Analysis of Datura Metel L.

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*ABSTRACT*- Medicinal plants contain some organic compounds which provide definite physiological action on the human body. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro. The Chromatographic, phytochemical investigation and Spectral study of Methanol Soluble Compounds of leaves of *Datura metel* showed that the plant contains terpenoids, saponins, tannins and alkaloids, flavonoides, glycosides and aminoacids. There are different types of carbohydrates were tested through Qualitative and qualitative test. The compounds varying inhibitory effects on *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *A. niger, Trichoderma virde* andconcluded thatplantcould be potentially use as chemotherapeutic agents.

Keywords: HPLC, FTIR, UV, Phytochemicals, Datura metel L. Potentialtoxicity, Rf Values.

# INTRODUCTION

*Datura metel* L. [1, 2] is an annual herb, native of India belongs to Solanaceae plant familyand it is an erect branched undershrub with long white leaves and spiny spherical fruits**.** It grows in the wild in all the warmer parts of the world, and is [cultivated](http://en.wikipedia.org/wiki/Plant_cultivation) worldwide for its chemical and ornamental properties. All parts of plants contain dangerous levels of [tropane alkaloids](http://en.wikipedia.org/wiki/Tropane_alkaloid) (highly poisonous) and may be fatal if ingested by humans or other animals, including livestock and pets.

The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct [3]. Medicinal plants are important for their pharmaceutically valuable secondary metabolites as like alkaloids, amino acids, antibiotics, various enzymes, steroids, flavonoids, terpenoids, glycosides etc. [4]**.** These are also important for their production of coloring agent, perfumes, insecticides and vitamins. Recently, research on the plant derived products has been initiated to evaluate the feasibility of using herbal medicines in disease management [5]. Because of the growing bacterial resistance against commercial standard and reserve antibiotics, the search for new active substances with antibacterial activity against pathogenic bacteria is of increasing importance. In India, 500 medicinal plant species are used to pathogenic bacteria. Plants have been used as traditional medicine since time immemorial to control bacterial [6]**.** Many of the spices and herbs used today have been valued for their antimicrobial effects and medicinal powers in addition their flavor and fragrance qualities [7]**.** Plants are rich in a variety of secondary metabolites many of these medicinal plants contain chemical constituents that could cause harmful effects to human if taken in large quantities. Alkaloids occurring in a large amount make these plants poisonous [8-11].

Earlier workers [12-14] have reported various constituents from this plant. In the present paper, we report the chromatographic separation and identification of compounds by various colour reactions and spectral analysis. The aim of this study is to investigate the bioactive constituents of *Datura metel* found in around G.I.F.S. Aurangabad by chemical reactions, UV-Vis and FTIR Spectral, and HPLC characterization method and study of antimicrobial activity of compounds*.*

# MATERIALS AND METHODS

##### Collection of plant materials

Leaves of *Datura metel* L. were collected from near around Govt. Institute of Forensic Science, Aurangabad in November 2012 and identified as taxonomically. The leaves of the plantwere shade dried and powdered.

**Test microorganisms:-** Pathogenic microorganisms, *viz*., *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *A. niger, Trichoderma virde* were used during the present study and were collected some from Ghati Medical College Aurangabad and some available in the Department of Microbiology and some were isolated from soil sample.

##### Preparation of plant extracts

1. Hot water extraction

50 gm of dried finely powdered leaf of *Datura metel* L. was taken in a beaker and 200ml of distilled water was added. The mixture was heated on a hot plate with continuous stirring at 45-50ºC for 30 minutes and filtered it by using Buchner funnel. Then the water extract was filtered using filter papers and the filtrate was evaporated through vacuum rotator evaporator. The dried component was kept for the phytochemical analysis.

1. Solvent Extraction

Crude plant extract was prepared by Soxhlet extraction method. About 250gm of powdered leaves of the plant was uniformly packed into a thimble and extracted with different solvents as like methanol, ethanol, and acetone and Petroleum ether separately. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 50-60ºC till all the solvent got evaporated. Dried extracts were dissolved and crystallized using vacuum rotatory evaporator in different solvents methanol, ethanol, acetone and chloroform and filtered with Whatmann filter paper no.42, respectively.

