 mg of calcium oxalate and 10 mg of the extract/standard were weighed and packed it together in semi- permeable memberane by suturing. This was allowed to suspend in a conical flask containing a 100 ml 0.1 M TRIS buffer. One group served as negative control (contained only 1 mg of calcium oxalate) Now, the conical flask of all groups in a incubator were placed, pre heated to 37 ˚C for 2 hours, for about 7-8 hours. Then, removed the contents of semi- permeable membrane from each group into a test tube. It was added 2 ml of 1 N sulphuric acid and titrated with 0.9494 N $KMnO_4$ till a light pink color end point obtained (1 ml of 0.9494 N $KMnO_4$

equivalent to 0.1898 mg of calcium).The amount of undissolved calcium oxalate is then subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate actually the test substance could dissolve [15-16]

EVALUATION OF ANTIOXIDANT ACTIVITY BY UV SPECTROPHOTOMETRIC METHOD USING DPPH RADICAL SCAVENGING ASSAY:

Free radical scavenging activity of different extracts of roots of *Hedychium coronarium* and *Ageratum conyzoides* Linn. plant species were measured by 1,1- diphenyl-2-picryl hydrazyl (DPPH). In brief, 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts in ethanol at different concentration (5,10,15, 20, 25, 30 µg/ml). The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm. by using spectrophotometer (UV-VIS Shimadzu). Reference standard compound being used was ascorbic acid and experiment was done in triplicate. The IC 50 value of the sample , which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated by using following equation:

$$\textbf{DPPH scavenging effect (\%) or Percent inhibition} = A_0 - A_1 / A_0 \times 100$$

Where A_0 was the Absorbance of control reaction and A_1 was the Absorbance in presence of test or standard sample(Ascorbic acid) [17-19]

ISOLATION OF PHYTOCONSTITUENTS

To perform this procedure first of all, extraction of given grounded plant material was done by hot continuous successive percolation method using Soxhlet apparatus and finally obtained alcoholic extract was concentrated and prepared as slurry for column chromatographic technique[20-21] This extract was used for isolation of phytoconstituents because it has lot of potential. Then, column and thin layer chromatographic techniques were followed and column was run by various ratios of n-hexane, chloroform and ethyl acetate. Finally, isolated samples in sufficient quantity were sent for various spectroscopic determination viz- IR, C13 NMR, 1H NMR and Mass spectroscopy [22-24] This spectroscopic study reveals the presence of 8a, hydroxy hedychilactone from underground roots part of *Hedychium* plant and Precocene-I & II from aerial leaves part of *Ageratum* plant species.

8a, hydroxy hedychilactone was isolated as a yellow semi solid from roots of *Hedychium* plant species compound revealed a molecular ion peak corresponding to (M+H) at *m/z* 331. 2 indicating the molecular formula $C_{20}H_{26}O_4$. The 1H NMR spectrum of compound showed all the features of labdane diterpene. The IR spectrum displayed absorption bands at 3400 cm-1 (OH), 1681 cm-1 (α, β-unsaturated C=O) and 1709 cm-1 ((α, β- unsaturated γ-lactone). The 1H NMR spectrum displayed four quaternary methyl signals each integrating for three protons as singlets at δ 0.78, δ 0.99, δ 1.03, and δ 1.74. It has displayed a singlet at δ 2.86 for one proton (H-9) indicating the presence of one methine adjacent to the carbonyl (C-7) carbon atom. A sharp *singlet* integrated for 1 H at δ 5.88 is due to methine proton (H-14) in the lactone ring. A characteristic doublet for one proton at δ 2.97 (d, *J*=7.0 Hz) indicating the presence of methine (H-9) group adjacent to olefinic double bond. The presence of one *trans* double bond at δ 5.83 (1H, d, *J*=10.84,) and δ 5.79 (1H, d, *J*=10.92Hz) was suggested by the 1H NMR. Another sharp *singlet* integrated for two protons at δ 4.69 (2H, s) is assigned to CH_2 group in the lactone ring, the 1H NMR spectrum also revealing that the *trans* double bond (C-11/C-12) is conjugated with lactone ring. The 13C NMR spectrum of compound C showed the presence of 20 C-atoms. The 13C NMR spectrum indicated the presence of α, β-unsaturated ketone (δ 207.49), trisubstituted olefin (δ 143.86 and 156.10) and four methyl signals (δ, 33.57,31.91,19.33 and21.87). Further, it also displayed signal at δ 167.84 is due to C=O of lactone ring, δ 128.83, 128.27 are corresponding to disubstituted olefin, and δ 69.71 is assignable to methylene carbon in the lactone ring.

