In Vitro Anti-Urolithiasis Activity Screening for Aqueous Extraction of Tridax Procumbens Linn. by Crystal Aggregation Assay

Mr. Rahul D. Kamble[1], Ms.Shraddha R. Patil[2], Ms. Namrata N. Shinde[3], Mr. Sagar S. Biravatkar[4], Ms. Arpita R. Borage[5], Dr. Abhijeet S. Kulkarni[6]

[1]*Assistant Professor, Ashokrao mane institute of pharmaceutical sciences and research, save*

[2,3,4,5] *Student, Ashokrao mane institute of pharmaceutical sciences and research, save*

[6]*Principal, Ashokrao mane institute of pharmaceutical sciences and research, save*

***Abstract*—Tridax procumbens is a commonly used medicinal plant which is popularly known as a widespread weed and pest plant distributed throughout the India. Traditional medicinal practitioners and peoples in ancient times use leaves of the plant against conjunctivitis, diarrhea and dysentery to wound healing and related inflammatory conditions. The chemical constituents of the plant showed that its leaves contain various alkaloids, flavonoids, carotenoids, fumaric acid etc. There leaves extract contain many pharmacological properties like anti-inflammatory activity, hepatoprotective, immunomodulatory, antimicrobial or antibacterial activity, antiseptic, anticancerous activity, antidiabetic, anti-urolithiasis activity, antioxidant effect, and bradycardiac effects etc. The leaf extract of the tridax procurements where used to check the anti-urolithiasis activity in vitro study. We have used the aqueous extract of leaves to check the activity which is prepared by the method of maceration. We have also prepared the alcoholic extract of leaves, methanolic extract and chloroform extract for evaluation of the chemical constituents present in the leaves of tridax procumbens.**

***Index Terms*—Tridax procumbens, Weed, Pharmacology, anti-urolithiasis activity.**

1. INTRODUCTION

The Asteraceae family, one of the biggest vascular plant groups, comprises 2250 genera and 25000 species worldwide[1]. Since the beginning of time, plants have provided food, raw materials for medicines, and countless other necessities for life itself [2]. People are interested in herbs and herbal medicines because of their effects, which have been scientifically verified[3]. *Tridax procumbens Linn* family Compositae, also known as "Ghamra" and "coat buttons" in English due to the appearance of the flowers, has been widely used in the Ayurvedic system of medicine for a variety of diseases [26]. The plant is indigenous to tropical America and has become a weed in subtropical Asia, Australia, and India. It is a wild herb found all over India. Additionally, coat buttons can be found in wastelands, dikes, railroads, riverbanks, meadows, and dunes. Its vast range and significance as a weed are caused by its prolific seed production and spreading stems [27]. Trida is a sparse, straggling plant that is between 12 and 24 cm long. It has a few leaves that are 6 to 8 cm long and very long, slender, solitary peduncles that can reach up to a foot in length. Simple, opposite, exstipulate, oval, acute, and inflorescence capitulum. Tridax has two types of flowers ray-florets and disk-florets. Basal placentation, fruit is cypsela"[28]. It has pharmacological effects that include those that are antibacterial, antifungal, antioxidant, hepatoprotective, anticancer, antidiabetic, immunomodulatory, wound healing, hypotensive, and more [37].

Many plants are utilised in the treating of urolithiasis in Ayurveda medicine.The condition known as urolithiasis, which derives from the Greek words for "stone" and "urine" (ouron, "urine," and lithos, "stone"), is one in which urinary stones can form or develop anywhere in the urinary tract. Most urinary stones in humans are mostly composed of calcium oxalate. Men make up a 80% of kidney stone patients. Men are more likely than women to have their first episode between 20 and a little later [42]. A

complicated series of processes, including the growth of crystals, which results in the development of stones, is the physical process of stone formation [43]. The volume of urine and the quantities of calcium, phosphate, oxalate, and sodium ions play a role in the formation of stones [44].

