

## Research Article



## Phytochemical Screening and *in vitro* Anti inflammatory Activity of Aerial Parts of *Tridax procumbens* L.

Kavitha. R, Prasanna. G\*

PG and Research Department of Biochemistry, Sengamala Thayaar Educational Trust Women's College, Sundarakkottai, Mannargudi, Thiruvavur Dt, Tamil Nadu, India.

\*Corresponding author's E-mail: [prasannakeertana@yahoo.in](mailto:prasannakeertana@yahoo.in)

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### ABSTRACT

The present investigation was aimed to screen the phytochemicals and *in vitro* anti inflammatory activity of aerial parts of *Tridax procumbens* L. Qualitative phytochemical analysis confirmed the presence of alkaloids, tannins, saponins, flavonoids, quinines, phlobatannins, terpenoids, phenols, coumarins, steroids and phytosteroids and absence of glycosides and anthraquinones. UV-Visible analysis represents the absorption spectrum at different wavelength. FT-IR analysis revealed the presence of functional groups like alcohol, alkanes, alkyl halide and aromatic group and alkenes. HPLC study confirmed the quantity of phenolics and flavonoids such as ferulic acid (0.090 mg/gm), quercetin (0.045 mg/gm), caffeic acid (0.005 mg/gm), rutin (0.002 mg/gm), gallic acid (0.002 mg/gm). *In vitro* anti-inflammatory property was also carried out in five different concentrations (20, 40, 60, 80 and 100 µg/ml) of aerial parts of plant using various methods such as HRBC membrane stabilization, protein denaturation inhibition, proteinase inhibitory activities. Among the different concentrations, 100 µg/ml of plant extract showed highest anti inflammatory activity for all three models. The obtained results were compared with standard aspirin. In conclusion, Aerial part of *Tridax procumbens* L. having remarkable anti-inflammatory activity which may be due to the presence of phenolic compounds and flavanoids.

**Keywords:** Aerial parts, Anti-inflammatory, FT-IR, HPLC, UV-Visible, Phytochemicals, *Tridax procumbens*.

### INTRODUCTION

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as the increase of vascular permeability, increase of protein denaturation and membrane alteration. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form of stress. Since inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion<sup>1</sup>. Inflammation is the response of vascular body tissue to injury or irritation. It is notable as the area becomes reddened in fair skin, darkening in dark skin, swollen and hot. It is often accompanied with pain or tenderness<sup>2</sup>.

Inflammations are treated by variety of anti inflammatory drugs. Most of the anti-inflammatory drugs, particularly steroids and cyclooxygenase inhibitors are often associated with adverse side effects including GI irritation, ulcers, hypertension and cardiac abnormalities. Drugs from plant sources have been used for the treatment of various disorders and diseases since ancient times. Nowadays, the use of herbal drugs to cure inflammation and pain is gaining popularity due to their effectiveness, fewer side effects, low cost and availability. Hence in the present study, *Tridax procumbens* L. belonging to the family of Asteracea was selected and evaluated for its anti inflammatory activity.

Phytochemical screening was also carried out by UV-Vis, FT-IR and HPLC analysis.

*Tridax procumbens* L. is a green perennial plant and is available in all seasons in many parts of India. It is listed as a weed and a pest plant<sup>3</sup>. It is commonly known as 'Ghamra' and in English popularly called 'coat buttons' because of appearance of flowers which has been extensively used in ayurvedic system of medicine<sup>4</sup>. Flavonoid was identified in the aerial parts of the plant<sup>5</sup>. Leaves possessed phytochemical such as alkaloid<sup>6</sup>, carotenoid<sup>7</sup>, hydroxyl cinnamate<sup>8</sup>, lignans<sup>9</sup>, benzoic acid<sup>10</sup>, phytosterols<sup>11</sup>, tannins<sup>12</sup> and flowers contain luteolin, glucoluteolin, quercetin and isoquercetin oxoesters<sup>13</sup>. The leaves of the *Tridax procumbens* L. as a remedy against conjunctivitis<sup>14</sup>. Leaf juice can be used to cure fresh wounds, to stop bleeding and liver disorders. It is traditionally used in the treatment of fever, typhoid fever, cough, asthma, epilepsy and diarrhea<sup>15</sup>. Plant is also used for the treatment of bronchial catarrh, dysentery, diarrhoea, malaria, stomachache, and headache and also marked depressant action on respiration etc. It is also reported to have antiseptic, insecticidal and parasitocidal properties<sup>16</sup>.