Characterization of Isolated Phytoconstituent HC-1

Physical state of constituents : Semi solid.
R_f value : 0.82 (n-hexane : ethyl acetate in 85:15 ratio)
Colour : Yellowish red

ISSN: 0975-8585

Spectral characteristics of Isolated Compound (HC-1)

Table 1: 1HNMR Interpretation of Compound HC-1

Position	δH multiplicity
C1	1.51,1,55(2H ,m)
C2	1.57,1.28(2H,m)
C3	0.78-0.99(6H,m)
C4	2.18 (1H,s)
C6	1.59-1.47(2H,m)
C8	1.74 (3H,m)
C9	2.86 (1H,s)
C11	5.76 (1H,d)
C12	5.7 (1H,d)
C13	6.46 (1H,d)
C16	4.11 – 4.95 (1H,s)

Table 2: 13 C NMR Interpretation of Compound HC-1

Position	Δc (ppm)
C1	19.33
C2	39.77
C3	33.5
C4	55.85
C5	22.91
C6	39.7
C7	207.49
C8	132.49
C9	128.1
C10	38.7
C11	130.93
C12	128.80
C13	167.84
C14	83.74
C15	167.84
C16	72.80
C17	33.5
C18	33.5
C19	14.05
C20	19.33

Table 3: IR Interpretation of Compound HC-1

The IR spectra (Nujol oil) showed α and β unsaturated lactone ring and α – β unsaturated C=O bonds

S.No.	Peak (cm^{-1})	Interpretation
1	721.84	Monosubstituted (C-H)
2	1072	C-O str.
3	1376.91	C-H bending
4	1460	N-H bending
5	1681.96	α -β unsaturated C=O
6	1709.19	α -β unsaturated lactone.
7	3400	O-H

ISSN: 0975-8585

Table 4: Mass spectra Interpretation of Compound HC-1

The Mass Spectra showed Mol. mass of compound HC-1 : 331.2

1.	**Base peak**	328
2.	**Parent peak (m/z)**	331.2
3.	**Fragmentations**	231.1, 287.1, 325.2

Proposed structure of isolated constituent depending upon interpretation of Mass, IR, NMR both C13 and 1H spectral analysis:

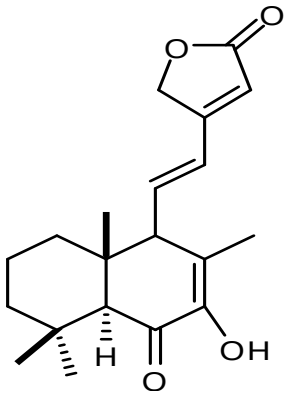


Figure 1: 8a, hydroxy hedychilactone

Like wise, Precocene -I & II Phytoconstituents from leaves of *Ageratum* plant species were isolated. **Precocene-I OR 7-methoxy ageratochromene** was isolated as a semisolid oily compound. (AC-1)

R_f value : 0.85 in the solvent system (Ethyl acetate: formic acid: Glacial acetic acid: Water (100:11:11:27)

Colour : Cream yellow coloured substance

Spectral Characteristics of Isolated Compound AC-1

Table 5: 1HNMR Interpretation of Compound AC-1

S.N.	δH multiplicity position
1.	1.6 (3H, s)
2.	1.6 (3H, s)
3.	1.91 (1H, d)
4.	2.75 (1H, d)
5.	7.2 (1H, d)
6.	6.28 (1H, s)
7.	4.2 (3H, s)
8.	6.2 (1H, trip)