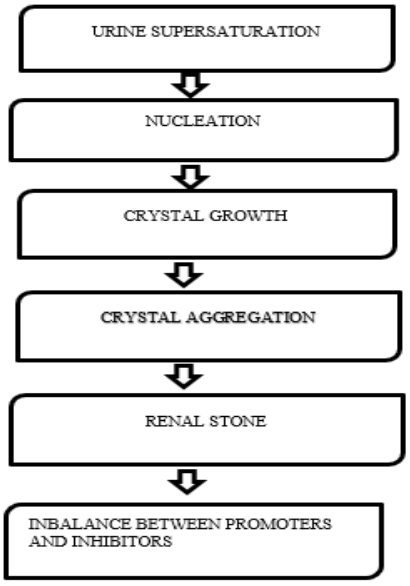

Figure 1.1-Kidney Stone Formation

HistoricalBackground- Forages, people all around the world have used complementary and alternative medicine (CAM). The majority of these practises rely on the regional biodiversity and use crude plant extracts or products made from various plant parts, which helps to create a local legacy[11]. For more than 5,000 years, CAM has been practised in India under several names, including Ayurveda, Siddha, Homoeopathy, and Unani. Tridax procumbens L. (T. procumbens), an Asian Ayurvedic plant with a long history of use, is a member of the Asteraceae family[12]. The World Health Organization (WHO) estimates that about 65% of people worldwide use herbal remedies as their main form of healthcare. Additionally, according to the National Health Interview Survey (NHIS) that the Centres for Disease Control and Prevention (CDC) conducted in 2007, 40% of adults in the United States used complementary and alternative medicine in some capacity, demonstrating that these medical practises are common even in developed countries[13]. T. procumbens has been used in traditional medicine for centuries to treat wounds, skin conditions, and to prevent blood clotting. It has antileishmanial, antioxidant, anticancer, immunomodulatory, insecticidal, anthelmintic, cardiovascular, antiseptic, antibacterial, and insecticidal effects. It also has anticoagulant and antileishmanial characteristics.

Plant Foarmation-

Plant was collected from the Shahuwadi region of Dist. Kolhapur (Maharashtra) for Identification. It was authenticated by Dr. S. Y. Jadhav, HOD Botany Department, Y. C. Warana Mahavidyalaya, and Warananagar. In the tropics, one form of typical grass is Tridax procumbens Linn. Tridax procumbens is also referred to as the "Coat button" and "Tridax daisy" in English, the "Mexican daisy" in Mexico, the "Ghamra" in Hindi, the "Dagadipala" in Marathi, and the "Vettukkaaya-thalai" in Tamil. It has pharmacological effects that include those that are antibacterial, antifungal, antioxidant, hepatoprotective, anticancer, antidiabetic, immunomodulatory, wound healing, hypotensive, and more [4].

Figure 1.1- Tridax procumbens Linn

Botanical name - *Tridax procumbens* Linn

Taxonomical Classification-

Divisions	Classing
Kingdom	Plantae-Plants
Sub kingdom	Tracheobionta- Vascular plant
Division	Spermatophyta
Sub - division	Magnoliophyta- Flowering plant
Class	Magnoliopsida- Dicotyledons
Subclass	Asteriodae
Order	Asterales
Family	Asteraceae-Aster family
Genus	Tridax Linn-Tridax
Species	Tridax procumbens Linn- Coat buttons

Table 1.1: Taxonomical Classification of *Tridax procumbens* Linn.

Vernacular Names: The plant is known by various names in different languages as follows:
English: Coat buttons/Tridax daisy
Hindi: Chamara
Sanskrit: Jayanti veda
Oriya: Bishalya karani
Marathi: Dagadi pala
Telugu: Gaddi chemanthi
Tamil: Thata poodu
Malayalam: Chiravanak
Spanish: Cadillip chica
French: Herbe Caille
Chinese: Kotobu kigiku
Latin: *Tridax aaaprocumbens* Linn [36]
The first and most important step in producing high-quality research results is the preparation of medicinal plants for experimental purposes. Before starting the planned biological testing, it involves extracting and determining the bioactive ingredients. The concept of preparing a medical plant plant for experimental purposes entails the correct and timely collection of the plant, authentication by a professional, suitable drying, and grinding. The bioactive component is then isolated, extracted, and fractionated as necessary [5,6,7].
Ayurveda, Siddha, and Unani are three types of Indian traditional medicine that heavily rely on plant-based remedies[8]. Diuretics are typically referred to as medications that boost the kidneys' production of urine. These medications increase the kidneys' capacity to excrete salt, chloride, or bicarbonate in the first place, and water in the second place [9]. Most illnesses, including those demonstrating oedema, such as congestive heart failure, nephritis, pregnancy toxaemia, premenstrual tension, and hypertension, may benefit from the use of diuretic chemicals that accelerate the excretion of water [10].