### MATERIALS AND METHODS

#### Collection of plant material

The aerial parts of *Tridax procumbens* L. were collected from Mathukur, Thanjavur district, Tamil Nadu. The plant material was identified and washed under running tap water followed by washing with distilled water to remove



the surface debris. Then they were shade dried and coarsely powdered.

### Preparation of plant extract

100g of plant powder was dissolved in 1000ml of water and incubated for 24 hrs. Then, it was filtered by using filter paper and the filtrate was boiled at 56°C up to semi solid consistency. Then the aqueous extract was used for the screening of phytochemical and *in vitro* anti-inflammatory activity.

### Preliminary phytochemical screening

Preliminary phytochemical screening was conducted as per standard procedures<sup>17</sup>.

### Spectral analysis

#### UV-Visible (UV-Vis) spectroscopic analysis

The aqueous plant extract was examined under UV-Visible spectral analysis. The extract was centrifuged at 3000 rpm for 10min and filtered through Whatmann No.1 filter paper. The sample was diluted to 1:10 with the same solvent. The extract was scanned in the wavelength ranging from 260-900nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected<sup>18</sup>.

#### Fourier Transform-Infrared (FT-IR) spectroscopic analysis

10mg of the dried extract powder was encapsulated in 100mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of extract was loaded in FTIR spectroscope (Shimadzu, Japan), with a scan range from 400 to 4000cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup><sup>19</sup>.

### HPLC analysis

#### Preparation of standards for HPLC

Standard stock solutions of flavonoids and phenolic compounds such as ferulic acid, quercetin, rutin, caffeic acid, gallic acid were prepared at concentrations of 2, 4, 6, 8, 10 µg/ml and filtered through HPLC filter 0.45 mm membrane filter (Millipore).

#### Preparation of extract

10g of aerial parts of *Tridax procumbens* L. powder were extracted with 15 ml 95% ethanol for 6 h. The resulting extract was suspended over water and partitioned using petroleum ether to remove waxes and impurities. The resulting extract was subjected to HPLC analysis.

#### HPLC analysis

The plant extract was analyzed for flavonoids and phenols using a RP-HPLC method<sup>20</sup>. Shimadzu Corp., Kyoto, consisting of a LC-10ATVp pump, SCL 10A system controller and a variable Shimadzu SPD-10ATVp UV VIS detector and a loop injector with a loop size of 20 µl was used. The peak area was calculated with CLASS VP software. Reverse phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250×4.6 mm i.d., particle size 5 µm, Luna 5

µ C-18; phenomenex, Torrance, CA, USA) at 25 °C. The gradient elution of solvent A (water-acetic acid; 25:1 v/v) and solvent B (methanol) had a significant effect on the resolution of compounds. Detection wavelength was 280 nm. Ferulic acid, quercetin, rutin, caffeic acid and gallic acid were used as internal and external standards. Phenolic acids and flavanoids present in sample were identified by comparing chromatographic peaks with the retention time (Rt) of individual standards. The amount of each phenolic and flavanoids is expressed as mg/gm.

### *In vitro* anti-inflammatory activity

*In vitro* anti-inflammatory activity of plant extract was carried out using various *in vitro* models such as HRBC membrane stabilization activity<sup>21</sup>, protein denaturation inhibitory activity<sup>22</sup> and proteinase inhibitory activity<sup>23</sup>. Standard drug was also subjected in order to compare the efficacy of anti inflammatory activity of plant extract.

