Asian Journal of Green Chemistry 8 (2024) 411-426

Asian Journal of Green Chemistry

Original Research Article View Article Online | View Journal

# Extraction, Phytochemical Screening, and Isolation of Active Fraction of *Turnera ulmifolia* Linn

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**ARTICLE INFORMATION**

Submitted: 2024-03-25
Revised: 2024-05-08
Accepted: 2024-05-23
Manuscript ID: AJGC-2404-1494
Checked for Plagiarism: Yes
Language Editor Checked: Yes

DOI:10.48309/AJGC.2024.454175.1494

**KEYWORDS**

*Turnera ulmifolia*
Extraction
Isolation
Spectral analysis

**ABSTRACT**

People used *Turnera ulmifolia* Linn. as an ancient remedy to treat dyspepsia and bronchitis, particularly as a tonic for various diseases such as weakness, fever, and cold. Indians commonly use the drug to treat pulmonary conditions in the thoracic zone, gastrointestinal diseases such as dyspepsia, hepatic disorders with repetitive bile release, and rheumatic diseases. *Turnera ulmifolia* (TU) was extracted using hydroalcoholic solvent. The obtained extract (ethanol: water; 70:30) was subjected for column fractionation using different solvents. After TLC analysis it was decided to further proceed with ethanol fraction for further characterization. The reason was, it displayed single fine spot in the TLC. The FT-IR, $^1$H NMR, and MS analysis was done on this fraction. Based on FTIR graph of Et: water fraction, it was concluded that the present compound may be alkane, cycloalkane with OH, and/or carbonyl functional group. For more precise analysis, the same fraction was subjected for $^1$H-NMR and Mass analysis. We have identified one active phytoconstituent. These findings are significant in light of the plant's prospective uses. Based on the present analysis, it was concluded that the active compound present in the isolated column fraction is 1-[(3*R*,5*S*,10*S*,13*S*,17*S*)-3-hydroxy-10,13-dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl]ethanone. We aimed to investigate this compound for its analgesic activity using *in vitro* and *in vivo* models and perform *in silico* screening of this compound on some potential targets of inflammation.



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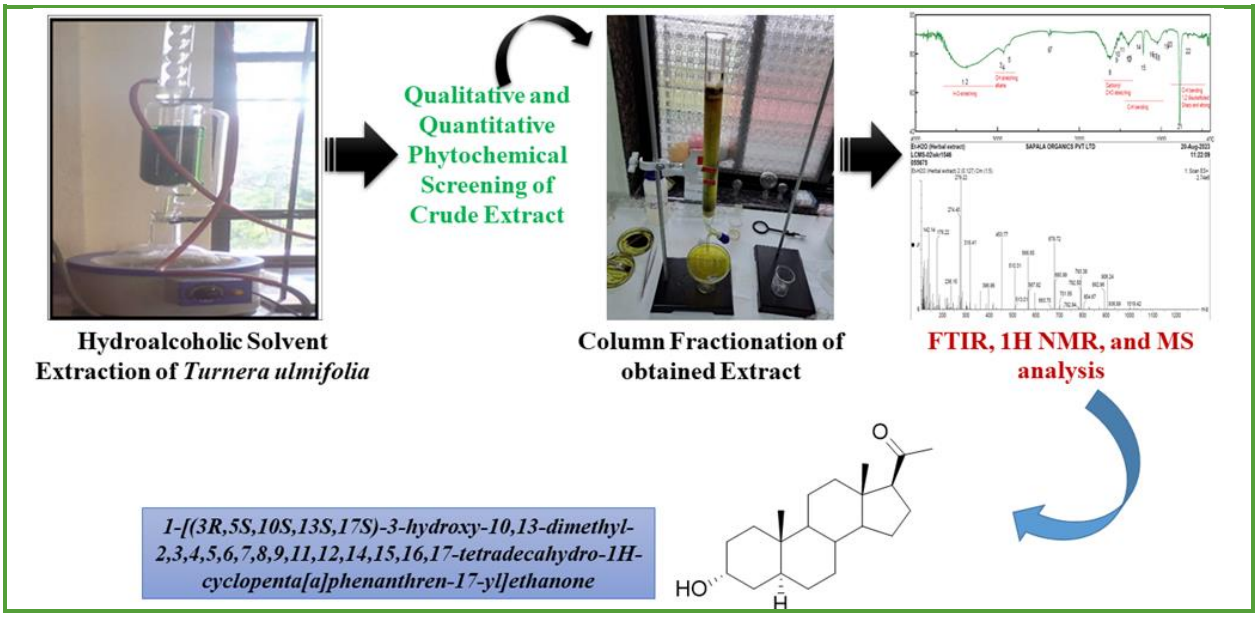
## Graphical Abstract