2. NEED OF INVESTIGATION

In the world's public healthcare system, herbal products have emerged as a crucial and essential component [22]. Studies on conventional and alternative medicine have shown that both are widely used [23]. Despite the fact that herbalists and believers do not require clinical trials, it is now necessary for their widespread acceptance and survival on the global market alongside contemporary treatments [24]. On the basis of risks and benefits, drug development has produced new medications, albeit more slowly than was anticipated. However, drug treatment outcomes have not improved as much as was anticipated. Herbal preparations made from herbs by extraction, decoction, purification, filtration, or other methods. The plant *Tridax procumbens* mostly found in tropical Africa, Asia, Australia and India. The investigation on that species is finished or is ongoing. In India mostly found the *Tridax procumbens* linn. These species show anti-urolithiasis activity properties and many more. To prepare medicinal medicines, it is required to determine the stability of plant extracts. Different excipients and additives are included in medicinal medicines made from herbal or other ingredients. There are numerous chemical components in the plant extract that either directly or indirectly affect stability. In this investigation we check the anti-urolithiasis activity of the aquous extract of *tridax procumbens* Linn. Urolithiasis is considered as the third most common disorder of the urinary tract. It is the solid-metallic minerals in the urinary tract. It is the consequence of an imbalance between promoters and inhibitors in kidney [25].

3. OBJECTIVES

1. Identification of herbal plant.
2. Extraction of herbal crude drug by Maceration and Soxhlet extraction method.
3. Extraction of *Tridax procumbens* Linn by using water, chloroform, petroleum ether and alcohol solvent.
4. Identification tests for evaluation of various chemical constituents.
5. To check anti-urolithiasis activity of aqueous extract of *Tridax procumbens* Linn by Crystal Aggregation Assay.

4. REVIEW OF LITERATURE

1) S.K. Khan et al. (2008) the study includes determination of herbal product. The article suggests the interest in herbs and herbal medicines because of their various uses. The study was conducted to estimate the diuretic activity of *tridax procumbens*. The *tridax procumbens* have been found it has antibacterial, antifungal, antioxidant, hepatoprotective,

anticancer, antidiabetic, immunomodulatory, wound healing, hypotensive, etc.

2) SuhailAsghar et al. (2011) In ayurvedic system of medicines there are many herbal plants are used for urolithiasis. Formation of urinary stones in located anywhere in the urinary system is called as urolithiasis. Oxalic acid combines with calcium to form calcium oxalate crystals, which deposit in the kidney. Many treatments are used since ages to treat kidney stones.
3) O.R. Alara et al. (2018) Many different natural sources have been employed with soxhlet extraction to obtain useful bioactive chemicals. The primary benefit of this approach is complete extraction with the use of the least amount of solvent, commonly known as the hot continuous extraction procedure or soxhlet extraction. The sample is initially put into a disposable thimble and placed inside the Soxhlet device. By refluxing the solvent, continuous extraction is accomplished. When the extraction chamber is full, the extract is transferred to the boiling flask.
4) N.N. Azwanida et al. Maceration, which is used to make tinctures, extracts, and concentrated infusions, implies "softening." This process of extracting crude drugs is efficient and recognised by the Indian Pharmacopoeia. In this procedure, the substance to be extracted is put in a closed container with appropriate menstruum, and the mixture is left for 7 days while being sometimes shaken. The solid residue is then compressed to extract as much of the solution as possible after the liquid has been filtered off. Filtration is used to combine and clean up the liquid.
5) C.K.Kokate et al. (2013) In the reference book of pharmacognosy investigated on the herbal plant, it's identification tests. It give pathway for the screening of photochemical nature of plant extract. The plant extract found rich source of carbohydrates, alkaloids, flavonoids, glycosides, tannins, etc.
6) .C. K. Kokate et al. (2007) The practical book determined the study of ash value. The ash value is calculated using a variety of techniques. The ash value of the medication in powder form was calculated. One identification test that shows the phytochemical in the plant extract is the ash value

5. PLAN OF WORK

1. Authentication of Plant
2. Collection And Processing of Plant
3. Extraction With Alcohol, Chloroform, Petroleum Ether and Water.
4. Phytochemical Evaluation of Different Extracts i.e.

a. Petroleum Ether
b. Chloroform
c. Alcoholic and Aqueous

5. In Vitro Anti-urolithiasis Activity Screening of Aqueous Extract

Crystal Aggregation Assay

6. MATERIAL AND METHODS

6.1) Authentication of Plant-

In present study, the plant reference was given from Flora of Kolhapur district pp. 258. The stem, bark and whole plant of *Tridax procumbens L.* were authenticated from botanist Dr. S.Y. Jadhav, Head of Department, Department of botany Y.C. Warana Mahavidylaya Warananagar, Maharashtra, India.