## RESULTS AND DISCUSSION

The term “medicinal plant” includes various types of plants used in herbalism. It is the use of plants for medicinal purposes, and the study of such uses. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. The plant constituents which possess pharmacological properties are called ‘active constituents’.

**Table 1:** Preliminary phytochemical analysis of *Tridax procumbens* L.

S.No.	Phytochemicals	Result
	Alkaloids	+
	Anthraquinones	-
	Carbohydrates	+
	Cardiac Glycosides	-
	Coumarins	-
	Flavonoids	+
	Glycosides	-
	Phenols	+
	Phlobatannins	+
	Quinines	-
	Saponins	+
	Steroids And Phytosteroids	+
	Tannins	+
	Terpenoids	+
	Triterpenoids	+

(+) indicate Positive; (-) indicate Negative

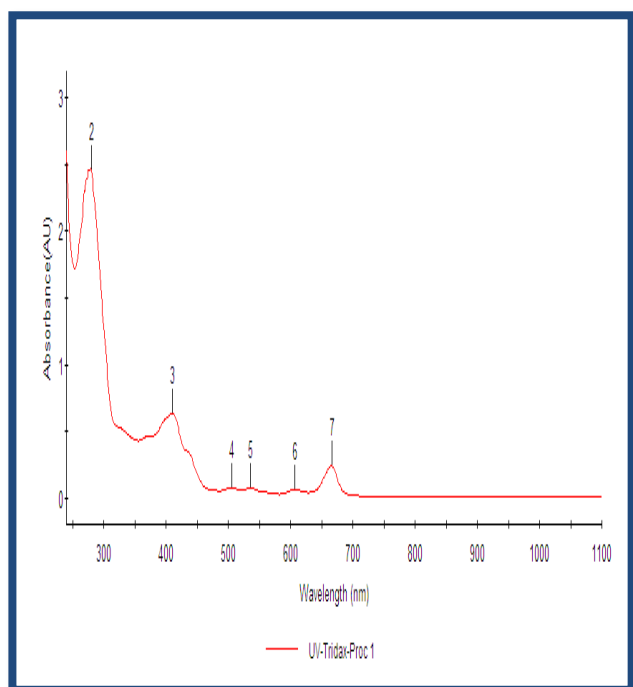


Phytochemistry is concerned with the chemical study of the plant constituents<sup>24</sup>. Therefore, the analysis of these bioactive constituents would help in determining various biological activities of plants. In the present study, qualitative phytochemical analysis revealed the presence of alkaloids, phenolics, flavonoids, steroids, terpenoids and saponins and absence of anthraquinones, cardiac glycosides, coumarins, glycosides and quinines (table 1).

UV-Vis spectrophotometer is related to the spectroscopy of photons in the UV-visible region. UV-Vis spectroscopy uses light in the visible ranges or its adjacent ranges. The color of the chemicals involved directly affects the absorption in the visible ranges. Molecules undergo electronic transitions in these ranges of the electromagnetic spectrum<sup>25</sup>. In the present study UV-Vis spectral profile showed the peaks 410, 505.6, 535.5, 607.4 and 665.7nm with the absorption 0.635, 0.075, 0.074, 0.061 and 0.239 respectively (table 2 and figure 1).

