International Journal of Chemical and Physical Sciences, ISSN:2319-6602
IJCPS Vol. 4 Special Issue – NCSC Jan-2015
www.ijcps.org

Isolation and Identification of Quercetin and Rutin from Leaves of Tridax Procumbens Linn by HPLC Analysis

P.S PANDE and M.N MISHRA

Department of Chemistry, Shankarlal Khandelwal Arts, Science and Commerce College, Akola 444 002, (M.S.) India

Abstract

Tridax procunbens (Linn) is an important medicinal plant belonging to the family Asteraceae. Number of chemical compounds of plant have been isolated and identified, these belongs to some major classes of substances namely alkaloids, flavonoids and essential oils. Quercetin and Rutin , the major flavonoids from the leaves of Tridax procumpens have been isolated and identified with the help of HPLC fingerprint analysis technique.

Keywords: Quercetin, Rutin, HPLC.

Introduction:

Tridax procumbens Linn is a weed having medicinal value.[1-2] In Ayurveda, the plant has extensively used as a medicine for number of diseases such as diabetic and liver disordered.[3-4] The plant has also been reported for various pharmacological activities such as anti-inflammatory, immunomodulatory, wound healing and antioxidant activity. [5-9] the plant has also effective against Gram positive and Gram-negative bacteria.[10] plant is reported to have a number of chemical constituent like alkaloid, tannins, flavonoids like luteolin, keampherol,saponins, carotenoids, and various acids like fumaric, lauric, myristic, palmitic, stearic, arachidic, benenic, palmitoloic, linoleic acid etc. [11]

Materials and Methods:

Chemicals : Quercetin, Rutin and all other chemicals and solvents were obtained from SD Fine chemicals (India).

Plant Material: The leaves of plant Tridax procumbens were collected from local market of hilly area near Melghat region of Maharashtra and identified.

Experimental:

1) Isolation of Rutin fraction : The leaves of *Tridax procumbens* L. were collected and washed with tape water then shade dried and after complete drying, coarse powder was prepared. Twenty grams of the powder was extracted by soxhlet apparatus with 250 ml of 80% ethanol till exhaustion. The extract was filtered and concentrated by evaporation under vacuum to about 10 ml then mixed with 25 ml distilled water and extracted with petroleum ether (50 x 3), then with chloroform (50 x 3).After extraction, the

WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 5.210
Volume 5, Issue 2, 461-466 Research Article ISSN 2278 – 4357

EVALUATION OF QULITATIVE AND QUANTITATIVE ANTIOXIDANT ACTIVITY OF DIFFERENT SOLVENT EXTRACTS ISOLATED FROM THE LEAVES OF TRIDAX PROCUMBENS

P. S. Pande*, V. D. Mane and M. N. Mishra

Shankarlal Khandelwal Arts, Science and Commerce College, Akola 444 002 (M.S), India.

Article Received on
26 Nov 2015,

Revised on 17 Dec 2015,
Accepted on 08 Jan 2016

***Correspondence for Author**
P. S. Pande
Shankarlal Khandelwal Arts, Science and Commerce College, Akola 444 002 (M.S), India.

ABSTRACT

Four different solvent extracts have been isolated from the leaves of tridax procumbens. The qualitative and quantitative evaluation of antioxidant activity was elucidated. All fractions showed moderate to good antioxidant activity assay for DPPH. IC_{50} values of four fractions have also been reported in the study.

KEYWORDS: Tridax procumbens, Antioxidant activity and IC_{50}.

INTRODUCTION

Nature is a source of medicinal plants and the number of modern drugs have been isolated from this natural resources. Medicinal plants have antioxidants components which have been used traditionally to terminate oxidation in food. This also minimize free radicals and stops the oxidation chain reactions this phenomena gives an answer to environmental and physiological stress, premature aging, atherosclerosis and cancer.[1] Tridax procumbens is known for several potential therapeutic activities like antiviral, anti oxidant, antibiotic efficacies, wound healing activity, insecticidal and anti-inflammatory activity. Some reports from tribal areas in India state that the leaf juice can be used to cure fresh wounds, to stop bleeding and as a hair tonic. Tridax procumbens is traditionally used in the treatment of fever, typhoid fever, cough, asthma, epilepsy and diarrrhoea.[2] The phytochemical screening revealed that the plant have presence of alkaloids, carotenoids, flavonoids (catechins and flavones) tannins, carotenoids and saponins. The proximate profile shows that the plant is rich in sodium, potassium and calcium.[3] Leaf of Tridax is reported to have croud proteins 26%,crude fiber 17% soluble carbohydrates 39% calcium oxide 5%, Luteolin, glucoluteolin, quercetin and isoquercetin.Whereas the fumaric acid, sitosterol and tannin has also been reported in the plant.[4] Oleanolic acid was obtained

in good amounts from Tridax and found to be a potential antidiabetic agent when tested against a glucosidase.[5] The literature survey reveals that Tridax procumbens plant posses good antioxidant activity.[6] keeping this medicinal value of Tridax in view, the present study is aimed to investigate the quantitative antioxidant activity of various leaf extracts.

MATERIALS AND METHODS

Sample leaves of tridax procumbens were obtained from local market of Melghat region of Maharastra and identified.

Extraction: leaves of Tridax procumbens were collected and washed with water and then shade dried and converted in to powder.

10 gm powder was taken in 25 ml of four different solvent each (Hexane, Diethyl ether, Dichloromethane, Dioxane) and filtered. the filtrate so obtain was used for qualitative and quantitative antioxidant evaluation.

Study of qualitative antioxidant activity by DPPH

After filtration the filtrate so obtained was taken into four different test tubes then one drop of each extract was taken on TLC plate and tested for antioxidant activity using DPPH reagent. The extract showed prominent bleaching of purple color of DPPH indicating presences of antioxidants.

Study of quantitative antioxidant activity by DPPH

The antioxidant activity of the four different extract was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The diluted working solutions of the test extracts were prepared in four different solvent. A solution of 0.002% of DPPH was prepared in same four solvent separately and 1 ml of this solution was mixed with 1 ml of sample solution. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV visible spectrophotometer. A mixture of solvent (pure 1 ml) with DPPH solution (0.002%, 1 ml) was used as blank for each solvent. The optical density was recorded and % inhibition was calculated using the formula given below

$$\text{Percent (\%)inhibition of DPPH (\%AA)} = \frac{A - B}{A} \times 100$$

RESULTS AND DISCUSSION

Sample preparation :-Eleven different solution of each extract were prepared having 1 mg/ml,1.1mg/ml,1.2mg/ml, to 2.0 mg/ml.1ml of each of this solution was mixed with 1ml of

0.002mg/ml DPPH solution and resulting solution was used as sample. Optical density of the sample was recorded by U. V. Visible spectrophotometer and the results obtained are reported in following tables.IC_{50} values have been determined for each extract.