After crystallization quantity of Methanol, ethanol and chloroform, soluble fraction was 1.6 gm, 0.4 gm, and 0.2gm obtained. Petroleum ether (40-600C) soluble fraction (0.8) contained mostly oily components. Acetone and n-hexane (1.5 gm) soluble fraction contain chlorophyll components. These crystallized components were kept at 10ºC for their future use in phytochemical analysis.

# STUDY OF METHANOL SOLUBLE FRACTION

The different types of colour reactions were used to perform the qualitative and quantitative analysis [15-23] of methanol soluble fraction.

* Test for carbohydrates

**(i)** **Molisch’s Test for carbohydrates:** In test tube, few drops of Molisch’s reagent (5% α-naphthol in 95% ethanol) were added into a 01 ml methanolic solution of compound and added concentrated H2SO4 from the wall side of the test tube, carefully. The violet color ring appeared at the junction of the two liquids was then observed.

**(ii)** **Iodine Reaction:** 06 mg of methanol soluble compounds added few drops of 0.01M iodine were added. This test was also performed on an unknown carbohydrate.

**(iii)** **Benedict’s test:** In aques solution ofcompounds, in a test tube added Benedict’s reagent on heating orange -red color appeared which indicate presence of the carbohydrates.

**Fehling’s test:** Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added in aqous solution of methanolic compound and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

|  |  |  |  |
| --- | --- | --- | --- |
| S. N. | Phytochemical Identified | Seeds | Leaves |
| 1 | Flavonoids | *+* | *++* |
| 2 | Alkaloids | *++* | *+* |
| 3 | Trepenodes | *+* | *±* |
| 4 | Glycosides | *++* | *+* |
| 5 | Saponins | *++* | *±* |

**(iv)** **Methods forDetermination of glycosides:** 10 mg of methanol soluble compoundswas introduced into two different beakers. To one of the beakers was added Sulphuric acid (1ml) while water (2ml) was added to the other beaker. The two beakers were heated for 02 minutes and the contents filtered into labeled test tubes. The filtrate was made alkaline with sodium hydroxide (0.5ml) and allowed to stand for three minutes. The presence of reddish brown precipitate in the filtrate was taken as positive for glycosides.

* Test for Flavonoids

**(i)** **Shinoda test:** In 08 mg of methanol soluble compoundswas added a small piece of magnesium ribbon, and then added drop wise addition of concentrated HCl. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids. Colors ranging from orange to red indicated flavones, red to crimson indicated flavonols, crimson to magenta indicated flavonones.

**(ii) Alkaline reagent test** In 08 mg of methanol soluble compoundswas mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

* Test for Alkaloids

Methanol soluble of compounds **(10 mg)** was mixed with 2ml of 1% HCl and heated gently on a water bath. The solution obtained was filtered and 1ml of the filtrate was treated with two drop of Mayer’s and Wagner’s reagents were then added to the mixture. The two solutions were mixed and made up to 50ml with distilled water. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

* Test for Saponins

10 mg of methanol soluble compoundswas stirred in a test tube, foaming which persisted on warming was taken as an evidence for the presence of saponins.

* Test for Tannins

10 mg compounds was stirred with 10ml of distilled water and then filtered. To the filtrate was added two drops of 5% Iron (III) Chloride (FeCl3) reagent. Blue–black or blue – green colouration or precipitate was taken as an indication of the presence of tannins.

* Test for proteins

**(i) Millon’s test:** 10 mg of methanolic compound was when mixed with 2ml of Millon’s reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

**(ii) Ninhydrin test:** 10 mg of methanolic compound when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

1. Phytochemical tests of Methanol soluble compoundsof *Datura* *metel* L.

*+ = Positive; ++ = strongly positive; ± = Trace; - = Not detected*

# CHROMATOGRAPHIC ANALYSIS OF METHANOL SOLUBLE FRACTION

1. Thin Layer Chromatography (TLC) Analysis

***TLC analysis of alkaloids:*** each chloroformic residue of the investigated plant at the

two habitats was dissolved in chloroform, then chromatographed on silica gel G TLC

as adsorbent using the following solvent systems.

a- Chloroform : methanol (8:2 v/v).

b- Chloroform : methanol (9:1 v/v).