6.2) Collection of Plant-

Tropical America is where the plant is native, while, tropical Africa, Asia, Australia and India have also adopted it as their own. The matured stem and bark of *Tridax procumbens* whole plant was collected from Shahuwadi region of Kolhapur district. Tridax is distributed throughout India and is also found along roadsides, grasslands, wastelands, railways, riverbanks, dams and dunes[14]. After authentication, all the stem, bark whole plant were dried at room temperature, until they were free from the moisture and subjected to physical evaluation study with different parameters.

6.3) Extraction procedure-

Soxhlet Extraction-

In this technique, the Soxhlet device' thimble chamber held the crushed material. Heat from the bottom flask causes the extraction solvent, chloroform, to vaporise into the sample thimble, condense in the condenser, and drip back. When the liquid reached the syphon arm, it poured back into the bottom flask and the procedure was repeated [30].The stem of the *Tridax procumbens*, which had been shade-dried, was ground into a fine powder and about 250 g of this powder was subjected to

successive hot continuous extraction (Soxhlet), using solvents in increasing order of their polarity, such as petroleum-ether, chloroform, and methanol to obtain the crude extracts and water for the maceration process. The powdered material will always be air dried in a hot air oven below 50°C before extracting with the next solvent [29,31].

6.3.1) Preparation of Petroleum ether extract-

In order to stop any enzymatic deterioration, fresh plant material was gathered and treated with alcohol. The parts that were recovered were carefully cleaned a minimum of three times with running tap water and once with sterile distilled water before being allowed to air dry at room temperature. These components were thoroughly powdered with a mixer after thorough drying. With the help of a Soxhlet extractor and solvents with different polarities, such as pet-ether (60–80°C), next extracted use new powder. The extraction process took 18 hours (approximately 45 cycles). After the solvent had evaporated, the extract had been concentrated. The solvent was redistilled after the extraction process was successful. The extract was then packed, weighed, and air dried before being placed in a refrigerator.

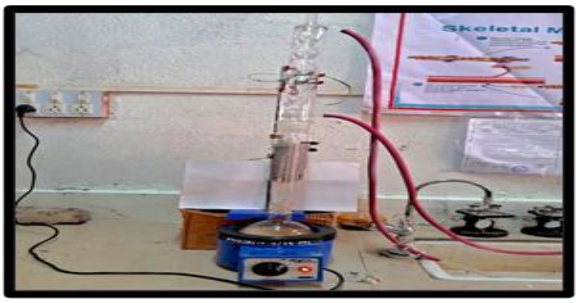

Figure 1.2- Soxhlet Extraction Method

6.3.2) Preparation of chloroform extract-

In order to stop any enzymatic deterioration, fresh plant material was gathered and treated with alcohol. The parts that were recovered were carefully cleaned a minimum of three times with running tap water and once with sterile distilled water before being allowed to air dry at room temperature. These components were thoroughly powdered with a mixer after thorough drying. With the help of a Soxhlet extractor and solvents with different polarities, such as chloroform (61–65°C), for next extract use new powder. The extraction process took 22 hours (approximately 45 cycles). After the solvent had evaporated, the extracts had been concentrated. The solvent was redistilled after the extraction process was successful. The extract was then packed, weighed, and air dried before being placed in a refrigerator.

6.3.3) Preparation of Alcoholic extract-

In order to stop any enzymatic deterioration, fresh plant material was gathered and treated with alcohol. The parts that were recovered were carefully cleaned a minimum of three times with running tap water and once with sterile distilled water before being allowed to air dry at room temperature. These components were thoroughly powdered with a mixer after thorough drying. With the help of a Soxhlet extractor and solvents with different polarities, such as chloroform (64°C), next extracted ues new powder. The extraction process carried out for 3 days (approximately 45 cycles). After the solvent had evaporated, the extracts had been concentrated. The solvent was redistilled after the extraction process was successful. The extract was then packed, weighed, and air dried before being placed in a refrigerator [38,39,40,41].