Table 6 : C13 NMR Interpretation of Compound AC-1

S.N.	Position	δc (ppm)
1.	C_2	48.1
2.	C_3	22.8
3.	C_4	125.9
4.	C_5	105.5
5.	C_6	159.2

ISSN: 0975-8585

6.	**C_7**	100.6
7.	**C_8**	169.8
8.	**C_9**	22.2
9.	**C_{10}**	56.0
10.	**C_{11}**	23.2
11.	**C_{12}**	24.0
12.	**C_{13}**	26.0

Table 7: IR Interpretation of Compound AC-1

S.No	Peak (cm^{-1})	Interpretation
1	2930, 2930	CH_3 Stretching
2	1710.85	C-O Stretching
3	1462.60	CH_3 Bending
4	1316.99	C-H def in Methyl
5	721.34	Due to Benzene
6.	1155.86	C-O Stretching
7.	1237.17	C-O Stretching
8.	910	C-O Stretching

Table 8: Mass spectra Interpretation of Compound AC-1

The Mass Spectra showed Molecular mass of compound : 192

1.	**Base peak**	175.1
2.	**Parent peak (m/z)**	192.1
3.	**Fragmentations**	192.1, 175, 160

Proposed structure of isolated constituent depending upon interpretation of NMR both 1H and C13, IR & Mass spectral analysis:

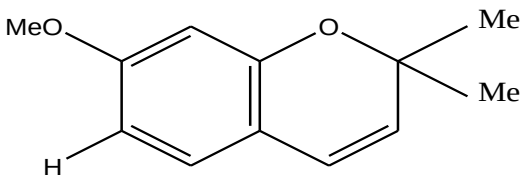


Figure 2 : 7-methoxyageratochromene (Precocene I)

Precocene –II OR 6,7 dimethoxy, 2,2-dimethyl chroman was isolated as a semisolid oily compound (AC-2)

Rf value : 0.90 in the solvent system (Ethyl acetate: formic acid: Glacial acetic acid: Water (100:11:11:27)

Colour : cream yellow coloured substance

ISSN: 0975-8585

Spectral Characteristics of Isolated Compound (AC-2)

Table 9: 1HNMR Interpretation of Compound AC-2

S.N.	δH multiplicity position
1.	1.65 (3H, s)
2.	1.67 (3H, s)
3.	5.7 (1 H, s) J=3
4.	6.90 (1 H, s) J=3
5.	6.6 (1 H, d)
6.	2.74 (3 H, s)
7.	2.73 (3 H, s)
8.	6.18 (1 H, d)

Table 10: C13 NMR Interpretation of Compound AC-2

S.N.	Position	δc (ppm)
1.	**C_2**	68.7
2.	**C_3**	132.4
3.	**C_4**	125.03
4.	**C_5**	105.03
5.	**C_6**	139.7
6.	**C_7**	135.2
7.	**C_8**	100
8.	**C_9**	149.21
9.	**C_{10}**	112
10.	**C_{11}**	27.7
11.	**C_{12}**	28.92
12.	**C_{13}**	60.18
13.	**C_{14}**	60.18

Table 11: IR Interpretation of Compound AC-2

S.No	Peak (cm^{-1})	**Interpretation**
1	2930, 2930	CH_3 Stretching
2	1710.85	C-O Stretching
3	1462.60	CH_3 Bending
4	1316.99	C-H def in Methyl
5	721.34	Due to Benzene
6.	1155.86	C-O Stretching
7.	1237.17	C-O Stretching
8.	910	C-O Stretching

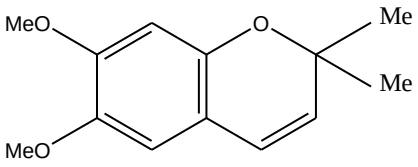
Table 12: Mass spectra Interpretation of Compound AC-2

The Mass Spectra showed Molecular mass of compound : 220.2

1.	**Base peak**	215
2.	**Parent peak (m/z)**	220
3.	**Fragmentations**	215, 175

ISSN: 0975-8585

Proposed structure of isolated constituent depending upon interpretation of NMR both 1H and C13, IR & Mass spectral analysis:



Ageratochromene (Precocene II)
OR
Figure 3: 6,7 dimethoxy,2,2-dimethyl chroman

RESULTS AND DISCUSSION

The alcoholic extract obtained by roots of *Hedychium* and leaves of *Ageratum* plant species at 10 mg concentration produced highest dissolution of calcium oxalate stones in comparison to other extracts . Cystone was to be equally effective found, when compared to alcoholic extract.