Table: 1 Optical density and percent antioxidant activity of Hexane extract

Conc.mg/ml	1mg/ml	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0
O.D of sample	0.253	0.250	0.246	0.244	0.242	0.239	0.236	0.235	0.234	0.233	0.231
% AA	31.06	31.88	32.97	33.51	34.05	34.87	35.69	35.96	36.23	36.51	37.05

O.D. of blank DPPH=0.367

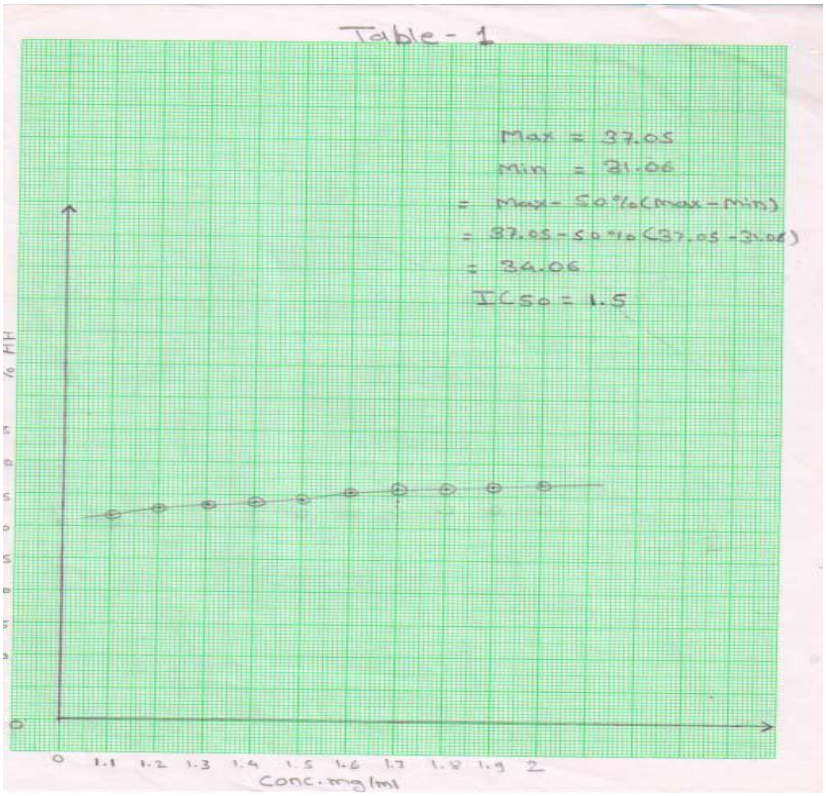


Table 2: Optical density and percent antioxidant activity of Dichloromethane extract

Conc.mg/ml	1mg/ml	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0
O.D of sample	0.242	0.236	0.230	0.228	0.228	0.224	0.219	0.212	0.207	0.201	0.192
% AA	37.14	38.70	40.25	40.51	40.51	41.81	43.11	44.93	46.23	47.79	50.12

O.D. of blank DPPH=0.385

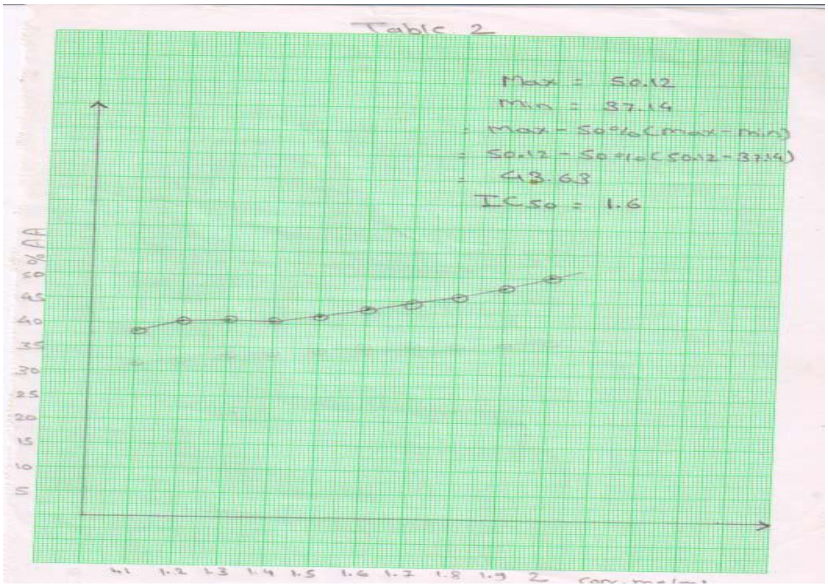


Table 3 Optical density and percent antioxidant activity of Dioxane extract

Conc.mg/ml	1mg/ml	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0
O.D of sample	0.169	0.160	0.159	0.150	0.150	0.149	0.147	0.140	0.135	0.135	0.130
% AA	47.51	50.31	50.62	53.41	53.41	53.72	54.34	56.52	58.07	58.07	59.62

O.D. of blank DPPH=0.322

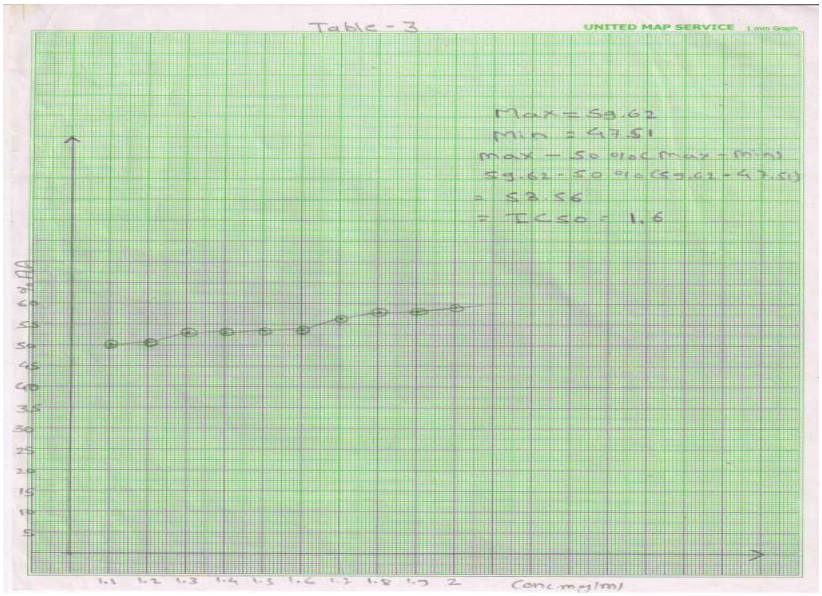
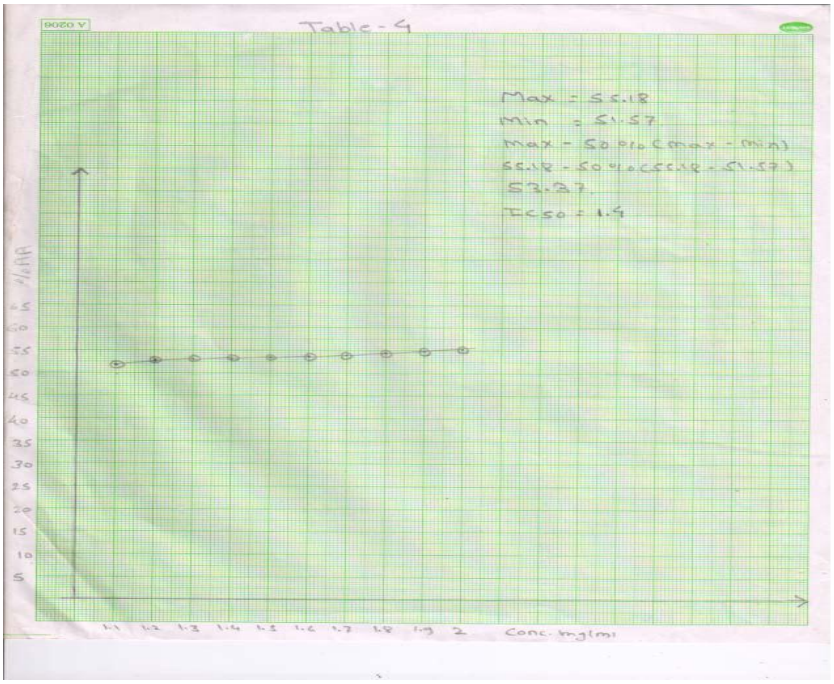


Table 4: Optical density and percent antioxidant activity of Diethyl ether extract

Conc.mg/ml	1mg/ml	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0
O.D of sample	0.215	0.213	0.210	0.209	0.208	0.206	0.205	0.203	0.201	0.200	0.199
% AA	51.57	52.02	52.70	52.92	53.15	53.60	53.82	54.27	54.72	54.95	55.18

O.D. of blank DPPH=0.444



Extract	IC_{50} value
Hexane	1.5mg/ml
D.C.M	1.6mg/ml
Dioxane	1.6mg/ml
Diethyl Ether	1.4mg/ml

CONCLUSION

Remarkable decrease in the O.D. values of sample for four different solvent extract were observed indicating antioxidant activity of the fractions. Four different solvent fractions viz Hexane, Dichloromethane, Dioxane ,Diethyl ether extracted from the leaves of tridax procumbens showed good to moderate antioxidant activity which is evident from the graph. The IC50 values for four different solvent fractions is as below.