**Table 2:** UV-Visible spectral analysis of *Tridax procumbens* L.

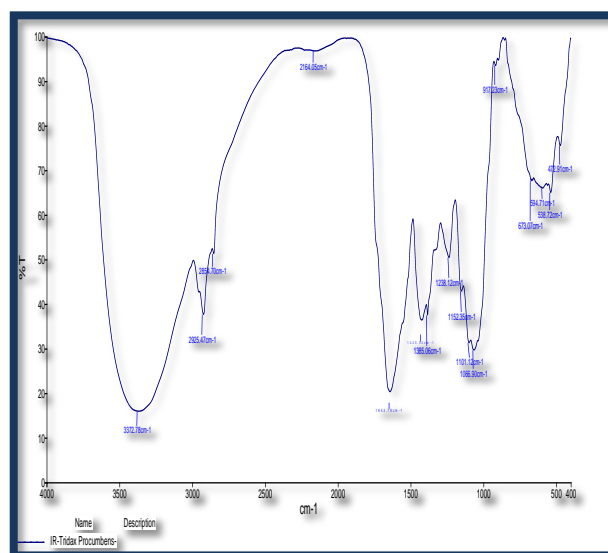
S.No	Absorption(AU)	Wavelength(nm)
1	0.635925467	410
2	0.075972752	505.6
3	0.074274481	535.5
4	0.061032074	607.4
5	0.239126541	665.7



**Figure 1:** UV-Visible spectral analysis of *Tridax procumbens* L.

**Table 3:** FT-IR spectrum analysis of *Tridax procumbens* L.

S. No.	Peak value (cm <sup>-1</sup> )	Bond/Strech	Functional group
1	3372.78	O-H Stretch	Alcohol
2	2925.47	C-H Stretch	Alkane
3	1642.78	C=C Stretch	Alkene
4	1238.12	C-F Stretch	Alkyl halide
5	673	C-Br Stretch	Halo compound
6	2854.70	C-H Stretch	Alkane
7	1152.35	-C-H Bending	Alkane
8	1425.92	C=C Stretch	Aromatic
9	1152.35	C-F Stretch	Alkyl halide
10	594	C-I Stretch	Halo compound
11	1101.12	C-F Stretch	Alkyl halide
12	2164.70	C-H Stretch	Alkane
13	538	C-I Stretch	Halo compound
14	1066.90	C-F Stretch	Alkyl halide



**Figure 2:** FT-IR spectral analysis of *Tridax procumbens* L.

The FT-IR has proven to be a valuable tool for characterization and identification of compounds or functional groups (chemical bonds) present<sup>26, 27</sup>. It allows the qualitative determination of organic compounds as the appearance of the bands in the infrared spectrum at a specific frequency, which is further influenced by the surrounding functional groups<sup>28</sup>. The results of FTIR peak values and functional groups were represented in table 3 and figure 2. When the plant extract was passed into the FTIR spectrum, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of functional groups such as alkanes (2925.47, 3372.78), alcohol (2831.50), alkyl halide (1238.12, 1152.12, 1101.35, 1066.90, 673, 594, and 538), alkene (1642.78) and aromatic compounds

(1425.92). Our study results correlates with previous study<sup>29</sup>.

HPLC is a versatile, robust and widely used technique for the isolation of natural products. HPLC can separate a mixture of compounds and is used in phytochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture<sup>30, 31</sup>. Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of herbal plants<sup>32</sup>.

In the present study, an aerial part of *Tridax procumbens* L. was subjected to HPLC analysis in order to find out some of phenolic and flavonoid compounds. Standard such as gallic acid, ferulic acid, caffeic acid, rutin and quercetin were used. The obtained result confirmed that phytochemicals at different retention time for ferulic acid (Rt 23.208), quercetin (Rt - 12.817), rutin (Rt - 10.792), caffeic acid (Rt - 9.042), gallic acid (Rt - 5.592)

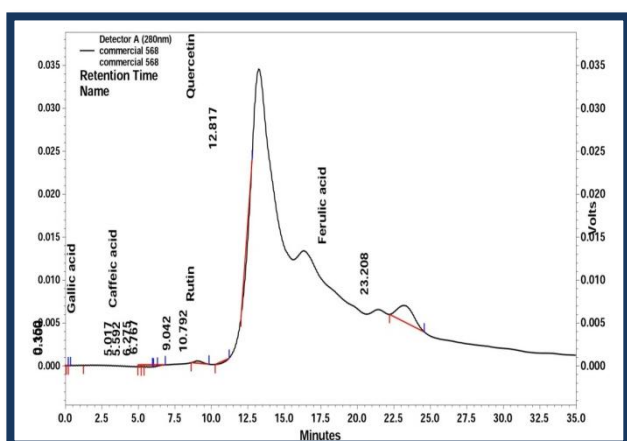
(table 4 and figure 3). Out of the five compounds, ferulic acid was found to be high concentration (0.090 mg/ gm) followed by quercetin (0.045 mg/ gm), caffeic acid (0.005 mg/ gm), gallic acid (0.002 mg/ gm) and rutin (0.002 mg/ gm).