The developed chromtograms were air-dried and sprayed with Dragendorrf ’ s

reagent, systems c and d gave best separation.

***Preparative TLC:*** preparative TLC silica gel G plate was used for isolation and purification of compounds using system (b). The band corresponding to alkaloidal compound were scraped off and eluted with methanol, the eluted bands were freed from solvent under vacuum; and then it was re-purified by the same manner.

1. Identified Alkaloids by TLC from Leaves *Datura Metel* L.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S. N.** | **Solvent System** | **R*f* value** | | **Component identified** |
| **Reported33** | **Found** |
| **1** | Chloroform: Methanol (7: 3) | **0.65** | **0.66** | β-carboline |
| **2** |  |  |  | hyoscine, |
| **3** | n-Butanol: Acetic acid : water  4:1:5 (v/v/v) |  |  | atropine |
| **4** | Chloroform : methanol (8:2) |  |  |  |
| **5** | 9:1 |  |  |  |

# Investigation of carbohydrates

Chaplin, M.F., Kennedy, J.F., (1994): Carbohydrate Analysis, A practical Approach. *Oxford*

Univ. Press, Oxford, New York, Tokyo. 2nd Ed., pp. 324.

1. Identification of Sugars by TLC from Leaves *Datura Metel* L.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.No.** | **Solvent system** | **R*f* value** | | **Sugar identified** |
| **Reported** | **Found** |
| 1 | *n-*butanol-acetic acid- | 0.28 | 0.26 | D-xylose |
|  | water (4:1:5) | 0.37 | 0.36 | L-rhamnose |
|  |  |  |  |  |
| 2 | Ethylacetate-water- | 0.38 | 0.36 | D-xylose |
|  | pyridine (2:2:1) | 0.48 | 0.47 | L-rhamnose |
|  |  |  |  |  |

**Study of free amino acids:**the free amino acids of *daturametel are* declared presence of aspartic acid, threonine, serine, glutamic acid, proline, methionine, isoleucine, leucine, tyrosine, histidine and arginine in the plants of the two habitats with different ranges of concentration. While glycine and phenyl alanine were present only in the plants

1. Identification of Aminoacids (Protein) by TLC from Leaves *Datura Metel* L.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.No.** | **Solvent system** | **R*f* value** | | **Aminoacids identified** |
| **Reported33** | **Found** |
| 1 | *n-*butanol-acetic acid- |  |  | aspartic acid |
|  | water (4:1:5) |  |  | threonine |
|  |  |  |  | serine |
|  |  |  |  | proline |
|  |  |  |  | methionine |
|  |  |  |  | isoleucine |
|  |  |  |  | tyrosine |
|  |  |  |  | histidine |
|  |  |  |  | arginine |

(ii) High Performance Liquid Chromatography (HPLC) Analysis

Intro……

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1. HPLChromatogram of *Datura Metel L.* Leaves

# UV-Visible spectral study of methanol soluble compounds

the pure material was dissolved in pure methanol then subjected to UV spectrophotometric measurements using Schimadzu UV 240 spectrophotometer. The UV- visible spectra of methanolic compounds of leaves were taken. The UV-visible spectra were performed to identify the compounds containing σ- bonds, π-bonds, and lone pair of electrons, chromophores and aromatic rings. Occurrence of peaks at 234-606 nm reveals the presents of alkaloids in the *Datura metel L*. On comparison of the spectra of leaves with standard [24], shows that these two compounds have some similar alkaloid, flavonoids, and glycosides compounds respectively [25-27]**.**

1. UV Spectrum of Methanolic Compounds of Leaves *Datura Metel* L.

|  |  |  |  |
| --- | --- | --- | --- |
| **S.No.** | **Solvent+(Shift reagent)** | **λmax values (nm)** | |
| Band II | **Band I** |
| 1. | MeOH | 268 | 320 |
| 2. | MeOH+AlCl3 | 278 | 345 |
| 3. | MeOH+AlCl3-HCl | 280 | 383 |
| 4. |  |  |  |

234-606 nm scopolamine and hyoscyamine

# FTIR spectral study of methanol soluble compounds

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of *Datura metel* L.leaves FTIR analysis confirmed the presence of amide, alkynes, alkanes, carboxylic acids, alkenes, aromatics, aliphatic amines and alkyl halides compounds which shows major peaks at 3654.12, 3307.55, 2918.44, 2849.92, 1643.73, 1454.46, 1054.13 and 510.34 respectively (Fig. 1A and B; Table-1).