ACKNOWLEDGEMENT

Authors are thankful to the Management and Principal of Shankarlal Khandelwal College, Akola for providing necessary facilities. Authors are also thankful to University Grants Commission for providing the financial support under major research project. Thanks are also due to Dr. S. P. Rothe, Department of Botany, Shri Shivaji College, Akola for identification of plant material**.**

REFERENCES

1. Sulekha Mandal, Satish Yadav, Sunita Yadav. Anti-oxidants. A Review. Journal of chemical and pharmaceutical research, 2009; 1: 102-104.

2. Nino J, Navaez DM, Mosquera OM, Correa YM., Antibacterial, antifungal and cytotoxic avtivities of eight Asteraceae and two Rubiaceae plants from Colombian biodiversity. Brazilian J Microbiol, 2006; 37: 566-570.
3. C. Ikewuchi Jude, C. Ikewuchi Catherine and M.Igboh Ngozi. Chemical Profile of Tridax procumbens Linn. Pakistan Journal of Nutrition, 2009; 8(5): 548-550.
4. R. K. Verma and M. M. Gupta. Lipid constituents of Tridax procumbens. Phytochemistry, 1988; 27(2): 459-163.
5. Muhammad Shaiq Ali, Muhammad Jahangir, Syed Shazad ul Hussan, Muhammad Iqbal Choudhary. Inhibition of a-glucosidase by oleanolic acid and its synthetic derivatives. Phytochemistry, 2002; 60: 295–299.
6. Nia, R.; Paper, D.H.; Essien, E.E.; Oladimeji, O.H.; Iyadi,K.C.; Franz, G: Investigations into in vitro radical scavenging and in-vivo anti-inflammatory potential of Tridax procumbens. Nig. J. of Physiological sciences, 2003; 18(1-2): 39-43.

International Journal of PharmTech Research
CODEN (USA: IJPRIF ISSN : 0974 -4304
Vol.5, No.4, pp 1640-1644, Oct-Dec 2013

Antioxidant Activity of Phytoconstituents Isolated from Leaves of Tridax procumbens

Prasanna S Pande[1]*, Vivek D Mane, Mayur N Mishra and S P Rothe[2]

**[1]Department of Chemistry,
Shankarlal Khandelwal Arts, Science and Commerce College, Akola 444002,India.**

[2] Department of Botany, Shri Shivaji College, Akola, India.

***Corres.author: pande.prasanna@rediffmail.com**

Abstract : Tridax is Mexican, Central American and south American genus of flowering plant with its greatest concentration of Species in Mexico[1]. *Tridax procumbens* is one of the species which is widely distributed in India. Literature survey beyond doubt proved the medicinal utility of the species Tridax procumbenes. In the present study, non polar flavanoids and saponins are isolated from leaves of tridax procumbenes. Antioxidant activity of each isolated fraction was tested by spraying the TLC chromatogram of with 2,2 diphenyl-1-picryl hydrazyl (DPPH .
Key words :*Tridax procumbens*, , non polar flavanoids, saponin, antioxidant.

Introduction:

India has a rich heritage of the phytochemical analysis and herbal formulations and their curative effects on various ailments in Ayurveda. In India, Ayurvedic medicines have been using many herbs such as turmeric from ancient time . The *Charaka Samhita* describes the utility of medicinal plants in details. Phytochemical study, especially Study on antioxidants has been attracting the world wide attention. Hence it is significant to identify the plants from nature for their medicinal use. *Tridax procumbens* is a weed found widely in India. The species has been extensively documented in the literature for its variety of medicinal properties. The leaves are reported to be employed in dysentery, and diarrhoea, and for restoring hair the leaf juice possesses antiseptic, insecticidal and parasiticidal properties.

The leaf extract of *Tridax procumbens* has been used since ancient times for healing of wounds. *Tridax procumbens* is known for several potential therapeutic activity, wound healing activity is also one of them[2]. This effect of this plant reported to promote wound healing in both normal and immune-compromised(steroid treated) rats in dead space wound model. The plant increased not only lysyl oxidase but also, protin and nuclic acid contain in the granulation tissue, due to a result of increase in glycosamine glycan content[3] .Traditionally in India, the fresh juice of *Tridax procumbens* leaves have been used since a long time as remedy for dermal wounds.[4 i]

Evaluation of wound healing property of *Tridax procumbens* in wister rats has been reported[5]. The wound healing potential of tropical formulation of leaf juice of *Tridax procumbens* in mice has been reported[6].

The wound healing activity of the ointment formulation having extract of *Tridax procumbens* has been evaluated experimentally upon wound in albino rats [7]and it was found that treated wound showed the faster rate of wound contraction in albino rats.The pharmacological screening of ethanolic extract of *Tridax procumbens* had been carried out on the paremeters like wound healing activity and leucocytes count. The extract showed significant increase in wound healing activity[8]

The extract of leaves of Tridax have been reported to exhibit the decrease in glucose level in the blood in model of alloxan induced diabetis in rat[9] Evaluation of hypoglycemic and anti hyperglycemic activity of *Tridax procumbenes* Linn has been documented [10]. Oral administration of acute and sub chronic of *Tridax procumbens* extract showed a significant reduction in fasting blood glucose level in diabetic rat. The extact of *Tridax procumbens* has been reported to produced a significant hypoglycemic effect in rat[11]. Further studies has been reported to show the solvent fraction containing non polar substances would be among the active principle for lowering blood sugar level. Pharmacognostical and pharmacological investigation on leaves of *Tridax procumbens* has been carried out[12] Owing to these diverse biological activities it was worth experimenting to isolate different phytoconstituents from leaves of tridax procumbenes and to study their antioxidant activity.

Material and Method

The leaves of the plant tridax procumbenes were collected from local market of the hilly area nearby Melghat region of Maharashtra and identified. The reagent DPPH 2,2 diphenyl -1-picrylhydrazyl Sig ma Aldrich was used for antioxidant assay.

A. Extraction of Non Polar flavanoids[18]

10 g powered and dried leaves taken as sample-

a The Sample was extracted with n-hexane in soxhlet apparatus. The extract was collected and evaporated.

b) The same sample was extracted with chloroform. The extract was collected and evaporated to dryness.

c Same sample was extracted with methanol, the extract was collected and evaporated to dryness.

The residue a, b, c were dried and collected and used for antioxidant assay.

B. Extraction of saponin[18]

10 g dried leaves were added to 100 ml 20% ethanol. It was heated in water bath for 4 hours with continuous stirring at about 55^0C then it was filtered and reextracted the residue over water bath at 90^0C and transferred to 250 ml separating funnel and added 20 ml diethyl ether and shacked vigorously. Aqueous layer was recovered and 60 ml n-butanol was added and combined extract washed twice with 10ml of 5% aqueous sodium chloride evaporated in water bath up to dryness. The residue so obtained was collected and used for antioxidant study.