## Introduction

Plants have historically provided pharmaceutical chemicals and bioactive molecules, providing a wealth of natural products with different therapeutic qualities. Plant extraction and phytochemical screening are essential to use these botanical wonders in traditional medicine, pharmaceutical drug discovery, nutraceutical development, cosmetics, and functional foods [1-5]. This vibrant area of research combines traditional herbal medicine with contemporary science to study plant chemistry [6]. To begin with, the extraction of plants began with the isolation of active constituents from the complicated matrix of plant cells that could be as structural as leaves, stems, roots, and fruit. We use maceration, solvent extraction, supercritical fluid extraction, and microwave-assisted extraction to separate and concentrate these essential components, but each method has its own set of disadvantages. Once we reach the extraction point, phytochemical screening becomes a crucial procedure. A thorough testing procedure for phytoelement determination helps understanding the chemical structure of plant extracts. Among phytochemicals, we will find alkaloids, flavonoids, terpenoids, phenolic compounds, saponins, etc. Chemicals in every category possess diverse properties that are very influential in health, agriculture, and manufacturing processes [7, 8].

The phytochemical screening of the phytochemical content of the plant extracts involves identifying the constituent elements using qualitative and quantitative test methods and analyses. Accumulation of TLC, HPLC, GC-MS, NMR, and UV-Visible spectrophotometry is needed to identify and quantify phytochemicals in extracts. Herbal extract and chemical separation are two essential steps for biodiversity exploration that can facilitate improvement our understanding of medicinal potential in plants and synthesize safe and effective herbal drugs and natural health products. Therefore, the multidimensional viewpoint plays one of the key roles in the

investigation of the green realm, in return, creating unlimited opportunities to develop friendly approaches to human health and comfort by means of the chemical diversity the nature provides. The next paragraphs will explain methods like plants extraction and characterization, the usefulness of the procedure, and applications, thus clarifying plants for holding medicinal value and biotechnology [9, 10]. The combination of green chemistry concepts and plant extracts is so strong and promising that it will definitely help in the sustainable development. Green chemistry is concerned with the design of chemical products and processes that are made so as to reduce or even totally get rid of the usage and generation of hazardous substances. The plant extracts which are rich in bioactive compounds are the sources of natural and renewable materials and substances for the production of eco-friendly chemicals and materials. Green chemistry can help to combine the power of plant-derived compounds through green chemistry methodologies and, thus, to create environmentally friendly alternatives to the synthetic chemicals, which will reduce the environmental impact and support the sustainable practices in various industries. This joint work is of great importance as it can lead to the creation of safer, more sustainable, and biodegradable products that will be the good for both human health and the environment [11-14].

The genus Turnera belongs to the family *Turneraceae* and is estimated to have approximately 85 different plant species occurring mostly in tropical and subtropical America, Africa, and Madagascar. These plants display different growth patterns; among them are herbs, sub-shrubs, shrubs, and even trees. Many Turnera species have multiple applications because they are rich in resources for treating various ailments. People used *Turnera ulmifolia* Linn. as an ancient remedy to treat dyspepsia and bronchitis, particularly as a tonic for various diseases such as weakness, fever, and cold. Indians commonly use the drug to treat pulmonary conditions in the thoracic zone, gastrointestinal diseases such as dyspepsia, hepatic disorders with repetitive bile release, and rheumatic diseases. The Dutch discourage the medicinal uses of abacá, or the Bahamian setting, as part of their everyday therapeutic approach. Moreover, clinicians often use this precise anatomic element to treat a wide range of medical ailments, such as balance disorders like vertigo and menstrual-related issues like dysmenorrhea, uterine bleeding, toothache, lumbago, metrorrhagia, and dyspepsia. This botanical specification has been used to treat dysentery in the Javanese community. Because of its expectorant properties, Mexicans use it as a medication for acidic digestive disorders and to treat coughs [15-19]. Given the limited research on the TU plant and its traditional applications, it is crucial to conduct scientific research on this plant to uncover additional potential applications. In the current work, we have extracted one active phytoconstituent from the TU plant. These findings are significant in light of the plant's prospective uses.