Phenolic compounds are famous group of secondary metabolites with wide pharmacological activities. Varied biological activities of phenolic acids were reported. Flavonoids are the largest group of phytonutrients, with more than 6,000 types. Gallic acid, a naturally occurring polyphenolic compound is extensively used in medicine and chemical research and other industries including pharmaceutical, cosmetic, and food industries<sup>33</sup>. Caffeic acid is believed to have potential anti-inflammatory and antioxidant properties. Ferulic acid has been reported to possess antimicrobial, anti-inflammatory, antiabetic, anticancer activities<sup>34</sup>.

**Table 4:** HPLC analysis of *Tridax procumbens* L.

UV Detector (280nm)				
Retention Time	Area	Height	Concentration (mg/ gm)	Name of the compound
5.592	9263	202	0.002	Gallic acid
9.042	9412	267	0.005	Caffeic acid
10.792	7283	173	0.002	Rutin
12.817	60916	116	0.045	Quercetin
23.208	149268	1887	0.090	Ferulic acid

Flavonoids are associated with a broad spectrum of health-promoting effects and are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is because of their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties<sup>35</sup>. Quercetin inhibits enzymes of the cytochrome P450 CYP1A, as well as CYP3A4, and is found to be beneficial in metabolizing a significant number of carcinogens and medications. Rutin is found effective in the treatment of inflammation during the chronic phase<sup>36</sup>.



**Figure 4:** HPLC analysis of *Tridax procumbens* L.

### ***In vitro* anti inflammatory activity**

#### **HRBC membrane stabilization activity**

In the present study, five different concentrations (20, 40, 60, 80, 100 µg/ml) of the aqueous extract of aerial parts of *Tridax procumbens* L. has been evaluated for the HRBC membrane stabilization activity. The percentage of inhibition was ranging from 60% to 93% at 20-100 µg/ml. Dose dependent inhibition for membrane stabilization was observed in plant extract. The percentage of inhibition for standard was 65%. The plant extract was effectively inhibiting the heat induced hemolysis when compared with standard. These results provided evidence for remarkable antiinflammatory activity of plant extract which might be due to the presence of phytochemicals such as phenolic and flavanoids<sup>37, 38</sup>. Our result consistent with previous report<sup>39, 40</sup>.

#### **Protein denaturation inhibition activity**

Protein denaturation is one of well documented causes of inflammation in conditions like rheumatoid arthritis<sup>41</sup>. Production of auto-antigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins<sup>42, 43</sup>.

Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. In the present study, the ability of plant extract

at different concentrations (20, 40, 60, 80, 100µg/ml) to inhibit protein denaturation was studied to assess the anti inflammatory activity.

**Table 5:** HRBC Membrane stabilization activity of *Tridax procumbens*

S. No.	Concentration (µg/ml)	% Stabilization
1	20	60±0.015
2	40	66.6±0.010
3	60	73.3±0.016
4	80	80±0.015
5	100	93±0.05
6	Aspirin (100µg/ml)	65±0.04

Values were represented as mean ± S.D for three determinations.

100µg/ml of the plant extract exhibited 88.9% of inhibition followed by other concentrations like 80 µg (77.8%), 60 µg (77.6%), 40 µg (55.45%) and 20 µg (44.6%). Standard drug aspirin showed 35% of inhibition. Plant extract showed the significant anti inflammatory activity when compared with standard aspirin. Our result stated that *Tridax procumbens* was capable of controlling the production of auto antigen and inhibits denaturation of protein during inflammation. Similar results were observed from previous study<sup>44</sup>.