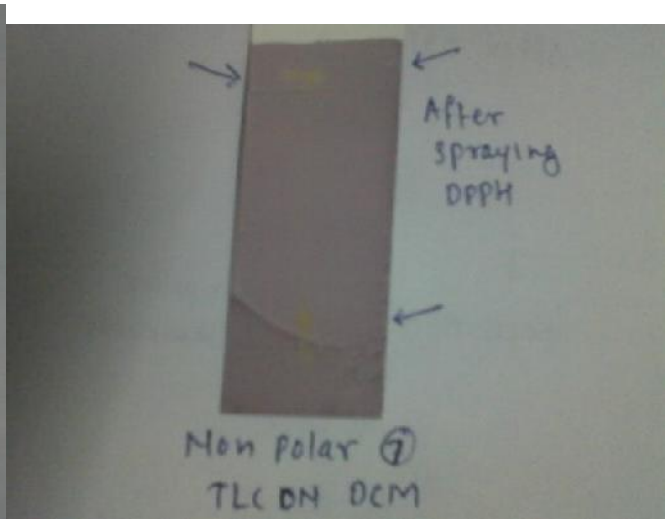
C. Extraction using solvents of different polarity

10 g powered and dried leaves were subjected to soxhlet extraction separately using solvents of different polarity as water, ethanol, dichloromethane and diethyl ether. The extracts were collected and tested for antioxidant activity using DPPH reagent. The ethanol extract showed prominent bleaching of purple color of DPPH indicating antioxidants.

Results and discussion

Antioxidant activity of non polar flavanoids : TLC chromatograph of non polar flavanoids plates 1A, 1B, 2A and 2B were developed using dichloromethane solvent. The TLC chromatograph was sprayed with DPPH 2, 2 diphenyl 1 -picrylhydrazyl reagent. The spots indicated by arrow bleached the purple color of DPPH indicating presence of antioxidants.

In the TLC chromatograph 1B, three spots bleached the color of DPPH and in 2B also three spots bleached the color of DPPH

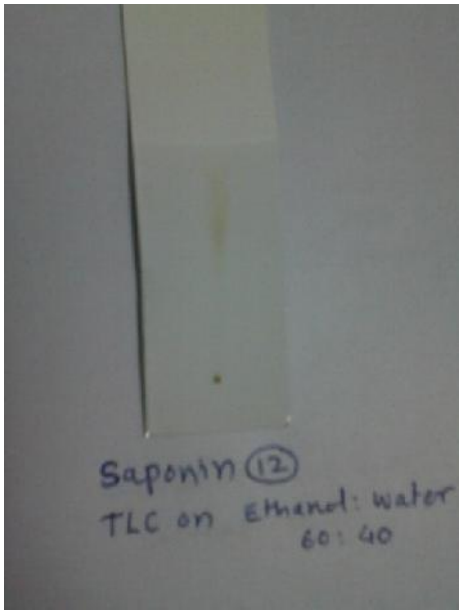


TLC on DCM plate 1A After DPPH spraying plate 1 B

TLC on DCM plate 2A After DPPH spraying plate 2 B

Antioxidant activity of Saponins

TLC chromatograph of saponins plate 3A and 3B was developed using ethanol : water mixture in 6:4 proportion. The TLC chromatograph was sprayed with DPPH 2, 2 diphenyl 1 -picrylhydrazyl reagent. The spot indicated by arrow bleached the purple color of DPPH up to considerable extent indicating presence of antioxidants.



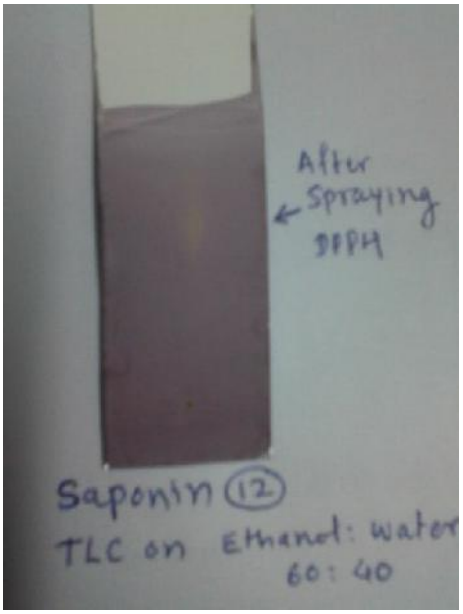


Plate 3A Plate 3B

Antioxidant activity of different solvent extracts

The four extracts viz water, ethanol dichloromethane and diethyl ether extracts were tested for antioxidant activity (plates 4A and 4B .

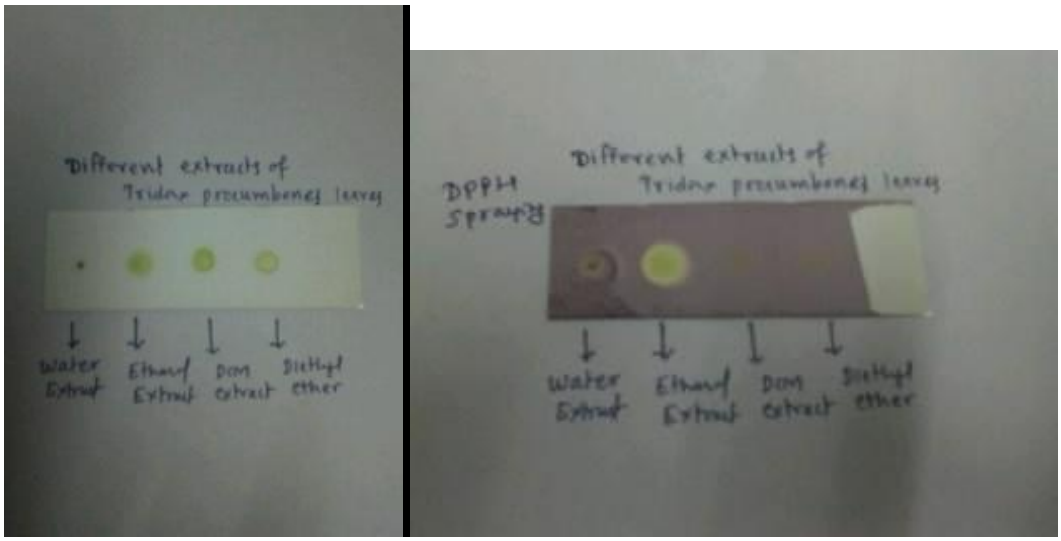


Plate 4A Plate 4B

Ethanol and water extract bleached the color of DPPH

Conclusion:

The ethanol extract of the tridax procumbenes showed highest amount of antioxidants as it bleached the color of DPPH to a maximum extent. Water extract also showed presence of antioxidants. Saponin fraction has also bleached the color of DPPH up to considerable extent which indicated the presence of antioxidants. Non polar flavanoids showed antioxidants in traces.

Acknowledgement

Authors are thankful to the Principal and Management of Shankarlal Khandelwal College, Akola for providing necessary facilities. Authors are also thankful to University Grants Commission for providing financial support.