## Experimental

### *Collection of plant material and authentication*

*Turnera ulmifolia* herb was collected in the month of May 2023 from local area of Korhur, Tirupati, Andhra Pradesh, India. From the collected plant material the herbarium was prepared and authenticated by Dr. K. Madhava Chetty, Head of Botanical Department of Sri Venkateswara University, Tirupati, 517502, Andhra Pradesh, India. A voucher specimen no. 0756 was deposited. After collecting

authentication certificate, the extraction procedure was performed.

*Soxhlet extraction using hydroalcoholic solvent*

For extraction, TU leaves and flowers were collected and washed them using distilled water to remove any dust or foreign particle, and then air-dried in the shade for a week at room temperature. It is essential to dry it in room temperature to avoid the loss of volatile phytoconstituents. About 500 g of dried plant material was grinded into a fine powder for further extraction. The resulting powder was subjected for Soxhlet extraction using Soxhlet apparatus by hydroalcoholic solvent (Ethanol: water; 70:30). Hydroalcoholic solvents, such as a mixture of ethanol and water, are commonly used for plant extraction due to several reasons. Firstly, ethanol enhances the extraction of a wide range of phytochemicals, including polar and non-polar compounds, thereby ensuring a comprehensive extraction of plant constituents. Secondly, the addition of water helps to mitigate the denaturation of thermolabile compounds and reduces the risk of irreversible structural changes during extraction. In addition, the use of a hydroalcoholic solvent provides a balance between polarity and non-polarity, making it suitable for extracting a diverse array of bioactive compounds from plant materials. Moreover, ethanol is relatively safe, cost-effective, and readily available, making it a preferred choice for plant extraction processes in both research and industrial settings. At least 74 hours were devoted to the extraction process, during which time 10 syphon cycles were completed. The dark green hue of the solvent indicated that the greatest amount of phytoconstituents had been extracted from the sample. The solvent was then evaporated off the obtained extract at room temperature. The crude extract derived from this procedure was subsequently used for further study [20-22].

*Organoleptic and physicochemical analysis of extract*

The extracted sample was subjected to different chemical and physical analysis such as color, odor, taste, pH of 1%, 10% solutions, foreign matter test, LOD, ash and extractive values, heavy metal estimation, and pesticide residues as per the procedure mentioned in literature [23-26].

*Preliminary phytochemical screening of extract*

The crude drug test sample was put through a series of qualitative tests to conduct preliminary phytochemical screening on it. Such assays are aimed to trace the presence of different classes of chemicals in the plant. I have gone through the qualitative tests like test for carbohydrates, test reducing sugars, test monosaccharides, protein test, amino acid test, fat and oil test, steroid test, test for cardiac glycosides, test for anthraquinone glycosides, saponin glycoside test, cyanogenetic glycoside test, and flavonoid test [27].

*Microbial content determination*

The solid samples were initially treated by preparing suspensions of 1 gram of samples in 9 mL of sterile distilled water. Liquid formulations were left to be dissolving or suspended by preparation of 9 mL sterile distilled water followed by addition of 1 mL of solution. The experiment consisted of serial dilutions which were evaluated using the plate pour technique in order to determine the living specimens. The plates were exposed to incubation at 37 °C for 24 hours during the test. The quadranticular by neatly in the colony counter appliance was attached, and the count of the quantity of colony forming units (CFUs) was taken. The average microbial content was calculated around these measurements by

correspondence of duplicate measurements. Adhesion and aggregation of microorganisms on food packaging surfaces were simulated using a collection of mediums such as Nutrient agar, Cetrimide Nutrient agar, Salt Nutrient agar, and MacConkey agar. The fungal growth in the sample was determined using the Sabouraud dextrose agar by pouring it into a dish and it should then be left to harden. Afterwards, a 1 ml of sample taken from each of the sample was applied, and then the agar surface was uniformly spread with it. The so grown plates were later placed in an incubator set on with-a-temperature of 27 °C for 72 hours in which bacterial and fungal counts were determined [28, 29].