Like wise, same alcoholic extract of both plants showed better antioxidant action on higher concentration as compare to standard ascorbic acid IC 50 value was obtained as for standard drug ascorbic acid approx 9.3 µg/ml and for alcoholic extract of leaves of *Ageratum* plant species approx 24.8 µg/ml Also for alcoholic extract of roots of *Hedychium* plant species approx 18.9 µg/ml.

Isolated well interpretated Phytoconstituents were 8a, hydroxy hedychilactone from 85:15 ratio of n-hexane and ethyl acetate from *Hedychium* species & Precocene – I & II from 95: 5 and 90:10 ratio of chloroform and ethyl acetate from *Ageratum* species. Their structure were confirmed then by spectral analysis.

Table 13: Calcium Oxalate dissolution by various extracts of *Hedychium coronarium* J. Koenig and Cystone

Group	Vol. of standard $KMnO_4$ (ml)	Wt. of Calcium Estimated (mg)	Wt. of Calcium Reduced (mg)	Percentage Dissolution (%)
CONTROL	4.6	0.8730	----------	----------
Standard (cystone)	2.8	0.5314	0.3416	39.12
Alcoholic extract of roots & rhizomes	2.8	0.5314	0.3416	39.00
Aqueous extract of roots & rhizomes	3.0	0.5694	0.3036	34.77

Correspond to 10 mg

Table 14: Calcium Oxalate dissolution by *Ageratum conyzoides* extracts and Cystone

Groups	Vol. of standard $KMnO_4$	Wt. of Calcium Estimated	Wt. of Calcium Reduced	Percentage Dissolution
CONTROL	4.6 ml	0.8730 mg	----------	----------
Standard(cystone)	2.4 ml	0.4555 mg	0.4175 mg	47.82 %
AlcE extract roots	3.9 ml	0.7402 mg	0.1328 mg	15.21 %
AqE extract roots	3.2 ml	0.6073 mg	0.2657 mg	30.43 %
AlcE extract leaves	2.6 ml	0.4934 mg	0.3796 mg	43.48 %
AqE extract leaves	2.8 ml	0.5314 mg	0.3416 mg	39.12 %

Correspond to 10 mg

ISSN: 0975-8585

Table 15: Showing absorbance of different extract of *Hedychium coronarium* J. Koenig with Ascorbic acid

CONCENTRATION (µg/ml)	ASCORBIC ACID (Abs)	PEE (Abs)	$CHCl_3$ E(Abs)	AlcE (Abs)
5	0.2380	0.2386	0.2343	0.2405
10	0.1719	0.2232	0.1720	0.2204
15	0.0469	0.2350	0.2420	0.1729
20	0.0415	0.2341	0.2245	0.1393
25	0.0410	0.2386	0.2348	0.0761
30	0.0390	0.2428	0.2128	0.0715