References

1. Brittonia, 17, 47-96, Jan 1965.
2. Susecla L, Sarsuathy A, Brindha P, Journal of phytological research 2002, 15 (2) , 141-147.
3. Udupa,SL;Udupa,AL;Kulkarni,DR1998 .Fitoterapia,69;507 -510.
4. Vpadhyay B, Praween,Dhakar A K ,Indian .J. Ethynopharmacol.2010;129:64-86.
5. Yogesh P Talekar ,et al Asian Journal of Pharma and Clinical Research54),2012,141 -145.
6. B.Yaduvanshi,Rajani Mathur, T.Velpandian ,Indian j.Pharma sci.2011 May-Jun,73(3):303-306.
7. Shikha Srivastava and Nidhi Mishra.Der pharma Lettre 2009,1(1);157-161.
8. Johnson D. Bento, Gorle Appalaraju, Research J of Pharmacy and technology, 2012, 5(2, 239 -242
9. D.A.Bhagwat,S.G.Killedar,R.S.Adnaik Intnl.J.Green pharma 2008,2,126-128.
10. Hemant Pareek,Sameer Sharma.et al.BMC Complementary and Alternative med.2009,9,48.
11. Kalaya Anulukanapakorn, Orasa Pancharoen,Vraiwan Permpipat. Thai.J.Pharm. Sci. 1997, vol.21, No.4 , pp211-221.
12. Sangeeta Kumara et al.IJPSR,2013 vol.4(2,792 -795.
13. Surendra.S.Agrawal,Gokul.S.Talele Sanjay.J.Surana.Jounral of Pharmacy Reasearch,2009,21 -71.
14. J.D.Habila,J.A.Bello,A.A.Dzikwi,H.Musa and N.Abubakar.African J.Pharmacy and Pharmacology 2010,vol.4(3,pp123 -126.
15. Sanjay M.Jachak, Raju Guatamet al.Fitoterapia Mar.2011,vol.82 issue 2 pp173-177.
16. A.Manjamalai and V.M.Berlin Grace.Int.J.Pharmacutical Analysis,ISSN 2051-2740 vol.37 Issue 10.pp261-271.
17. B.Sailaja, K.Bharti and K.V.R.G Prasad Indian .J. Of Natural Products and Resources.vol.3 4)2012 pp.535-540.
18. Sheetal B Kale and R N Gacche J. Microb. World 9 2), 2007, pp 229 -232.

*****

INTERNATIONAL JOURNAL
OF PHARMA AND BIO SCIENCES
IJPBS

Internationally indexed journal

Indexed in Chemical Abstract Services (USA), Index coppernicus, Ulrichs Directory of Periodicals, Google scholar, CABI ,DOAJ , PSOAR, EBSCO , Open J gate , Proquest , SCOPUS , EMBASE ,etc.

Rapid and Easy Publishing

The "International Journal of Pharma and Bio Sciences" (IJPBS) is an international journal in English published quarterly. The aim of IJPBS is to publish peer reviewed research and review articles rapidly without delay in the developing field of pharmaceutical and biological sciences

Pharmaceutical Sciences

- Pharmaceutics
- Novel drug delivery system
- Nanotechnology
- Pharmacology
- Pharmacognosy
- Analytical chemistry
- Pharmacy practice
- Pharmacogenomics
- Polymer sciences
- Biomaterial sciences
- Medicinal chemistry
- Natural chemistry
- Biotechnology
- Pharmacoinformatics
- Biopharmaceutics

Biological Sciences

- Biochemistry
- Biotechnology
- Bioinformatics
- Cell biology
- Microbiology
- Molecular biology
- Neurobiology
- Cytology
- Pathology
- Immunobiology

Indexed in Elsevier Bibliographic Database (Scopus and EMBASE)
SCImago Journal Rank 0.129
Impact factor 0.67*

*Instruction to Authors visit **www.ijpbs.net**

For any Queries, visit "contact" of www.ijpbs.net

Int J Pharm Bio Sci 2014 Jan; 5(1): (P) 396 - 400

Research Article **Medicinal Chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EVALUATION OF ANTIOXIDANT ACTIVITY OF SAPONIN AND TANNIN FRACTIONS ISOLATED FROM THE LEAVES OF *TRIDAX PROCUMBENS*

P. S. PANDE*, V. D. MANE AND M. N. MISHRA

Shankarlal Khandelwal Arts, Science and Commerce College, Akola 444 002 (M.S), India

ABSTRACT

Saponin and tannin fractions have been isolated from the leaves of *tridax procumbens.* The quantitative evaluation of antioxidant activity was elucidated. Both fractions showed moderate to good antioxidant activity assay for DPPH. IC_{50} values of both fractions have also been reported in the study.

KEYWORDS: Saponnin, Tannin, Antioxidant activity and IC_{50}

P. S. PANDE
Shankarlal Khandelwal Arts, Science and Commerce College, Akola 444 002 (M.S), India

INTRODUCTION

Antioxidants are the substances that inhibit the action of free radicals. Free radical and reactive oxygen species (ROS) play important role in many pathological conditions such as cancer, arthritis, cardiovascular diseases and liver diseases hence much attention is paid to it in recent years[1,2,3] . The products from plants have been used in different system of medicines viz. Unani, Ayurveda and Homeopathy. Charak Samhita and Sushrut Samhita include 700 plants as sources of drugs. A study of *Tridax procumbens* with special references to wound healing properties has a wide scope for searching various chemicals ingredients with high therapeutic activity. Some poly hydroxy flavones have been proved to have a high grade of antioxidant activity. Many researchers have published reviews on antioxidant activity of plant origin. *Tridax procumbens* has been extensively documented in the literature for its pharmacological activity. The ethanolic extracts of *tridax procumbens* has been reported to possesses immunomodulatory activity.[4] Leaves of *tridax procumbens* have been documented to be useful for dysentery, diarrhoea and preventing hair fall[5,6,7]. The whole plant of *tridax procumbens* has also shown good antimicrobial activity.[8] The extract of flower of *tridax procumbens* have been reported to exhibit some anti cancer activity as well.[9] Hence it is worth extracting the phytoconstiuents from *tridax procumbenes* and to study their antioxidant activity. In the present study saponin and tannin fractions have been extracted from the leaves of *tridax procumbenes* and their antioxidant activity has been determined quantitatively.

MATERIALS AND METHODS

Sample leaves of *tridax procumbens* were obtained from local market of Melghat region of Maharastra and authenticated from the Department of Botany, Shri Shivaji College, Akola

Extraction of saponin10 g of coarse powered of dried leaves was added to 100 ml 20% ethanol and heated in water bath for 4 hours with continuous stirring at about 55^0 C then it was filtrated and re extracted the residue over water bath at 90^0C and transferred to 250 ml separating funnel and added 20ml diethyl ether and shacked vigorously. Aqueous layer was recovered and discarded the diethyl ether layer. 60 ml of n-butanol was added and combined extract washed twice with 10 ml of 5% aqueous sodium chloride and evaporated in water bath up to dryness and completely dried in oven. The residue so obtained was collected and preserved for further investigation.

Extraction of tannin

10 g of coarse powered of dried leaves was taken in beaker containing approximately 100 ml distilled water and boiled for 30 min, filtered and the filtrate was centrifuged at 2000 rpm and supernatant was collected. Then sample was dried, collected and preserved for further investigation.

Study of antioxidant activity by DPPH

The antioxidant activity of the isolated saponin and tannin fractions was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl
(DPPH). The diluted working solutions of the test extracts were prepared in methanol.
0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV visible spectrophotometer. Methanol (1 ml)
with DPPH solution (0.002%, 1 ml) was used as blank.The optical density was recorded and % inhibition was calculated using the formula given below

$$\text{Percent (\%) inhibition of DPPH (\%AA)} = \frac{A - B}{A} \times 100$$

Where A = optical density of the blank and B = optical density of the sample.