*Tests for specific microorganisms*

A key requirement in the implementation of the tests designed to screen plant extracts is the microorganisms specific detection which is a determinant factor in the safety, purity, and compliance to production standards of the products. These issues are among the most important of the consumer-related ones driving a future where safer and reliable products are being offered while noticing the ethical and responsible use of plant-based materials during agricultural, industrial and every other sector of our economy is possible. After the organisms have been swabbed from meat, the tests for *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* contamination were taken per the procedure reported in the study [28–32].

*Quantitative phytochemical analysis*

The employment of the phytoscreening data for quantitative phytochemical analysis has a dramatic impact on number of fields including pharmacology, herbal medicine, food science, and agriculture. Analytical approach of this approach provides specific info about bioactive compounds present in herb ingredient. As a result, a respect and influence research as well as the producers of products acquire is made possible. The extract of this plant was tested in the laboratory for different quantitative phytochemical screening, which was Saponin screening, Alkaloid screening, Carbohydrate screening, Steroid screening, Glycosides screening, Sulfonic acid screening, Flavonoid screening, Total phenols content screening, and Tannin screening [33-35]. The phytoconstituents, the technique as well as the standards are presented in Table 1 in the appendices.

*Column fractionation of extract*

The obtained extract (ethanol: water; 70:30) was subjected for column fractionation using different solvents. The fractionation is generally depend on the polarity of the solvent system therefore we have tried different kind of solvent with different proportions. The fractionation was done till the complete vanishing of extract sample kept on the top of the column. A column with sintered disc of 300 mL capacity with 18 mm bore size have been used. The slurry of silica gel (mesh size: 60-120) was prepared in water and it was poured in the column. The cotton bolus was kept at the top of the column was kept and the extract sample of about 5-8 gram was kept on the top and again one cotton bolus kept over it to avoid unequal distribution of extract. The different solvent was used for extraction are litsed in Table 2 along with the details of fractions obtained through it. The total run time of fractionation was about 48 hours. The working photograph of the column fractionation is depicted in Figure 1S (Supporting information).

**Table 1.** The phytoconstituents, the technique, and the standards

| Phytoconstituent | Methods | Standard |
|---|---|---|
| Carbohydrate | Phenol sulphuric acid method | Glucose |
| Protein | Barford method | Albumin |
| Saponin | Simple solubility method | - |
| Steroids | Liberman-Burchard reaction method | Diosgenin |
| Alkaloids | Bromocresol green reagent | Atropin |
| Flavonoids | Aluminum chloride colorimetric method | Quercetin |
| Tannins | Foilin denis reagent | Tannic acid |
| Total Phenolic | Folin ciocalteu method | Gallic acid |

**Table 2.** The solvents used for fractionation

| Sr. No. | Solvent | Run time | Fraction quantity | Color |
|---|---|---|---|---|
| 1 | Ethyl acetate (100%) | 4-5 h | 28 mL | Light yellow |
| 2 | Benzene: Ethyl acetate (60:40) | 4 h | 24 mL | Yellowish green |
| 3 | Benzene: Ethyl acetate (80:20) | 3 h | 27 mL | Greenish brown |
| 4 | Chloroform (100%) | 7 h | 19 mL | Yellow |
| 6 | Chloroform (100%) | 5 h | 16 mL | Yellowish brown |
| 6 | Acetone (100%) | 8-9 h | 31 mL | Pale yellow |
| 7 | Acetone (100%) | 6-7 h | 25 mL | Greenish |
| 8 | Ethanol: Water (70:30) | 12-15 h[a] | 80 mL | Yellowish green |

[a] *Till get vanished completely*

Here, we used different solvents of varying polarity range from non-polar to polar just to make sure for the separation of any kind of compound present in the crude extract.

## Results and Discussion

### *Organoleptic and physicochemical analysis of extracts*

TU was extracted using hydroalcoholic solvent. The organoleptic properties of the obtained extract are provided in Table 3. The results of physicochemical analysis are summarized in Table 4.

Organoleptic testing includes judging how a thing tastes, smells, looks, and feels in the mouth. Products should undergo organoleptic testing to ensure they are up to par with what the company and the customer have agreed upon. Assessing the organoleptic qualities of a product is crucial to establishing its commercial viability and storage life. It is important to look at the sensory experience of a product even if research shows that it is safe and meets nutritional promises. All of the senses (taste, smell, touch, sight, and hearing) are involved. The extract met the criteria for acceptable organoleptic qualities.

