Phytochemical Studies, GC-MS Analysis and In vivo Analgesic, Anti-inflammatory and Antidiarrheal Activity of Chaerophyllum villosum Wall. ex Dc. and Achillia millefolium L

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Phytochemical Studies, GC-MS Analysis and *In vivo* Analgesic, Anti-inflammatory and Antidiarrheal Activity of *Chaerophyllum villosum* Wall. ex Dc. and *Achillia millefolium* L.

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ABSTRACT

Background:

Chaerophyllum villosum (Ganjari) and *Achillea millefolium* L. (Yarrow) are highly medicinal plants widely used in both folk and official medicine.

Materials and Methods:

The analgesic activities of *C. villosum* and *A. millefolium* were evaluated by hot plate method and acetic acid-induced writhing test, the anti-inflammatory activities were evaluated by carrageenan-induced paw oedema method and antidiarrheal activity was carried out by charcoal meal test.

Results:

The quantitative phytochemical analysis and gas chromatography-mass spectrometry analysis of both plants showed several phytoconstituents including alkaloids, phenols, tannins, flavonoids, saponin, terpenoids with highest concentration of Tri tetracontane (7.52%) and methyl stearate (8.5%) in methanolic and chloroformic extract of *C. Villosum* respectively. Octadecanal (6.21%) and n-hexadecanoic acid (10.6%) were showing maximum concentration in methanolic and chloroformic extract of *A. millefolium* respectively. The chloroformic extract of *A. millefolium* showed higher (25.33±4.72) peripheral analgesic activities at 300 mg/kg. The chloroformic extract of *Chaerophyllum villosum* exhibited higher (11.56±0.15) central analgesic activities at 300 mg/kg. In antidiarrheal activity the methanolic extract of *Chaerophyllum villosum* highly (17.06±1.5) inhibited charcoal movement at 300 mg/kg. In anti-inflammatory activities the chloroformic extract of *Chaerophyllum villosum* at 300 mg/kg showed high inhibition in the paw volume (1.09±0.04) after 5 hours of induction of carrageenan.

Conclusion:

The results suggested that *Chaerophyllum villosum* and *Acheilia millefolium* exhibited significant peripheral and central analgesic activity, anti-inflammatory and antidiarrheal activity which elucidated its use in the treatment of pain, inflammation and gastrointestinal disorders.

Keywords: *Chaerophyllum villosum; Achillia millefolium;* GC-MS; anti-inflammatory, analgesic, antidiarrheal

1. Introduction:

Different medicinal plants have been utilized as traditional medicines for therapeutic purposes from the beginning of mankind. Plants are rich sources of several chemical compounds that are both biologically fascinating and useful [1]. The World Health Organization (WHO) estimates that conventional medicine serves the primary healthcare requirements of about 80% of the world's population. Due to their accessibility and safety, herbal medicines have been utilized extensively since the prehistoric era [2] An unpleasant feeling called pain can range from minor localized discomfort to excruciating anguish. It consists of both emotional and physical elements. Nerve stimulation causes the physical component of pain. Pain can be localized, as in the case of an in-jury, or it can be widespread, as in the case of conditions like fibromyalgia. The transmission of pain impulses to the brain, where they can be modified by a variety of variables, is mediated by

certain nerve fibers. By working on the sensory nerve system, analgesic drugs delay or eliminate analgesia or pain without appreciably affecting awareness [3].

The immune system participates in the physiological process of inflammation. The primary function of inflammation is to protect organisms from microbial infections, while it can also occasionally serve as a physiological defense mechanism against diseases like cancer. But under specific settings, inflammation can become dangerous and result in life-threatening clinical diseases [4]. The treatment of inflammation and illnesses linked to it involves the use of many therapeutic medication classes. These drug classes include biologics, nonsteroidal anti-inflammatory medicines, and corticosteroids (NSAIDs) [5]. But using these medications has been linked to a number of problems. Glaucoma, hyperglycemia, heightened susceptibility to infection, hypertension, muscle wasting, and Cushing's habitus are some adverse effects associated with the usage of corticosteroids. The necessity to find and create substitute medications has arisen due to the different issues and negative side effects linked with these medications [6].