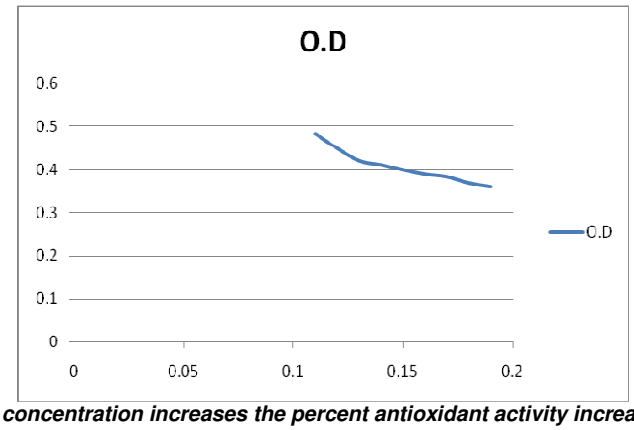
RESULTS AND DISCUSSION

The stock solution 1mg/ml of isolated saponin fraction was prepared using water as solvent. The required dilutions 0.11mg/ml to 0.19 mg/ml were prepared by appropriate dilutions. The optical density and percent antioxidant activity was calculated and reported table 1

Table 1
Optical density and percent antioxidant activity for saponin fraction O.D. of blank DPPH = 0.565

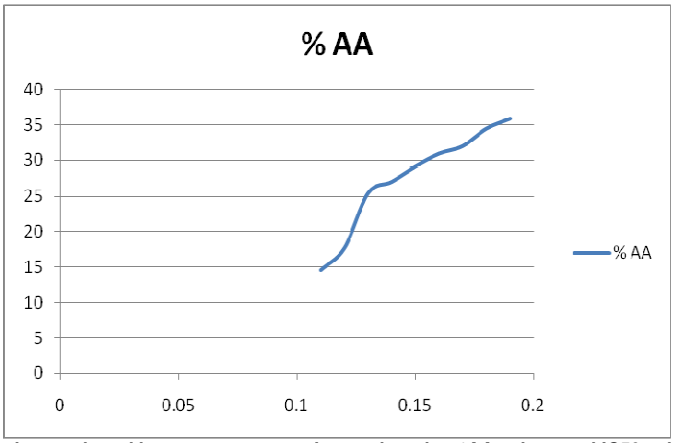
Conc. Mg/ml	0.11	0.12	0.13	0.14	0.15	0.16	0.17	0.18	0.19
O.D of sample	0.483	0.449	0.421	0.412	0.400	0.390	0.384	0.370	0.362
% AA	14.51	17.68	25.48	27.07	29.20	30.97	32.03	34.51	35.92

Decrease in O.D. of sample with increase in concentration of saponin fraction



As concentration increases the percent antioxidant activity increases.

Increase in percent antioxidant activity with increase in concentration of saponin

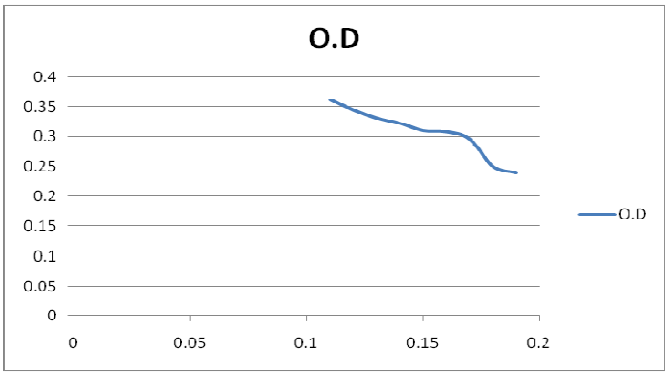


A graph was plotted between concentration against the %AA values and IC50 vale was calculated from graph. The IC50 vale for saponin component was found to be = 0.13mg/ml

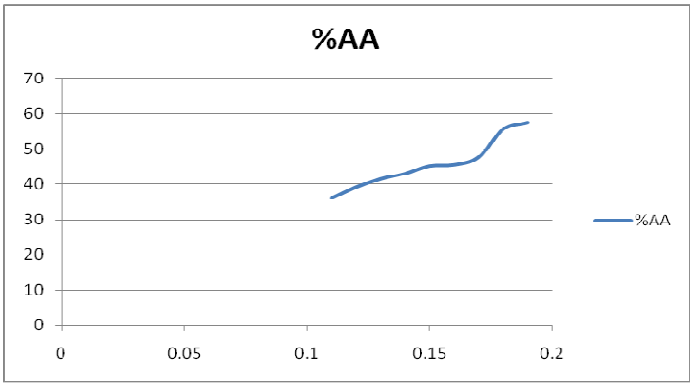
Table 2
Optical density and percent antioxidant activity for tannin fraction O.D. of blank DPPH = 0.565

Conc.Mg/ml	0.11	0.12	0.13	0.14	0.15	0.16	0.17	0.18	0.19
O.D of sample	0.361	0.344	0.331	0.322	0.310	0.308	0.296	0.251	0.240
%AA	36.10	39.11	41.41	43.00	45.13	45.48	47.61	55.57	57.52

Decrease in O.D. of mixture with increase in concentration of tannin



Increase in percent antioxidant activity with increase in concentration of tannin



IC50 vale for tannin =0.165mg/ml

CONCLUSION

Remarkable decrease in the O.D. values of sample for both the isolated fractions was observed indicating antioxidant activity of the fractions. Both the fraction viz saponin and tannin extracted from the leaves of *tridax procumbens* showed good to moderate antioxidant activity which is evident from the graph. The IC_{50} values for saponin and tannin fractions were found to be 0.13mg/ml and 0.165mg/ml respectively.

ACKNOWLEDGEMENT

Authors are thankful to the Management and Principal of Shankarlal Khandelwal College, Akola for providing necessary facilities. Authors are also thankful to University Grants Commission for providing the financial support under major research project. Thanks are also due to Dr. S. P. Rothe, Department of Botany, Shri Shivaji College, Akola for identification of plant material.

REFERENCES

1. Fejes S., Blazovics A., Lemberkovics E., Petri G. and Kery A., *J Ethnopharmacol* (2000); 69: 259-265.
2. Chopda, M.Z.: Wound healing agents of plant origin, A Thesis, North Maharashatra University, Jalgaon, M.S. India.(2009).
3. Namrata V. Nemade, Manojkumar Z. Chopda and Raghunath T. Mahajan *Academic Scienses, International J of Pharmacy and Pharmaceutical Science* Vol 3(4),(2011)
4. M.K. Oladunmoye. Immunomodulatory *International journal of tropical medicine,* (2006), 1 (4), 152-155.
5. V. K. Saxena and S. Albert. *J. Chem. Sci.*,(2005), 117(3), 263–266.
6. V. Rathi, J. C. Rathi, S. Tamizharasia and A. K. Pathak.. *Pharmacognosy Review*, (2008), 2(3), 185- 186.
7. Mohammed Ali, Earla Ravinder, Ramidi Ramachandram, (2001), *Fitoterapia*, 72, 313-315.
8. R.B. Mahato and R.P. Chaudhary. *Scientific World*, (2005), 3(3), 26-31.
9. Vishnu Priya P , Radhika K , Siva Kumar R , Sri Ramchandra M, Prameela Devi Y and A.Srinivas Rao , *Pharmanest - An International Journal of Advances In Pharmaceutical Sciences* Vol. 2 (1) January - February (2011)

E-ISSN: 2278-3229

IJGHC, September 2014 – November-2014; Sec. A; Vol.3, No.4, 1351-1354.

International Journal of Green and Herbal Chemistry

An International Peer Review E-3 Journal of Sciences

Available online atwww.ijghc.com

Section A: Green Chemistry

Research Notes

CODEN (USA): IJGHAY

GCMS Analysis of Various Extracts of Leaves of *Tridax Procumbens*

P.S.Pande*, M.N.Mishra*, V.D.Mane*and **S.P.Rothe**[1]

*Department of Chemistry, Shankarlal Khandelwal Art, Science and Commerce College,Akola 444002, India.

[1]Department of Botany, Shri Shivaji College, Akola, Maharashtra, India

Received: 06 August 2014**; Revised:** 23 August 2014**; Accepted:** 04 September 2014

Abstract: In the present study,various extracts of leaves of Tridax procumbens were subjected to G.C.M.S analysis .The G.C.M.S analysis revealed several important bioactive compounds.These bioactive compounds were searched in the literature which revealed the biological activities of the identified compounds.

Key words:Tridax procumbens ,G.C.M.S analysis, Bioactive compounds.