Physicochemical analysis was used to investigate the following chemical properties of the test substances, all of which have been recognised as major structural components contributing to penetration, irritation, or sensitization: Instead of incept molecules, the physicochemical possession of active plant matters needs to be authenticated to ensure the drug is not tampered with or contaminated. The essence of determining the total ash content of different drugs lies simply in realizing how potent or pure an ingredient is. Such material is referred as the "foreign organic matter" and typically includes one or more component of the above components.

**Table 3.** The organoleptic properties of TU extract

| Parameters | Observation |
|---|---|
| Color | Dark Greenish or Greenish Black |
| Odor | Agreeable slightly like Henna |
| Taste | Bitter mixed with Pungent |
| Texture | Semisolid |
| Yield (%) | 8.2 |

**Table 4.** The physicochemical analysis of *Turnera ulmifolia* extract

| Parameters | Observation |
|---|---|
| pH | |
| 1% Solution | 6.6 |
| 10% Solution | 5.1 |
| Foreign content | 0% |
| LOD | 5.19% |
| Ash values | |
| Total Ash value | 7.2% |
| Acid insoluble ash value | 0.91% |
| Water soluble ash value | 3.78% |
| Sulphated ash value | 1.80% |
| Extractive values | |
| Alcohol-soluble extractive | 16.21% |
| Water-soluble extractive | 13.43% |
| Heavy metals estimation (present) | Absent |
| Pesticide residues | Absent |

Along with the defined components and description given, or the specified limit by the monograph in the specific organ or organs of the organism from where the preparation is provided, there may also be other components that the organ or organs being pulled from. Inherently, the presence of ash in herbal medicines reflects the relative level of inorganic material.

Ammonium and nitrogen are two of the most common organic waste products. It is the qualities of both action and normative that assess the appropriateness of herbal medicines. Given that case, one of the determinants to prohibit heavy metals is to do frequent testing and assessing of the level of these metals, particularly in the plant setting. Metal poisoning in the body has been linked to brain and central nervous system problems, low organ function, changes in blood composition as well as pulmonary, renal/urinary system, hepatic/liver, and other organs' damage. Pesticide residues in food have been related to cancer, liver and nervous system diseases, and even blindness. The long-term effects might include a drop in sperm count and fertility, a rise in cholesterol levels, a higher risk of infant death, and a slew of metabolic and genetic disorders. All the parameters were under the acceptable limits and favorable to be considered as drug candidate in pharmaceutical stream.

*Microbial content determination*

It is vital to develop adequate criteria for the microorganisms that may be found in herbal remedies in order to limit the hazards that are posed to the health of patients. The existence of moulds and feces-associated coliforms indicates

that there is a possibility for contamination. An analysis was done to assess the microbiological contamination of herbal medications. It was observed that all the values were below the permitted limits and no any presence of microbial contents observed [36-38].

*Preliminary phytochemical screening of extract*

The phytochemical screening method is a well-known to the scientific method of analyzing, studying, extracting, and testing specific classes (phyto constituents) contained in different parts of the roots as a new medication discovery source. With that being the case, the base would be able to get rid of its active integral for further investigations. The discovery of the bioactive compounds extracted from medicinal plants is aimed at identifying the compounds, which in turn is a critical step towards the development of new medicines thus, the initial phase of this process starts with the screening of the phytochemicals. Table 5 indicates the outcome of phytochemicals analysis. Duo to the presence of different phytochemicals in the extract, it may be noted it is much in different disease.

**Table 5.** The results of preliminary phytochemical screening of TU extract

| Chemical Test | Observations |
|---|---|
| Carbohydrates | Present |
| Reducing sugars | Absent |
| Monosaccharides | Absent |
| Proteins | Present |
| Amino acids | Present |
| *Test for Fats and Oil* | |
| i) Solubility test | Partially in Chloroform & Benzene |
| ii) Saponification test | Present |
| Steroids | Absent |
| Cardiac Glycosides | Present |
| Anthraquinone Glycosides | Absent |
| Saponin Glycoside | Present |
| Alkaloids | Absent |
| Tannins and Phenolic compounds | Present |
| Flavonoids | Present |

**Table 6.** Quantitative evidence of different phytotonutrients from TU extract

| Parameters | Results (%) |
|---|---|
| Carbohydrate | 21.67±1.23 |
| Protein | 0.93±0.02 |
| Saponin | 3.64±0.71 |
| Steroids | 1.02±0.21 |
| Alkaloids | 0.99±0.11 |
| Flavonoids | 19.39±1.15 |
| Tannin | 17.83±1.09 |
| Total Phenolics | 15.78±1.03 |

*The values are expressed as mean±S.D*

*Quantitative estimation of phytoconstituents*

The amount of a phytochemical in addition to its level of concentration, detected in the extract, is determined through the process of quantitative analysis. The analysis with the use of quantitative techniques produces the results that are listed in Table 6.