Maceration aqueous extract –

This fairly straightforward extraction technique has the drawbacks of a lengthy extraction time and poor extraction effectiveness [32]. The maceration procedure includes separating the parts of the crude medicines that have therapeutic effects. Based on the bulk of unprocessed pharmaceuticals in a solvent or menstrual fluid. In that process, an amount of kg of *Tridax procumbens L.* powder was immersed in 500ml of distilled water and solvent chloroform 10ml for 7 days at room temperature and if placed inside conical flask (show in fig 1.3) it should be shaking time to time to ensure complete extraction. up until the soluble material has broken down. After pressing the moist solid material), the combination is next filtered, and the combined liquids are clarified by decantation or muslin-cloth filtering [33,34,35].

Figure 1.3- Maceration Method

6.5) Crystal Aggregation assay-

The rate of aggregation of the CaOx crystals was determined by the method of Atmani and Khan [20] with slight modifications. The COM crystals were prepared by mixing both the solutions of calcium chloride and sodium oxalate at 50 mmol/L. The solutions were equilibrated to 60° C in water bath, cooled to 37^0C and kept overnight. Then the solution was centrifuged and evaporated at 37^0C. CaOx crystals were used at a final concentration of 0.8 mg/mL, buffered with 0.05 mol/L Tris-HCl and 0.15 mol/L sodium chloride at pH 6.5. The experiment was conducted at 37^0C in the presence and absence of pet ether and Methanol extract at 10mg /mL. The absorbance was recorded at 620 nm for a period of one hour for every 10 minutes time interval. All samples were assayed in triplicate. Cystone was used as positive control. Percentage inhibition of aggregation rate was then calculated by comparing the turbidity slope of different concentrations of cystone /Ethanol extract with the turbidity slope of the control by the following formula.

[1-(Tsi/ Tsc)] × 100

Where Tsi was the turbidity slope of aggregation in the presence of inhibitor sample i.e, cystone / plant extract (Ethanol) and Tsc was the turbidity slope of aggregation in the absence of inhibitor.

$$\%Inhibition = \frac{Control-Test}{Control} \times 100$$

7. RESULT AND DISCUSSION-

8.1) Percentage Yield of Extraction-

Sr.no	Extract	Nature of Extract	Wt.(g)	% yield w/w
1	Pet-ether	Semisolid viscous	29.40	20.97%
2	Chloroform	Semisolid viscous	26.529	21.36%
3	Ethanol	Semisolid viscous	14.687	13.96%
4	Aqueous	Solid	11.11	13.88%

Table 1.2- % Yield of Extracts of *Tridax* procumbens *L.*

$$\% \text{ Yield} = \frac{weight\ of\ extract}{weight\ of\ powder} \times 100$$

Physicochemical Analysis-

Sr. no	Parameters	Literature	Observation
1	Physical Test		
	Nature	Coarse Powder	Coarse Powder
	Colour	Green	Green
	Odour	Pungent	Pungent
2	Extractive Value		
	Petroleum Ether	-	20.97%
	Chloroform	-	18.36%
	Ethanolic	-	13.96%
	Aqueous	-	13.88%
3	Loss of Drying	-	-
4	Ash Value		
	Total Ash	-	0.395
	Acid Insoluble Ash	-	0.307
	Water Soluble Ash	-	0.385

Table 1.3: Physicochemical analysis of *Tridax procumbens* Linn.

8.3) Activity Analysis-

8.3.1) COM crystal:

Std drug cystone tab conc. (5mg/ml)	Optical Density (O.D.)	%inhibition
control	0.22	-
5min	0.19	13.63
10min	0.15	31.81

15min	0.12	45.45
20min	0.08	63.63
25min	0.04	81.81

Table 1.5- %inhibition of std. drug (Cystone tab)

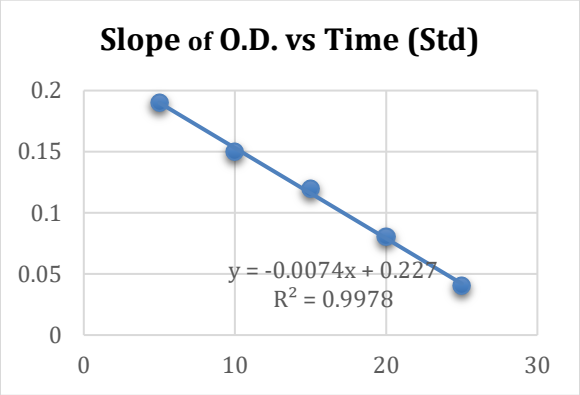

Figure 1.8-Percentage Inhibition of Calcium Oxalate Crystal by std drug (Cystone tab)