FTIR-spectra [30-35]of the methanol soluble compounds of leaves of *Datura* *metel* L shows a peak at 3400-3800 cm-1, this is due to the vibration mode of OH groups present in the compounds. Since the stretching (symmetric & anti-symmetric) the OH-groups occurs in this region. We observed that particular peak in seeds and leaves at 3320- 3540 cm-1 revealed the presence of OH- groups in compound. The peaks 2365 cm-1, 2092 cm-1 and 2085 cm-1 confirmed the asymmetric stretching of C-H. The band 1652 cm-1 shows the N-H bend of primary amines. The absorption bands at 1640 cm-1 and 1680 cm-1 revealed the stretching vibration of C=O due to the rise in amide. IR peaks at 405-3432 cm-1 shows the presence of alkaloids. Peaks at 1660- 1750 cm-1 shows the ester group in seeds, peaks at 1642-1713 cm-1 shows the ester group in leaves.

A peak at 1200-1450 cm-1 shows the presence of benzene rings, that peaks is observed in spectra of seeds at 1218-1394 cm-1 and in leaves at 1289-1409 cm-1. Peak at 2000- 3000 cm-1 shows the presence methyl group occurs, that region is observed in spectra of seeds at 2924-2981 cm-1, in leaves at 2090 cm-1. Presence of phenyl group generally ranged between 400-1500 cm-1 in seeds it is observed at 405-1046 cm-1, in leaves at 403-769 cm-1. The FTIR values showed presence of alkanoids, particularly the peak at 1635-1650 cm-1. Study of peaks in spectra of leaves are shows that both have some similar compounds as like alkaloid, flavonoids, glycosides, saponins, terpeniods.

1. FTIR Spectrum of Methanolic Compounds of Leaves *Datura Metel* L.

|  |  |  |
| --- | --- | --- |
| **S. N.** | **Wave Number (cm-1)**  **(Vmax ,KBr)** | **Assigned Functional group** |
| 1 | 3415 cm-1, | OH |
| 2 | 3368 cm-1 | C-H stretch |
| 3 | 1683 cm-1, | C=C aromatic |
| 4 | 1382 cm-1, | CN |
| 5 | 1224 cm-1 | C-O |
| 6 | 1520 | phenyl group |
| 7 | 2090 | methyl group |
| 8 | 1642-1713 cm-1 | ester group |
| 9 | 1216-1402 cm-1 . | benzene rings |
| 10 |  |  |
| 11 |  |  |
| 12 |  |  |

***Identification of flavonol glycosides:*** the flavonol glycosides compounds were characterized as isorhamnetin 3-o-glucopyranoside (1), rutin-4`, 7-dimethyl ether (2) and kaempferol-3,7-dirhaminoside-4`-methyloxide (3). Acid hydrolysis of compounds (1,2) using 2N HCl yielded isorhamntein as aglycone and glucose as sugar moiety for compound (1) while compound (2) yielded quercetin-4`, 7-dimethyl ether and the sugars glucose and rhamnose on the other hand compound (3) yielded kaempferol-4`- methyl ether and sugar rhamnose.

UV spectral date showed absorption maxima in methanol, band I (355, 352 and 350nm) for compounds (1,2,3) indicating that these compound are flavonol with 3-OH substitution, addition of NaOAc indicated the presence of free OH group at C-7 in compound (1) only, while addition of boric acid indicated the absence of 3`,4` dihydroxy groups in the three compounds. Addition of NaOMe cause a bathochromic shift in compound (1) only indicating the presence of free OH at C-4` in this compound (Harborne, 1984; Liu, et al., 1989).