The kinetic study was carried out by a spectrophotometer at an absorption spectrum that was obtained by gradually increasing the fraction concentration from 100 mg/ml to 10 mg/ml. A diet high in carbs should not underestimate the importance they do in ensuring a healthy body. Through its regulation, a healthy digestive system is guaranteed and this it saves you from digestion problems. Carbohydrates are therefore fundamental to biological life in that they feature as constituents of glucose that may then be transformed into energy used by the body to power physiological processes and to enable physical activity. Energy is the main carbohydrate function in the organisms: they contribute to providing necessary fuel to organs such as brain and kidneys as well as to heart muscles and parts of our central nervous system. To illustrate, fiber is the kind of carbohydrate that not only does it help with digestion but it also makes one fuller real quick stale and brings the cholesterol in blood back again to normal levels [39, 40]. The total carbohydrate content in extract was found to be 21.67%. Unlike animal proteins but according to the regular protein functions, plant proteins can find the applications in enzymatic, structural, and functional body actions (photosynthesis, biosynthesis, transport, immunity, etc.). Apart from that, they represent the media of storage to fill he needs of peachlings with respect to developing and feeding. In the total proteins of the extract, 0.93% content was detected [41].

Saponin content in legumes cause a decline in the count of lipids in the blood that converts into a reduction in the chances of cancer and the glucose response in the blood. The prevention of dental cavities and blood platelet aggregation, hypocalciuria therapy in humans and atagous disease in dwellers of polluted territories are some of the potential areas for the application of diet saponins. The sum of the quantity of saponins in the tincture turned out to be 3.64% [42].

Among various chemical compounds found in both the animal and plant kingdoms, plant steroids is a particular group all of its own. Glucocorticoids are steroidal medication secreted in inflammatory diseases. The extraction total yield of steroids was 1.02%. Accordingly, this plant may have therapeutical values only on non-inflammatory diseases [43]. Alkaloids serve two purposes in plants: these secondary chemical make the distasteful due to their bitter taste and their effect on the plant's development. In the field of medicine, alkaloids play the most essential role for anesthesia, protective system of the heart, as well as the anti-inflammatory and pharmaceutical drugs. For example, morphine, strychnine, quinine, ephedrine, and nicotine have long been known for their alkaloid nature and commonly utilized in medical context. The total alkaloid content of the extracts was about 0.99% [44].

Disease risks can be decreased for medical care providers and heavily stressed individuals by employing natural remedies such as herbs and fruits with high flavonoid contents. Several studies have demonstrated the causality between their consumptions and endothelium function through the activation of protein kinase B (Akt). This endothelial nitric oxide synthase stimulates the production of a molecule named nitric oxide which enhances the movement of oxygen to the different body

organs. The total extraction was the sum of about 19.39% flavonoids [45].

Tannins could be present in barks of trees, wood, leaves, elder buds, stems, fruits, seeds, roots, and a fissure in young leaves that mainly cause on leaves of oak trees. The tannin compounds of plants show a property of protecting various plant structures such as xylems, epidermis, and vascular cells apart from those listed. Tannin is the substance in the wood of the tree that does not allow harmful bacteria or fungus to enter into the tree, unless the tannin is kept in the bark. Along with wood, tannins may also be sourced. A total tannins amount in soaked dried fruit was determined to be 17.83% units [46].

Naturally occurring substances called phenolic compounds are present in plants, and those antioxidant compounds have redox qualities. In this case, these qualities are responsible for the plant's antioxidant action. Plant extracts operate through the hydroxyl bonds formation (which involve the hydroxyl groups of plants) that are used in the capture of free radicals. The effect was approximately 15.78% by the total phenol content in extract. The elemental analysis via above screening appliances showed an optimum level of different phytochemicals that may be effective in relieving variety of diseases [47-49].