Tridax procumbens Solid 5mg/ml	Optical Density (O.D.)	%inhibition
Control	0.22	-
5min	0.17	22.72
10min	0.15	31.81
15min	0.12	45.45
20min	0.09	59.09
25min	0.07	68.18

Table 1.6-%inhibition of *Tridax procumbens L.* solid sample

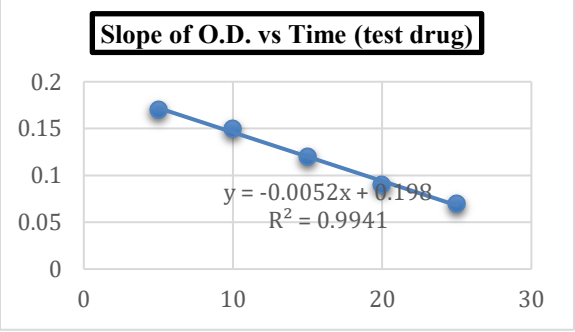

Figure 1.9 -Percentage Inhibition of Calcium Oxalate Crystal by *Tridax procumbens L.*

Figure 1.10- Evaluation tests of Crystal Aggregation Assay

8. DISCUSSION

The plant materials used for study it was identified, authenticated and collected properly**.** analytical grade solvents and reagents were used for the extraction and other tests. In the phytochemical investigations of the plant extract to identify the secondary plant metabolites or bioactive ingredients carried out on the petroleum ether extract, chloroform extract, alcoholic extract and aqueous extract of *Tridax procumbens*. The test results are found the presence of carbohydrates, alkaloids, tannins and phenolic compounds in petroleum ether extract and chloroform extract. In the alcoholic extract there is present carbohydrates, monosaccharides, alkaloid, glycosides, tannins and phenolic. In the aquous carbohydrates, alkalloid, glycosides, tannins and phenolic compounds are present.

In vitro anti urolithiasis activity analysis of aqueous extract of *Tridax procumbens* Linn by the Crystal aggregation assay method. There is comparison between the % inhibition of the standard drug ie. Cystone tablet with aqueous plant extract. And the % inhibition values of the std drug and plant extract are found to be similar hence, the aqueous extract of *tridax procumbens* shows the ant-urolithiasis activity.

9. CONCLUSION

Thus, from the above tests and results, it was concluded that the aqueous extract of plant *Tridax procumbens* Linn shows the anti-urolithiasis activity and Different types of phytochemicals such as

alkaloids, glycosides, carbohydrates, tannins and phenolic compounds present in plant.

In this study the aqueous extract of plant was used to check anti-urolithiasis activity. As compared to standard the aqueous extract of *Tridax procumbens* extract showed good in vitro antiurolithiasis activity hence dissolves the calcium oxalate crystals and can be used in kidney stone.

REFERENCE

[1] Shabana, H. A., Gairola, S., & Mahmoud, T. (2020). Tridax procumbens L. (Asterales Asteraceae), a new record to the flora of the United Arab Emirates. Biodiversity Journal, 11(4), 889-896.

[2] Sumayya sikandari and Prathima Mathad(2015). In vitro antiurolithiatic activity of Butea monosperma Lam. and Nigella sativa Linn.seeds. Ukaaz-Annals of Phytomedicine,4(1):105-107.

[3] Sanjay kumar Gupta,Madhav singh baghel,Chaturbhuja Bhuyan,B. Ravi Shankar. Ashok.BK.Panchakshari D Patil (2012). Evaluation of anti-urolithiatic activity of Pashanabhedadi Ghrita against experimentally induced renal calculi in rats. AYU (An international Quaterly journal of Research in Ayurveda.33(5):429-434.

[4] Safina, K., & Singh, D. C. (2017). A COMPREHENSIVE REVIEW OF A HEALING HERB: TRIDAX PROCUMBENS LINN. International Journal of Ayurveda and Pharma Research.