INTRODUCTION

The work on medicinal plants has been attracting the worldwide attention owing to their divine medicinal properties because they are natural product and no side effects. *Tridax procumbens L.* is one such plant which shows various pharmacological, anti-diabetic, anti-inflammatory, analgesic and marked depressant action on respiration[1-6] various studies have been carried out using plant extract of Tridax procumbens in different solvent extraction such as antioxidant activity[7].Therefore it is worth studying the further medicinal use of the plant.

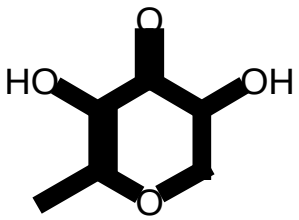
EXPERIMENTAL

The leaves of the plant Tridax procumbens were collected from local market of hilly area nearby Melghat region of Maharashtra and identified.

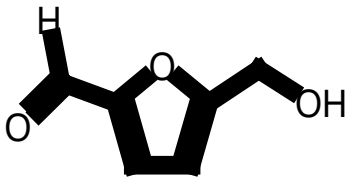
1. **Procedure:** 10 gm dried leaves of tridax procumbens were crushed in 100 ml distill water and filtered. The filtrate so obtained was allowed to stand overnight.The suspended impurities so developed were filtered off.The process is repeated until no impurities are formed.The clear solution so obtained was subjected to column chromatographic separation using Ethylacetate ethanol 1:1 solution. The first component so obtained was collected and tested for antioxidant activity by DPPH. The fraction showed good antioxidant activity .The sample was analyzed by G.C.M.S analysis.2. **Procedure:**10gm of dried coarse powder of leaves of tridax procumbens was subjected to soxhlet extraction with n- hexane.The extract was removed and the marc was further extracted with DCM,The extract was removed the marc so obtained was extracted with ethyl acetate and the marc obtain was again extracted with n-Butanol. The extracted sample was analyzed by G.C.M.S. **Procedure -3)**10 gm of dried coarse powder of leaves of tridax procumbens was subjected to soxhlet. Extraction with n- hexane.The extract so obtained was analyzed by G.C.M.S.

RESULTS AND DISCUSSION

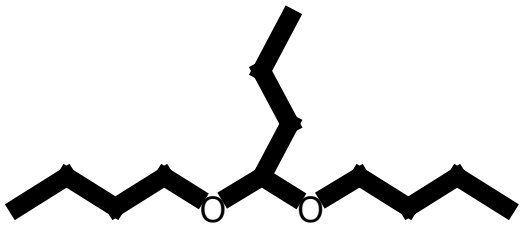
1.The G.C.M.S Analysis of sample extracted by procedure(1) revealed several peaks. Out of these the peak of 9.2min has been identifiedas 4H-pyrane-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl is in 96.8% probability limit.when compared by massM\Z(144,134,126,115,101,87,73,58,55,53,43)



The literature survey reveals that the compound possesses antioxidant activities. Another compound identified with probability limit of 94.4% when compared with mass spectrum M\Z(126,123,109,97,95,93,84,81,75,69,61,58,55,53,43,41) is 5-hydroxymethyl-2-fururaidehyde and is a reported antioxidant in grape and apple juice.[8]

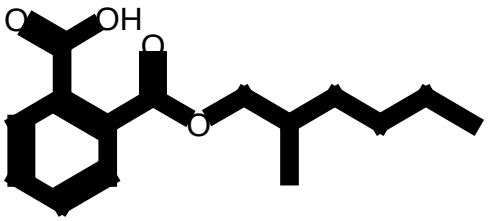


2. The G.C.M.S. analysis of sample extracted by procedure (2) revealed a peak at 6.9 min and identified as 1,1-dibutoxy butane with 93.5% probability. When compared with mass spectrum M\Z(159,141,129,113,103,99,89,85,79,73,67,64,57,55,53)



3.The G.C.M.S analysis of sample extracted by procedure (3) revealed several peaks out of which peak of 19.9 minute was predominant and identified by library search as n-hexadecanoic acid with probability of 80.4%.when compared by mass spectrum M\Z(256,239,227,213,199,185,171,157,143,129,115,97,83,73,60,57) It is reported to possess some antioxidant properties.[9]

Another peak obtained at 28.2 min was identified as 1,2-Benzenedicarboxylic acid mono(2-ethylhexel)ester with probability limit of 50.1% when compared with mass spectrum M\Z(279,261,180,167,149,132,122,113,104,93,83,76,71,57) and known to possess some antimicrobial activity.



CONCLUSION

The GCMS analysis of different extracts of Tridax procumbens revealed important bioactive molecules.The structures are identified by mass spectra of library search with high probability limit.

ACKNOWLEDGEMENT

Authors are thankful to the Management and Principal of Shankarlal Khandelwal College, Akola for providing necessary facilities. Authors are also thankful to University Grants commission for providing the financial support under major research project.Thanks are also due to Dr. S.P.Rothe, Department of Botany, Shri Shivaji College, Akola for identification of plant material.

REFERENCES

1. V.Ravikumar,K.S.Shivashangari and T.Devaki,*Mol.Cell Biochem*,2005,**269(1-2),**131-6
2. Vyas P Suresh, Tiwari Umesh, RastogiBhawna, and Singh Paramjit, *Journal ofEthnopharmacologoly*,2004,**92,**113-119
3. BhagwatDurgacharan A, Killedar, Suresh G and Adnaik, Rahul S,*International Journal ofGreen Pharmacy*, 2010,**2(2),** 126-128.
4. ReddipalliHemalatha, *Int. J. of Green Pharmacy*, 2008,**1.2(3)**,164-169.
5. Diwan Prakash V; IravatiKarwande, I. Margaret and Sattur P.B, Indian J of Pharmacology, 1989**, 21,**1-7.
6. Jain Hitesh,*Indian J of Nat. Prod*, 2006, 23**(1),** 31-33.

7. Sneha mundada, Ruchishivahare, Review. International Journal of Pharm Tech Research. 2010; **2(2):**1391-1394
8. Kom.k.et al Agric Bio 1 chem 88, 53.
9. Dr.Duke,sPhytochimical and ethnobotanical database.

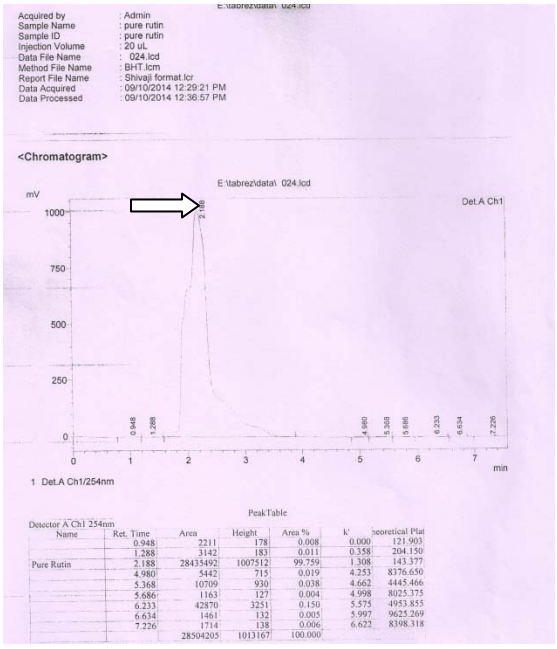
Corresponding author: Dr. P.S.Pande,
Department of Chemistry, Shankarlal Khandelwal College Akola
pande.prasanna@rediffmail.com

aqueous layer was collected and left to stand in a cold place for 72 hours; a yellow precipitate separated out of the solution .the precipitate was dissolved in ethanol and used for HPLC analysis.