Harborne, J.B., (1984): Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 2nd edn,., *Chapman and Hall, London and New York.*

Harborne, J.B., Mabry, T.J., (1982): The flavonoids: Advances in Research. *Chapman and Hall, London.*

# Antimicrobial Study of methanol soluble compounds

The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in mm). For the antimicrobial study agar diffusion method was used**.** The antimicrobial activity of methanol soluble compounds of seeds and leaves of *Datura metel* was determined by Filter Paper Disc diffusion method. The various bacterial species were first incubated at 45o C for 48 hrs. The sterile filter paper discs (6mm) were soaked with standard antibacterial agent and various test samples and were dried at 50oC. The disc were then placed on soft nutrient agar (2%) petri plates previously seeded with suspension of each bacterial species. The diameter of zone of inhibitions was measured at 37 ± 1oC after 24 hrs. For fungal activity; Saborauds broth media with 4% agar was used for the preparation of plates and incubated with spores and mycelium suspension of fungi obtained from one week old culture. The diameters of zones of inhibition were measures at 28 ± 1oC after 48 hrs. The various results of these test recorded in Table-2 and Table-3[36-39]**.**

1. Antibacterial study of of methanol soluble compoundsof *Datura* *metel* L.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Name of Microrganisms | Diameter of Zone of Inhibition (mm) | | | | | | Standard  100% |
| Seeds compounds | | | Leaves compounds | | |
| 50% | 75% | 100% | 50% | 75% | 100% |
| *P. aeruginosa* | 7.8 | 8.2 | 10 | 6.0 | 8.4 | 10.5 | 15.8 |
| *K.pneumonia* | 6.5 | 7.5 | 8.9 | 7.6 | 8.9 | 9.8 | 16.0 |
| *Escherichia coli* | 7.0 | 10 | 10.5 | 6.9 | 7.0 | 9.8. | 15.5 |

1. Antifungal study of of methanol soluble compoundsof *Datura* *metel* L.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Name of Microorganisms | Diameter of Zone of Inhibition (mm) | | | | | | Standard  100% |
| Seeds compounds | | | Leaves compounds | | |
| 50% | 75% | 100% | 50% | 75% | 100% |
| *A. niger* | 7.0 | 8.5 | 15.5 | 6.8 | 9.5 | 13.0 | 18.5 |
| *Trichoderma virde* | 7.9 | 10.2 | 12.7 | 7.3 | 9.7 | 12.5 | 17.0 |

# Results and Discussion

Environmental factors affect the production of biologically active constituents by plants at different habitats. The higher production of alkaloids were confirmed by using FTIR – spectra and UV–visible spectra and chemical reactions. The phytochemical analysis of the compounds revealed the present of saponins, tannins and alkaloids, glycosides, flavonoids and also tested that tannins, glycosides and akaloids were present and that the methanolic compounds of the plant. Compounds were active against *E. coli, P. aeruginosa K.pneumonia Escherichia coli , Trichoderma virde* and *A. niger etc,* medically important organisms. The result is summarized in table-2 and table-3. It was concluded that an increase in the concentration showed higher activity recorded by the diameter of zone of inhibition. The fact that organisms may need higher concentration of compounds to inhibit growth. Saponins are a special class of glycosides which have soapy characteristics. It has also been shown that saponins are active antifungal agents [40]**.** Classes of alkaloids are among the major powerful poisons known. Apart from being poisonous, some alkaloids have also been proved to be useful in correcting renal disorders [41-43]**,** it therefore, means that the alkaloids may be a poison that can be tried on lower or higher organisms. Therefore this study supports the earlier finding that compounds of the plants used in the present work may be useful in the chemotherapy of mycotic infections.

##### Conclusion

Alkaloids, terpenoids, saponins, tannins and glycosides were identified in methanol soluble compoundsof Seeds and leaves of *Datura* *metel,* whichshowed potential antibacterial activity against *P. aeruginosa K. pneumonia and E. coli.* Possible antimicrobial components contain compounds included alkaloids, saponins, tannins, flavonoids and glycosides. Study suggests that *seeds and leaves* compoundsof the plant could be used as a source of drugs useful in the chemotherapy of some microbial infections. Chromatographic investigation of bioactive constituents of plant revealed the presence of flavonol, flavanone, flavone and isoflavone glycosides beside the presence of alkaloid compounds, fatty acids, sterols, amino acids and combined sugars which possesses medicinal and physiological activity.