*Identification of active compound from column fraction*

*Thin-layer chromatography (TLC)*

The obtained fractions were subjected for TLC analysis to check for the number of chemical compounds present in the fraction. The TLC images, Rf values, and observation are presented in Table S1 (Supporting information). The solvent system used for TLC was benzene: ethyle acetate: ethanol (4:4:2).

*Spectral analysis of fraction*

After TLC analysis, it was decided to further proceed with ethanol: water (70:30) fraction for further characterization. The reason was it displayed single fine spot in the TLC. The FT-IR, $^{1}H$ NMR, and MS analysis was done on this fraction. The broad peak at 3200 to 3400 $cm^{-1}$ confirms the presence of –OH stretching.

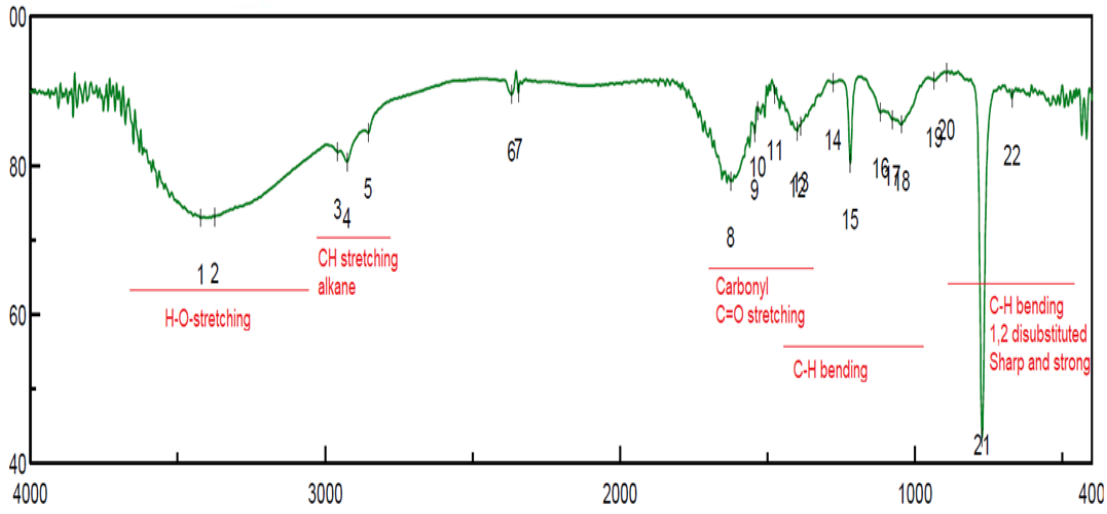
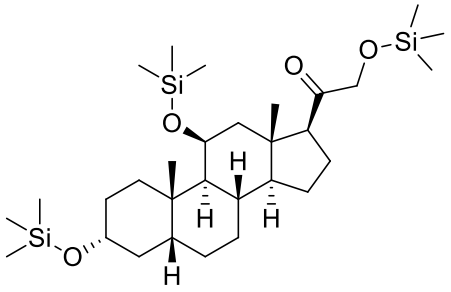
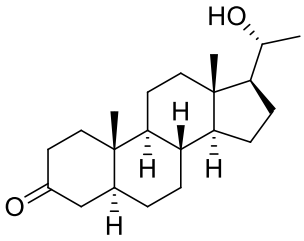
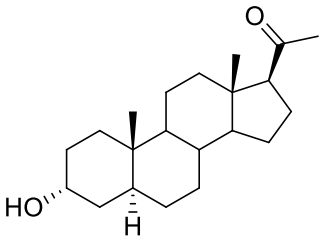
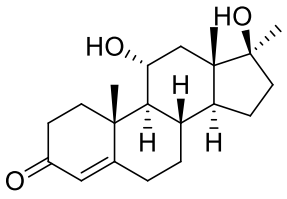