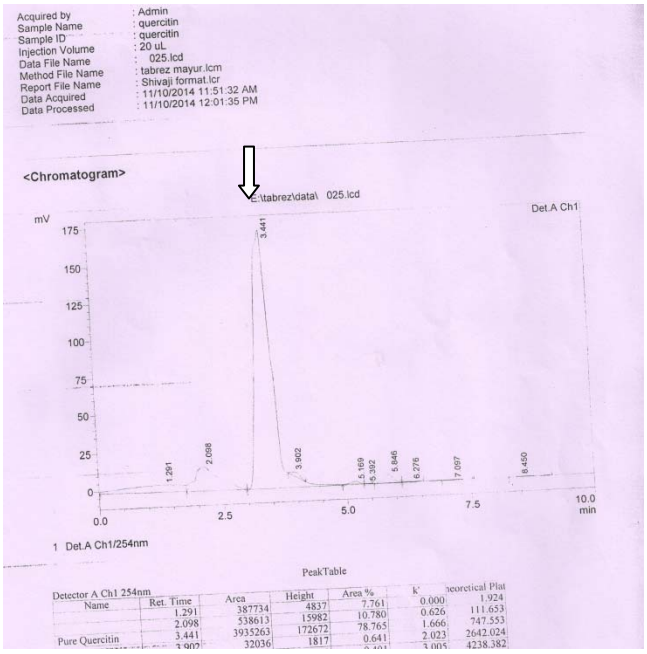
2) Isolation of Quercetin fraction: The leaves of Tridax procumbens L. were collected and washed with tap water then shade dried and after complete drying coarse powder was prepared. Twenty grams of the powdered was successively soxhlet extracted with petroleum ether ,chloroform and methanol respectively. Each of solvent was used for a period of 24 hours. The methanolic fraction was concentrated to obtain a semisolid consistency. The same semisolid fraction was successively extracted with 50 ml of petroleum ether (fraction I) , 50 ml of ethyl acetate (fraction II) and 50 ml of ethyl acetate (Fraction III) with the help of separating funnel. Each extraction was repeated three times to ensure complete extraction in each case .This was done for the entire methanolic extract. Fraction III was used for HPLC analysis.

Result and Discussion:

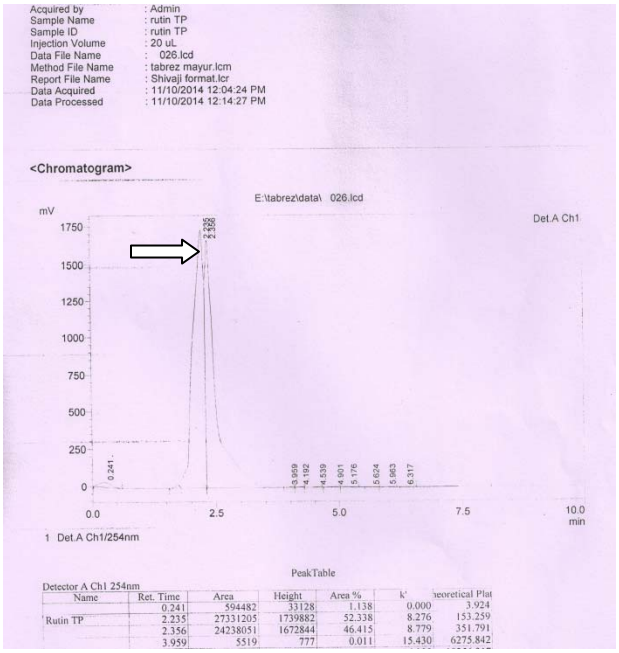
The HPLC chromatogram of the sample containing rutin fraction (isolated by procedure 1) was compared with that of pure rutin, the peaks for rutin coincided indicating rutin in the extract of leaves of tridax procumbens, analogously the HPLC chromatogram of the sample containing quercetin fraction (isolated by procedure 2) was compared with that of pure quercetin, the peaks for quercetin coincided indicating quercetin in the extract of leaves of tridax procumbens.



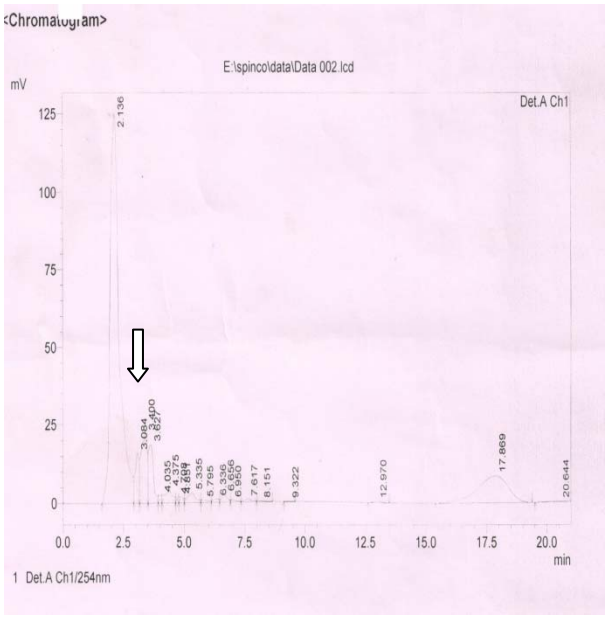
HPLC chromatogram of pure rutin



HPLC chromatogram of pure quercetin



HPLC chromatogram of rutin in sample



HPLC chromatogram of quercetin in sample

Conclusion:

HPLC analysis showed presence of rutin and quercetin in the leaf extract of tridax procumbens.

Acknowledgement:

Authors are thankful to the Management and Principal of Shankarlal Khandelwal College, Akola for providing necessary facilities. Authors are also thankful to University Grand Commission for providing the financial support under major research project. Thanks are also to Dr. S. P Rothe, Department of Botany, Shri Shivaji College, Akola for identification of plant material.

References

[1]. Udupa,SL ;Udupa,AL;Kulkarni,DR (1998).Fitoterapia,69;507-510.

[2]. Sanhavi et al./IJPBS, 2014; Vol.5(4): 1454 - 1459.

[3]. Pathak, A.K., Saraf, S., Dixit, V.K., Hepatoprotective activity of Tridax procumbens - Part I. Fitoterapia 1991, 62, 307-13.

[4]. Bhagwat, D.A., Killedar, S.G., Adnaik, R.S., Antidiabetic Activity of leaf extract of Tridax procumbens. International Journal of Green Pharmacy 2008, 2, 126-128

[5]. Salahdeen, H,M., Yemitan, O.K., Alada, A.R., Effect of aqueous leaf extract of Tridax procumbens on blood pressure and heart rate in rats, African Journal of Biomedical Analysis 2004, 7, 27-29.

[6]. Saraf, S., Pathak, A.K., Dixit, V.K., Hair growth promoting activity of Tridax procumbens Fitoterapia 1991, 62, 495-498.

[7]. Margaret, I., Srinivasa, P.R., Jamil, K., Anti-inflammatory profile of Tridax procumbens in animal and fibroblast cell model,Phytotherapy 1998, 12, 285-287.

[8]. Agrawal, S.S., Talele, G.S., Surana, S.J., Antioxidant activity of fractions from Tridax procumbens, Journal of Pharmacy Research2009, 2, 7173.

[9]. Vyas, S.P., Tiwari, U., Rastogi, B., Singh, P., Saraf, D.K., Immunomodulatory effects of aqueous extract of Tridax procumbens in Experimental animals., Journal of Ethno pharmacology 2004, 92, 113-119.

[10]. Mahato, R.B., and Chaudhary, R.P., Ethnomedicinal study and antibacterial activities of selected plants of Palpa district, Nepal. Scientific World 2005, 3(3), 26-31.

11) Kusum Singh, Vinita Ahirwar, Acute and chronic toxicity study of Tridax procumbens on haemoglobin percent and blood sugar level of Sprague Dawley rats, Journal of Pharmacology and Toxicology 2010; 1(1):1-6.