[5] Ingle KP, Deshmukh AG, Padole DA, Dudhare MS, Moharil MP, Khelurkar VC. Phytochemicals: Extraction methods, identification, and detection of bioactive compounds from plant extracts. J Pharmacogn Phytochem. 2017; 6:32–6. [Google Scholar]

[6] Azwanida NN. A review on the extraction methods uses in medicinal plants, principle, strength, and limitation. Med Aromat Plants. 2015; 4:196. [Google Scholar]

[7] Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. J Pharmacogn Phytochem. 2014; 2:115–9. [Google Scholar]

[8] Chauhan C etal. Germination, emergence, and dormancy of Mimosa pudica. Weed Biology and Management 2009; 9(1): 38–45.

[9] Barrar FSK. Text book of pharmacology. New Delhi: S. Chand; 2003, p298.

[10] Sravani P, Mohana Lakshmi S, Kumar Saravana A. Evaluation of diuretic activity of Xanthium strumarium L. Int J Preclin Pharm Res. 2010;1(1):31–4. [Google Scholar]

[11] Purohit, S. S., and Vyas, S. P. (2004b). Medicinal Plants: Cultivation A Scientific Approach. Jodhpur: Agrobios (India).

[12] Cowan, M. M. (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12, 564–582.

[13] Barnes, P. M., Bloom, B., and Nahin, R. L., (2008). Complementary and alternative medicine use among adults and children: United States, 2007. Natl. Health Stat. Report. 10, 1–23.

[14] Chauhan, B.S. and D.E. Johnson. (2008). Ecology of Two Troublesome

[15] Asteraceae Species of Rainfed Rice: Siam Weed (Chromolaena odorata) and Coat Buttons (Tridax procumbens) Johnson Weed. Science. 56: 567–573.

[16] Chatterjee, T.K. Herbal Options Eastern Traders Calcutta, 1996, p. 1, 7.

[17] Ekka, NR.: Namdeo, K.P.; Samal, P.K. Standardization Strategies for Herbal Drugs-An Overview. Res. J.Pharm. &Tech. 2008,1(4), 1. 19) Mukherjee, P.K.; Rai., S.; Bhattacharyya, S.; Debnath, P.K.; Biswas, T.K.; Jana, U.;

[18] Pandit, S.; Saha, B.P: Paul,P.K. ClinicalStudy Of 'Triphala' A Well Known Phytomedicine from India. Iran. J. Pharm. & Therap.

[19] 2005, 1,51-54. 20) Mukharjee, P.K. Quality control of herbal drugs an approachto evaluation of

[20] botanicals Business Horizons Pharmaceuticals Publications New Delhi, 2002.

[21] Kokate, C.K.; Purohit, A.P.; Gokhale, S.B. Practical PharmacognosyVallabh Prakashan New Delhi. 1996, p. 123-125.Kokoski, J.; Kokoski, R.: Salma, F.J. Fluorescence of powderedvegetable drugs under ultraviolet radiation. J. Amer. Pharm. Assoc. 1958, 47, 715-717.

[22] Lachmann, L; Lieberman, H.A.; Kanig, L.A. The Theory and Practice of Industrial Pharmacy

Varghese Publsihing House Bombay, 1976, pp. 66-70, 77.

[23] Lai SL. Ch 1. Guangdong: People's Publishing House; 2000. Clinical Trials of Traditional Chinese Materia Medica. [Google Scholar]

[24] Eisenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL. Unconventional medicine in the United States. Prevalence, costs, and patterns of use. N Engl J Med. 1993; 328:246–52. [PubMed] [Google Scholar]

[25] Mills' S. Clinical Research in Complementary Therapies: Principles, Problems and Solutions. In: Lewith GT, Jonas WB, Walach H, editors. Elsevier Science: Churchill Livingstone; 2003. pp. 211–27.

[26] agannath. N,Somashekara S.Chikkannasetty Govindadas D,Devasankaraiah G (2012). Study of anti urolithiatic activity of Asparagus racemosus on albino rats. Indian.

[27] D. A. Bhagwat, S. G. Killedar, R. S. Adnaik. Anti- diabetic activity of leaf extract of Tridax procumbens. Intnl. J. Green Pharma, 2008, 2, 126- 128.

[28] B. S. Chauhan and D. E. Germination. Ecology of Two Troublesome Asteraceae Species of Rainfed Rice: Siam Weed (Chromolaena odorata) and Coat Buttons (Tridax procumbens) Johnson Weed Science 2008, 56, 567–573.