**Figure 1.** FT-IR of Et: water fraction indicating presence of non-aromatic compound

**Table 7.** Predicted compounds from Mass analysis

| Compound name with Structure | Code | Exact mass | Observed peak in spectrum |
|---|---|---|---|
|  1-[(3R,5R,8S,9S,10S,11S,13S,14S,17S)-10,13-dimethyl-3,11-bis(trimethylsilyloxy)-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl]-2-trimethylsilyloxyethanone | UK-1 | 566.36 | 566.65 |
|  Pregnan-3-one, 20-hydroxy-, (5a,20R)- | UK-2 | 318.49 | 318.41 |
|  1-[(3R,5S,10S,13S,17S)-3-hydroxy-10,13-dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl]ethanone | UK-3 | 318.49 | 318.41 |
|  11a-Hydroxy-17a-methyl testosterone | UK-4 | 318.45 | 318.41 |

The small or weak peaks at 2800 to 2900 $cm^{-1}$ shows the –CH stretching of alkanes. A moderate sharp peak at 1450 to 1750 $cm^{-1}$ indicate the presence of –C=O stretching whereas, sharp peak at 1200 to 1300 $cm^{-1}$ shows the presence of –CH bending. The peak at 600 to 850 $cm^{-1}$ indicate the –CH bending of aliphatic substituted alkanes (Figure 2).

Based on FT-IR graph of Et: water fraction, it was concluded that the present compound may be alkane, cycloalkane with OH and/or carbonyl functional group. For more precise analysis, the same fraction was subjected for Mass analysis by LC-MS. From analysis we have predicted few compounds and short listed them considering the FT-IR analysis. The mass spectrum is depicted in Figure S2 (Supporting information). By searching different compounds having similar kind of molecular weights as obtained from mass spectrum on https://webbook.nist.gov/chemistry/mw-ser/ we got almost 45-60 compounds. Out of them, we have selected best matching non-aromatic compounds, as listed in Table 7. In mass spectra, we found many peaks but the peak at 318.41 resembles with the structure UK-3 ($C_{21}H_{34}O_2$) in the Table 7. One peak was observed at 301 which exactly matched with the probable fragment of UK-3 i.e. $C_{21}H_{33}O^+$. The peak at 274.40 resembles with second fragment peak of UK-3 i.e. $C_{19}H_{29}O^+$.

To confirm the structure of this compounds, we have compared the 1H NMR and fortunately it is matching perfectly with UK-3. Accordingly, we concluded that the isolated compound may be UK-3. In Figure S3 (Supporting informatiom), the experimental $^1$H-NMR and predicted $^1$H-NMR is given. Therefore, based on the present analysis, it was concluded that the active compound present in the isolated column fraction is 1-[(3R,5S,10S,13S,17S)-3-hydroxy-10,13-dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl]ethanone. We aimed to investigate this compound for its analgesic activity using *in vitro* and *in vivo* models and perform *in silico* screening of this compound on some potential targets of inflammation.

## Conclusion

TU was extracted using hydroalcoholic solvent. The sensorial properties with physicochemical analyses of the produce extract was carried out and all parameters were ideal. In microbial content determination, it was observed that all the values were below the permitted limits and no any presence of microbial contents observed. Throughout the qualitative and quantitative assessment, we were able to discover that the extract keeps the right proportions of the different phytoconstituents, which have been related to the anti-inflammatory effects of phyto drugs. The obtained extract (ethanol: water; 70:30) was subjected for column fractionation using different solvents. After TLC analysis, it was decided to further proceed with ethanol: water (70:30) fraction for further characterization. The reason was that it displayed single fine spot in the TLC. The FTIR, $^1$H-NMR, and MS analysis was done on this fraction. FTIR graph of Et: water fraction it was concluded that the present compound may be alkane, cycloalkane with OH, and/or carbonyl functional group. For more precise analysis, the same fraction was subjected for Mass analysis by LC-MS. From the present analysis, it was concluded that the active compound present in the isolated column fraction is 1-[(3R,5S,10S,13S,17S)-3-hydroxy-10,13-dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl]ethanone. Our objective was to examine the analgesic efficacy of this drug via the use of *in vitro* and *in vivo* models. In addition, our objective is to conduct *in silico* screening of this drug against putative inflammatory targets.

## Disclosure Statement

No potential conflict of interest was reported by the authors.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Authors' contributions**

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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**How to cite this manuscript**: Vuppula Sruthi*, G. Baba Shankar Rao. Extraction, Phytochemical Screening, and Isolation of Active Fraction of *Turnera ulmifolia* Linn. *Asian Journal of Green Chemistry*, 8(4) 2024, 411-426. DOI: 10.48309/AJGC.2024.454175.1494