**Table 6:** Protein denaturation inhibitory activity of *Tridax procumbens*

S.No.	Concentration (µg/ml)	% Inhibition
1	20	44.6±0.015
2	40	55.4±0.020
3	60	77.6±0.026
4	80	77.8±0.025
5	100	88.9±0.026
6	Aspirin (100µg/ml)	35±0.01

Values were represents as mean ± S.D for three determinations

#### Proteinase inhibitory activity

Neutrophils are known to be a rich source of proteinase and are localized at lysozyme. It was previously reported that proteinases of leukocytes play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors<sup>45</sup>. In the present study, the aerial part of *Tridax procumbens* L. was subjected to determine the proteinase inhibitory activity. Our plant extract showed significant anti proteinase activity ranging from 16.6-75% at different concentrations (20-100µg/ml). Dose dependent inhibitory activity was observed from plant extract that is the highest value was found to be 75% at 100 µg/ml and it was significantly higher than that of standard aspirin (62%).

**Table 7:** Proteinase inhibitory activity of *Tridax procumbens*

S.No.	Concentration (µg/ml)	% Inhibition
1	20	16.6±0.025
2	40	31±0.020
3	60	50±0.015
4	80	66±0.016
5	100	75±0.015
6	Aspirin (100µg/ml)	62±0.01

Values were represents as mean ± S.D for three determinations

#### CONCLUSION

From the above result, it was concluded that the aqueous extract of aerial part of *Tridax procumbens* L. showed significant anti inflammatory activity in a dose dependent manner which might be due to the presence of phytochemicals such as phenolics and flavonoids. The current study provides evidence for the traditional use of *Tridax procumbens* L. against inflammatory disorders and thus, further research are needed for the isolation of bioactive components responsible for anti-inflammatory activity.

#### REFERENCES

1. Tortora, Gerard J, and Sandra Reynolds Grabowski, *Principles of Anatomy and Physiology*, 7<sup>th</sup> ed, 1993.
2. Kumar, Rukmini, Clermont, Gilles, Vodovotz, Yoram, Chow, Carson C, The dynamics of acute inflammation, *Journal of Theoretical Biology*, 230(2), 2009, 145–155.
3. Pai Chitra, Kulkarni Ujjwala, Borde Manjusha, Murali Sowmy Mrudula P, Deshmukh Yashwant, Antibacterial activity of *Tridax procumbens* with special reference to Nosocomial pathogens, *British Journal of Pharmaceutical Research*, 1, 2011, 164-173.
4. Bhagwat DA, Killedar SG, and Adnaik RS, Antidiabetic activity of leaf extract of *Tridax procumbens*, *International Journal of Green Pharmacy*, 2, 2008, 126- 128.
5. Diwan PV, Tillo LD, Kulkarni DR, Steroid depressed wound healing and *Tridax procumbens*, *Indian Journal of Physiol Pharmacol*, 27, 1983, 32-36.
6. Tram NTC, Mitova M, Bankova V, Handjieva N, and Popov SS, GC-MS of *Crinum latifolium* L. alkaloids, *Zeitschrift für Naturforschung*, 57c, 2002, 239-242.
7. Rodriguez-Amaya DB, and Kimura M, Harvest plus handbook for carotenoid analysis, Harvest plus Technical Monograph 2. Washington, DC and Cali: *International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture*, 2004.
8. Ortan A, Popescu ML, Gaita AL, Dinu Pîrvu C, Câmpeanu GH, Contributions to the pharmacognostical study on *Anethum graveolens*, Dill (*Apiaceae*), *Romanian Biotechnology Letter*, 14, 2009, 4342-4348.
9. Chapman JM, Knoy C, Kindscher K, Brown RCD, Niemann S, Identification of antineoplastic and neurotrophic lignans in medicinal prairie plants by liquid chromatography electron impact mass spectrometry, Reprint of Poster from Kansas City Life Sciences Day, 2006.
10. Ndoumou DO, Ndzomo GT, Djougoue PF, Changes in carbohydrate, amino Acid and phenol contents in cocoa pods from three clones