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##### References

1. Mundt, S., S. Kreitlow and R. Jansen. Fatty acids with antibacterial activity from the Cyanobacteria *Oscillatoria redekei* HUB 051. *Journal of Applied Phycology*, 15: 263-276, 2003.
2. Cousins D, *Int Zoo Yearbook*, 40, 341-350, 2006.
3. Parekh, J., D. Jadeja and S. Chanda. Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. *Turkey Journal of Biology*, 29: 203-210, 2005
4. Kuganathan, N. and Ganeshalingam, S., Chemical Analysis of *Datura Metel,* Leaves and Investigation of the Acute, Toxicity on Grasshoppers and Red Ants, E-Journal of Chemistry, 2011, 8(1),107-112.
5. Abutbul, S., A. Golan-Goldhirsh, O. Barazani, R. Ofir and D. Zilberg. Screening of desert plants for use against bacterial pathogens in fish. *Isr. J. Aquacult. Bamidgeh*, 57: 71-80, 2005.
6. Bhuvaneswari, R. and R. Balasundaram. Traditional Indian herbal extracts used *in vitro* against growth of thepathogenic bacteria - *Aeromonas hydrophila*. *Isr. J. Aquacult. Bamidgeh*, 58(2): 89-96, 2006.
7. De, A.K. Spices: Traditional Uses and Medicinal Properties. Daryaganj: Asian Books Pvt. Ltd., 7-8, 2004.
8. Grisez, L. and F. Ollevier. *Vibrio* (Listonella) *anguillarum* infection in marine fish larviculture. In: P. Lavens, E. Jaspers and I. Roelande, Eds, 91- Fish and Crustacean Larviculture Symposium, European Aquaculture Society, Gent, Special Publication, 24: 497, 1995.
9. Service, R.F. Antibiotics that resist resistance. Science, 270: 724-727, 1995.
10. Doaigey A R, *Am J Bot*., 78, 1608-1616, 1991.
11. Creasy R, The edible flower garden. Periplus Editions (HK) Ltd., Singapore, 1999.
12. S.Siva Sakthi, P.Saranraj\* and M.Geetha., Antibacterial Evaluation and Phytochemical Screening of *Datura metel* Leaf Extracts against Bacterial Pathogens, International Journal of Pharmaceutical & Biological Archives 2011; 2(4):1130-1136. *ISSN 0976 – 3333*.
13. YANG Bing-You, XIA Yong-Gang, CHEN Dong, KUANG Hai-Xue chemical constituents from *Datura metel L, Chinese Journal of Natural Medicines* 2010, **8**(6): 429−432.
14. Navaratna Rajah Kuganathan#\* and Sashikesh Ganeshalingam, Chemical Analysis of *Datura Metel* Leaves and Investigation of the Acute Toxicity on Grasshoppers and Red Ants, E-Journal of Chemistry, 2011, 8(1), 107-112, ISSN: 0973-4945.
15. Ravi Kumar R., P. Krishnamoorthy, the callus infection and isolation of alkaloid from Datura metel L. International Journal Of Pharmacy&Technology (IJPT), Dec-Vol. 2 Issue No.4, 945-959, 2010.
16. Jain, S.K., Medicinal plants.National book trusts, New Delhi., 29-30, 1968.
17. Jaggi, R.K. ,Cardre, J. S. and Kapoor, V.K., Tissue culture techniques in the production of the plants of the family Solanaceae, *Indian Drugs,* 27, 270-280, 1989.
18. Chessbrough, M. Medical laboratory manual for Tropical countries, Linacre House, Jordan Hill, Oxford, 2000.
19. Johnson, C.R., Miller, M.J., Pasto, D.J. (1998). Experiments and Techniques in Biochemistry. United States of America: Prentice Hall Inc.
20. Bettelheim, F. A. and March, J. (1998). Organic and Biochemistry. 3rd ed. New York: Harcout Brace College Publishers.
21. Evans, W.C. Trease and Evan’s Pharmacognosy. 5thedition, Haarcourt Brace and Company, 2002: 336.