AVG. CONTROL READING : 0.2444

$$\% \, Inhibition = (Abs_{control} - Abs_{test}) / Abs_{control} \times 100$$

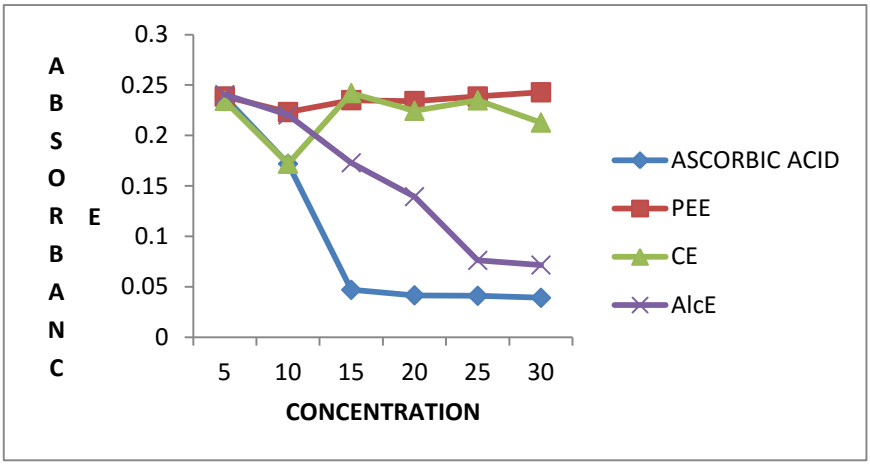


Figure 4: Showing absorbance in different concentration

Table 16: Showing % inhibition of different extract of *Hedychium Coronarium* J. Koenig.

Concentration (µg/ml)	ASCORBIC ACID (% Inhibition)	PEE (% Inhibition)	$CHCl_3$ E (% Inhibition)	AlcE (% Inhibition)
5	2.61%	2.37%	4.13%	1.59%
10	29.66%	8.67%	29.62%	9.81%
15	80.8%	3.84%	0.98%	29.25%
20	83.01%	4.21%	8.14%	43%
25	83.22%	2.37%	3.92%	68.86%
30	84.02%	0.65%	12.92%	70.7%

ISSN: 0975-8585

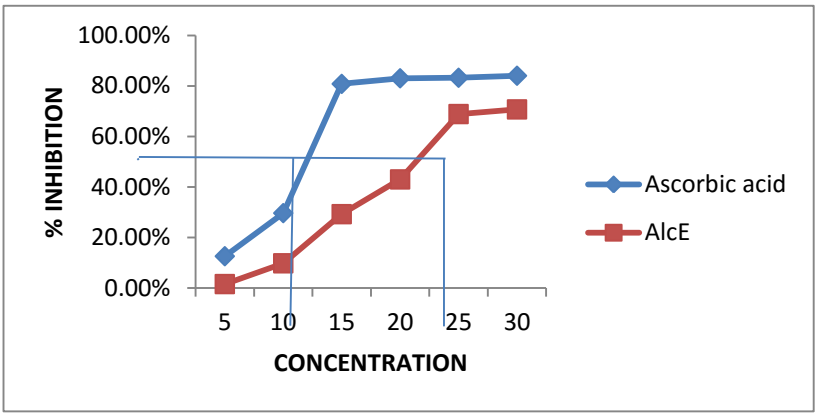
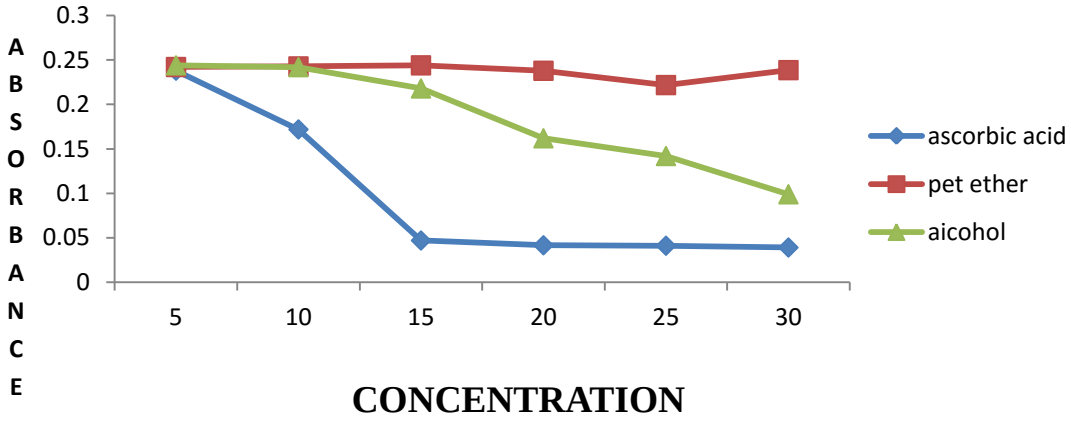