- after infection with *phytophthora megakarya* Bra. and Grif. *Annals of Botany*, 77, 1996, 153-158.
11. Horwitz, Official methods of analysis of the AOAC, 2006; 1<sup>8th</sup> Eds.
  12. Luthar Z, Polyphenol classification and tannin content of buckwheat seeds (*Fagopyrum esculentum* Moench), *Fagopyrum*, 12, 1992, 36-42.
  13. Ali M, Ravinder E, Ramachandran R, A new flavonoid from the aerial parts of *Tridax procumbens*, *Fitoterapia*, 72, 2009, 313–315.
  14. Nia R, Paper DH, Essien EE, Oladimeji OH, Iyadi KC, Franz G, Investigation into *in-vitro* radical scavenging and *in-vivo* anti-inflammatory potential of *Tridax procumbens*, *Nigerian journal of physiological science*, 18, 2003, 39-43.
  15. Mann A, Abdulkadir NU, Muhammad G, Medicinal and economic plants of Nupe Land, *Juber Evans Books and Publication*, 2003; 78.
  16. Behera KK, Purandra Mandal, Dayanidhi Mahapatra, Green leaves for diarrhoeal diseases used by the tribals of *Kenjhar* and *Mayurbhanj*, *International Web Journal on Ethnobotany*, 5(4), 2006, 519-528.
  17. Harborne, JB, Phytochemical methods, London Chapman and Hall Ltd., 1984, pp 49-188.
  18. Iqbal Hussain, Lajber Khan, Murad Ali Khan, Farman Ullah Khan, Sultan Ayaz and Farid Ullah Khan, UV-VIS spectroscopic analysis profile of ascorbic acid in medicinal plants of Pakistan, *World Appl. Sci. J.*, 9(7), 2010, 800-803.
  19. Liu H, Sun S, Lv G, and Chan, KKC, Study on Angelica and its different extracts by fourier transform infrared spectroscopy and two-dimensional correlation IR spectroscopy, *Spectrochimica Acta Part A*, 64(2): 2006, 321-326.
  20. Samee W, Vorarat S, Simultaneous determination of Gallic acid, Catechin, rutin, Ellagic acid and Quercetin in flower extracts of *Michelia alba*, *Caesalpinia pulcherrima* and *Nelumbo nucifera* by HPLC, *The Pharm Health Sci J*, 2(2), 2007, 131-137.
  21. Sakat S, Juvekar AR, Gambhire MN, *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn, *International Journal of Pharma and pharmacological Sciences*, 2(1), 2010, 146-155.
  22. Mizushima Y, Kobayashi M, Interaction of anti-inflammatory drugs with serum proteins especially with some biologically active proteins, *Journal of Pharma Pharmacology*, 20, 1968, 169-173.
  23. Oyedepo OO, Femurewa AJ, Anti-protease and membrane stabilizing activities of extracts of *Fagra zanthoxiloides*, *Olaxsubscorpioides* and *Tetra pleuratetraptera*, *Int J Pharmacog*, 33, 1995, 65-69.
  24. Evans WC, Trease and Evans Pharmacognosy, WB Saunders Company Ltd, London. 2002, 15th edn: 230-240.
  25. Gunasekaran S, UV-Vis spectroscopic analysis of blood serum, *Asian Journal of Microbiology Biotech and Environmental Science*, 5(4), 2003, 581-582.
  26. Aysal P, Ambrus AD, Lehotay SJ, Cannavan A, Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction, *Journal of Environmental Science and Health*, 42, 2007, 481-490.
  27. Ibrahim M, Hameed AJ, and Jalbout A, Molecular spectroscopic study of river Nile sediment in the greater Cairo region, *Appl. Spectrosc.*, 62(3), 2008, 306-311.
  28. Schulz H, Schrader B, Quilitzsch R, Pfeffer S, Krüger H, Rapid classification of basil chemotypes by various vibrational spectroscopy methods, *J Agric Food Chem*, 51(9), 2003, 2475-81.