22. Siddiqui, A.A., and M. Ali. Practical pharmaceutical chemistry. First edition, CBS Publishers and distributors, New Delhi, 1997: 126-131.
23. Iyengar, M.A. Study of drugs. 8thedition, Manipal Power Press, Manipal, India, 1995: 2.
24. Shinoda J., Color reactions of flavone and flavonol derivatives and the like., *J. Pharm. Soc. Jpn.* (1928) 48, 214-220.
25. Jasper C., Maruzzella J. C. and Henry P. A, **The antimicrobial activity of perfume oils,** *J. Am Pharm. Asso.,* 47,471-476, 1958.
26. Vincent J. C. and Vincent H. W., Annual Review of Medicine, *Proc. Soc. Exp. Biol. Med.*, 55, 55-62, 1944
27. Sofowora, A., 1993. Medicinal Plants and Traditional Medicine in Afric. John Wiley and son Ltd., 150-153.
28. Harborne J. B., 1965, Plant Polyphenols: Characterisation of Flavonoid Glycosides by acidic and enzymic hydrolysis, *Phytochem.,* 4, 107-120
29. Suzuki,T., Yoshioka, T., Tabata, M., and Fujita, Y.,(1987).Potential Datura innoxa cell suspension cultures for glycosylating quinine. Plant cell Rep,6:275-278.
30. Zehra, M., Banerjee, S., Sharma, S. and kumar, S., (1999). Planta Medica. 65: 62-63.
31. Jaggi, R.K.,Cardre, J.S. and Kapoor, V.K., (1989). Tissue culture techniques in the production of the plants of the family Solanaceae. Indian Drugs. 27: 270-280.
32. Silverstein M., Identification of Organic Compounds, by spectroscopic methods, 2005.
33. Bhat S.V., Nagasampagi B.A., Shivkumar M., Chemistry of Natural Products, Narosa, publishing house, New Delhi, fourth reprint, 2008, 237-316.
34. Ganapathi, G., and F. Kargi,(1990). Recent advances in indole alkaloid production by Catharanthus rose’s (periwinkle). J.Exp.Bot.41:259-267.
35. Flick, C.E., D.A.Evans and W.R.Sharp(1983) In:Handbook of plant cell culture (eds.) Evans, D.A., W.R . Sharp, P.V. Ammirato and Y. Yamada,Collier Macmillan Publishers, London, 1:13-18
36. Trease, G.E. and W.C. Evans, 1989. Pharmacognosy 11th Edn. Brailliar Tiridel and Macmillian Publishers London.
37. Herborne, J.B., Phytochemical Methods 3rd Edn. Chapman and Hall Ltd., London, pp: 135-203, 1973.
38. P.A. Egwaikhide and C.E. GimbaAnalysis of the Phytochemical Content and Anti-microbial Activity of *Plectranthus glandulosis* Whole Plant,Middle-East Journal of Scientific Research 2 (3-4): 135-138, 2007 ISSN 1990-9233, © IDOSI Publications, 2007.
39. Parekh, J., D. Jadeja and S. Chanda. Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. *Turkey Journal of Biology*, 29: 203-210, 2005.
40. Rathish R. Nair and V. Sumitra Chandra. *Pucinia granatum* - A potential source as antibacterial drug. *Asian journal of Microbiology, Biotechnology and Environmental Science*, 7: 625 – 628, 2005.
41. Agrawal, P., V. Rai and R.B. Singh. Randomized, placebo controlled single-blind trial of holy basil leaves in patients with noninsulin-dependent Diabetes mellitus. *International Journal of Clinical Pharmacology and Therapeutics*, 34:406-409, 1996.
42. Sundaram Ravikumar, Gopi Palani Selvam and Anitha Anandha Gracelin. Antimicrobial activity of medicinal plants along Kanyakumari Cost, Tamil nadu, India. *African Journal of Basic and Applied Sciences*, 2 (6): 153-157, 2010.
43. Parekh, J., D. Jadeja and S. Chanda. Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. *Turkey Journal of Biology*, 29: 203-210, 2005.