[29] S.K. Khan, A.H.M.M. Rahman, M.S. Alam, Ferdous Ahmed, A.K.M. Rafiul Islam, and M. Matiur Rahman. Taxonomic Studies on the Family Asteraceae (Compositae) of the Rajshahi Division. Research Journal of Agriculture and Biological Sciences, 2008, 4(2), 134-140.

[30] Hanumantappa. Phytochemical and pharmacological activities of Portulacaoleracea L. in albino rats; 2014. [Google Scholar] (http://scholar.google.com/scholar_lookup?&title=Phytochemical%20and%20pharmacological%20activities%20of%20Portulacaoleracea%20L.%20in%20albino%20rats&publication_year=2014&author=Hanumantappa%2C)

[31] Jorgensen J. H. and Turnidge (2007), Susceptibility Test methods: Dilution and Disk diffusion methods, In Manual of clinical Microbiology (Volume II), Ed. Murray P. R., Baron E. J., Jorgensen J. H., Landry M. L. Pfaller M. A. 1152-1173. [Google Scholar] (http://scholar.google.com/scholar_lookup?&title=Susceptibility%20Test%20methods%3A%20Dilution%20and%20Disk%20diffusion%20methods&pages=1152-1173&publication_year=2007&author=Jorgensen%2CJH&author=Turnidge%2C&author=Murray%2CPR&author=Baron%2CEJ&author=Jorgensen%2CJH&author=Landry%2CML)

[32] Van-Burden TP, Robinson WC. Formation of Complexes between protein and tannin acid. J Agric Food Chem. 1981; 1:77.

[33] Ćujić N, Šavikin K, Janković T, Pljevljakušić D, Zdunić G, Ibrić S. Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. Food Chem. 2016; 194: 135–42.

[34] Azwanida NN. A review on the extraction methods suse in medicinal plants, principle, strength and limitation. Medicinal & Aromatic Plants 2015; 4:3. Doi: 10.4172/2167-0412.1000196

[35] Zhang Z and Li G. A review of advances and new developments in the analysis of biological volatile organic compounds. Microchemical Journal 2010; 95(2):127-139

[36] Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction Technologies for Medicinal and Aromatic Plants, (1stedn), no. 66. Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology. 2008.

[37] Ankita, J., & Jain, A. (2012). Tridax procumbens (L.): A weed with immense medicinal importance: A review. International journal of pharma and bio sciences, 3(1), 544-552.

[38] Sawant, R. S., & Godghate, A. G. (2013). Preliminary phytochemical analysis of leaves of Tridax procumbens Linn. International Journal of Science, Environment and Technology, 2(3), 388-394.

[39] Handa SS, Khanuja SPS, Longo G, Rakesh DD (2008) Extraction Technologies for Medicinal and Aromatic Plants, (1stedn), no. 66. Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology.

[40] N.N. Azwanida A review on the extraction methods uses in medicinal plants, principle, strength and limitation Med. Aromat. Plants, 4 (2015), p. 196.

[41] A. Rezvankhah, Z. Emam-Djomeh, M. Safari, G. Askari, M. Salami Microwave-sassisted extraction of hempseed oil: studying and comparing of fatty acid composition, antioxidant activity, physiochemical and thermal properties with Soxhlet extraction J. Food Sci. Technol. (2019),

[42] O.R. Alara, N.H. Abdurahman, C.I. Ukaegbu Soxhlet extraction of phenolic compounds from Vernonia cinerea leaves and its antioxidant activity J. Appl. Res. Med. Aromat. Plants (2018),

[43] SuhailAsghar, Showkat Ahmad Patni, Elias Edwin Jarald, Pankaj Kushwah, et.al. Effect of unex on ethylene glycol induced urolithiasis in rats. Int J Pharmacol.2011; 43(4): 466-468.

[44] Kok DJ, lestra JA, Doorenbos CJ, et al. The effects of dietary excess in animal protein and in sodium on the composition and the crystallization kinetics of calcium oxalate monohydrate in urines of healthy men. J Clin Endocrinol Metab.1990:4: 861-7.

[45] Mandel NS, Mandel GS. Urinary tract stone disease in the United States veteran population. II. Geographical analysis of variations in composition. J Urol; 1989:142: 1516-1.=