  29. Prasanna G, Anuradha R, Ultraviolet - Visible and Fourier Transform-Infrared Spectroscopic studies on *Drynaria quercifolia* L. rhizome, *Asian J Pharm Clin Res*, 9(3), 2016, 85-88.
  30. Cannell RJP, Natural products isolation. Human Press Inc. New Jersey, 1998, 165-208.
  31. Piana M, Zadra M, de Brum TF, Boligon AA, Gonçalves AF, da Cruz RC, de Freitas RB, do Canto GS, Athayde ML, Analysis of rutin in the extract and gel of *Viola tricolor*, *Journal of Chromatography Science*, 51, 2013, 406-411.
  32. Fan C, Yan J, Qian Y, Wo X, Gao L, Regulation of lipoprotein lipase expression by effect of Hawthorn flavonoids on peroxisome proliferators' response element pathway. *J Pharmacol Sci*, 100, 2006, 51–8.
  33. Spizzirri UG, Iemma F, Puoci F, Cirillo G, Curcio M, Parisi OI, Picci, N, Synthesis of antioxidant polymers by grafting of gallic acid and catechin on gelatin, *Biomacromolecules*, 10(7), 2009, 1923-1930.
  34. Batista R, Uses and potential applications of ferulic acid. In: Warren, B. (ed). Ferulic acid: antioxidant properties, uses and potential health benefits. 1ed edn. Nova Science Publishers, Inc., New York, USA, 2014, 39–70,
  35. Panche AN, Diwan AD, and Chandra SR, Flavonoids: an overview, *J Nutr Sci*, 5, 2016, e47.
  36. Doshi GM, and Une HM, Quantification of Quercetin and Rutin from *Benincasa hispida* Seeds and *Carissa Congesta* Roots by High-performance Thin Layer Chromatography and High-performance Liquid Chromatography, *Pharmacognosy Res*, 8(1), 2016, 37–42.
  37. Roy SP, Niranjana CM, Jyothi TM, Shankrayya MM, Vishawanath KM, Prabhu K, Gouda VA, Setty RS, Antiulcer and anti-inflammatory activity of aerial parts *Enicostemma littorale* Blume, *Pharmacology*, 2(4), 2010; 369-373.
  38. Garg VKR, Jain M, Sharma PKR, Garg G, Anti inflammatory activity of *Spinacia oleracea*. *International Journal of Pharma Professional's Research*, 1(1), 2010, 1-4.
  39. Nitin gupta, Ankit R. Patel and R.P. Ravindra, Design of akkalkara (*Spilanthes acmella*) formulations for antimicrobial and topical anti-inflammatory activities, *International Journal of Pharma and Bio Sciences*, 2012, 161-170.
  40. Prasanna G, Chitra M, *In vitro* anti-inflammatory activity of *Drynaria quercifolia* rhizome. *Research J of Pharmacognosy and Phytochemistry*, 7(1), 2015, 6-8.
  41. Mizushima Y, Kobayashi M, Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins, *J Pharm Pharmacol*, 20(3), 1968, 169-73.
  42. Singh M, Soni P, Upmanyu N and Shivhare Y, *In-vitro* Anti-arthritis activity of *Manilkara zapota* Linn. *Asian J Pharm Tech*, 1, 2011, 123-124.
  43. Kokila N, Radha R, Jayshree N, *In vitro* antioxidant and antiarthritic activity of polyherbal formulation, *IJPI'S Journal of Pharmacognosy and Herbal Formulation*, 13(3), 2013, 10-15.
  44. Sakat S, Juvekar AR, Gambhire MN, *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn, *International Journal of Pharma and Pharmacological Sciences*, 2(1), 2010, 146-155.
  45. Das SN, Chatterjee S, Long term toxicity study of ART-400. *Indian Indg Medicine*, 16(2), 1995, 117-123.

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