Figure No. 05:- Showing % inhibition in different concentration

Table 17: Absorbance of Different extracts of *Ageratum conyzoides* Linn. with Ascorbic acid
CONTROL : 0.2444

CONCENTRATION (µg/ml)	ASCORBIC ACID (Abs)	PET ETHER (Abs)	AlcE (Abs)
5	0.2380	0.2421	0.2440
10	0.1719	0.2428	0.2420
15	0.0469	0.2440	0.2180
20	0.0415	0.2379	0.1619
25	0.0410	0.2218	0.1420
30	0.0390	0.2386	0.0989

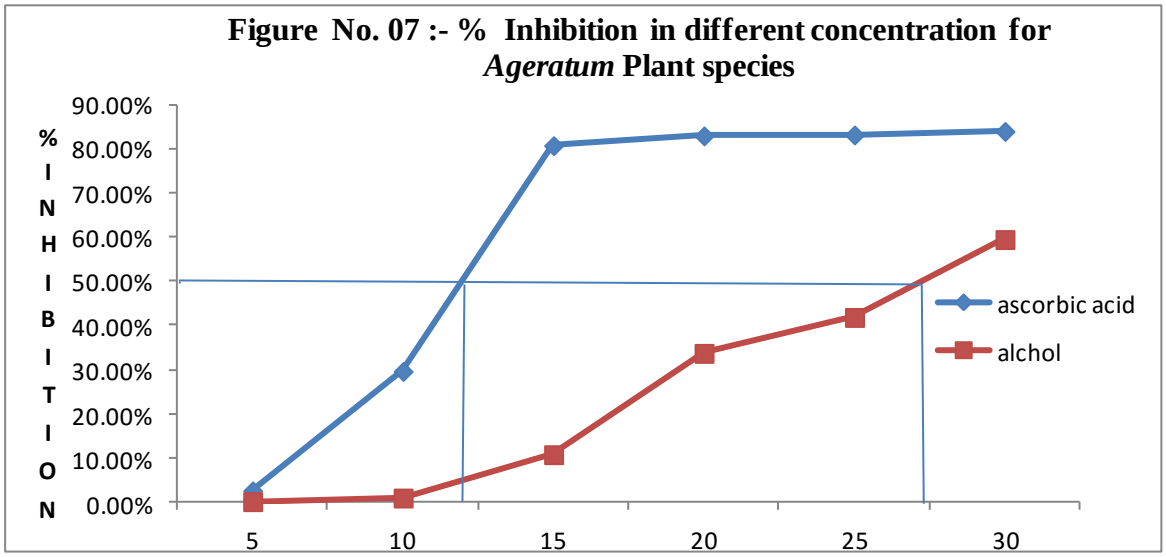
Figure No. 06 : Absorbance in different concentration for *Ageratum* Plant species



ISSN: 0975-8585

Table 18: % inhibition of Different extracts of *Ageratum conyzoides* Linn. with Ascorbic acid.

CONCENTRATION (µg/ml)	ASCORBIC ACID (% Inhibition)	PET. ETHER EXT. (% Inhibition)	ALC. EXT. (% Inhibition)
5	2.61%	0.99%	0.16%
10	29.66%	0.65%	0.98%
15	80.8%	0.16%	10.80%
20	83.01%	2.65%	33.75%
25	83.22%	9.24%	41.89%
30	84.02%	2.37%	59.53%



ACKNOWLEDGEMENTS

The authors are thankful to the Management and Staff members of SGRR Institute of technology & science, Patel nagar, Dehradun specially Dr. Archana Gahtori and Dr. Meenu Choudhary mam for their support and Mahant Shri Devendra Das Ji Maharaj, Chairman, SGRR Education Mission, Dehradun for providing me necessary facilities. We are also thankful to the Management and Staff members of B.N. Institute of Pharmaceutical Sciences, Udaipur (Rajasthan) for their invaluable support and guidance.

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