In the intestinal system, diarrhea is caused by an imbalance between the absorptive and secretory mechanisms, which leads to an excessive loss of fluid in the faeces [7]. It results in more than 58 million deaths annually among newborns and young children under the age of five [8]. The synthetic drugs used to treat diarrhea have many side effects [9]. The medicinal plants are abundant in several phytoconstituents that have antidiarrheal potential and can be utilized to treat digestive issues with fewer negative effects [10].

With 1600–1700 genera and 24,000–30,000 species, the Asteraceae is the world's most diverse vascular plant family. With over 650 species spread throughout 15 tribes, it is also the largest plant family in Pakistan [11]. The Asteraceae family's *Achillea* genus has between 110 and 140 species and has a significant amount of plant resources. All over the world, *Achillea* species are used as a natural treatment for wounds, bleeding, gastrointestinal problems, headache, inflammation, aches, and spasmodic diseases [12]. *Achillea millefolium*, also known as Khati-Booti, Baranjasif, and Jarri, is herbaceous, perennial plant that is erect and 0.2 to 0.9 meters tall [13]. It has been utilized in the pharmaceutical sector to create herbal medications that use the stem, leaves, and flowers as therapeutic components [14]. Leucorrhea, influenza, pneumonia, ulcers, diarrhea, cancer, tumors, warts, and other conditions have all been successfully treated with

yarrow. It is also used as an anti-hematic, contraceptive, analgesic, anti-inflammatory, antipyretic, anthelmintic, antibacterial, antifungal, anticancer, antioxidant, and anti-edematous agent [15]. Alkaloids, flavonoids, steroids, saponins, phenols, and tannins were among the phytochemicals reported in *A. millefolium* [16].

The family Apiaceae includes 434 genera and 3780 species. Members of the apiaceae family are primarily found in the northern hemisphere. It constitutes about 56 genera and 167 species in Pakistan [17]. The apiaceae family Includes species that are economically significant and employed in several pharmaceutical and cosmetic industries. About 110 different species are present in the genus *Chaerophyllum,* which belongs to apiaceae family and is found in temperate and sub-temperate regions of Asia, Africa, and Europe [18]. Studies on the phytochemistry of *Chaerophyllum* species have found that they include secondary plant metabolites like lignins, phenylpropanoids, polyacetylenes, phenolic acids, and related substances called flavonoid glycosides [19]. In many countries, the *Chaerophyllum* species are also employed in traditional medical procedures. Plants belonging to Apiaceae family are good source of metabolites such as terpenoids, saponins, coumarins, flavonoids and steroids. Fresh stems and leaves of *Chaerophyllum macropodum* can occasionally be added to salads, and dried leaves and roots can be brewed into a tea to treat coughs, allergies, and sore throats [20]. The perennial plant *Chaerophyllum villosum* has a height limit of 0.30 meters. It has elongated roots, hairy stem which bear 2-3 pinnate velvety leaves. It is locally used as food and can be used to relieve stomach pain, cold, and cough. Jangli gajar is the common name of *Chaerophyllum villosum* [21]. It is extensively distributed in East Asia Himalayas, which includes Nepal, China, India, and Bhutan, and grows in humid and cold climates to a height of 2100–3500 m [18].

Although traditional healers in various regions of the world commonly mention the antinociceptive, anti-inflammatory, and anti-diarrheal properties of *A. millefolium* and *C. villosum*, there have yet to be any scientific publications about their analgesic and anti-inflammatory properties. Therefore, it is very important to assess the analgesic, anti-diarrheal and anti-inflammatory properties of both plants scientifically. The objective of the current investigation was to examine the analgesic, anti-diarrheal and anti-inflammatory effects of methanol and chloroform extracts of *A. millefolium* and *C. villosum.*

2. Material and Methods

2.1 Plant Collection

Fresh parts of *Achillea millefolium* and *Chaerophyllum villosum* including roots, stem and leaves were collected in August 2020 from the Miranjani peak (2,992 m), NathiaGali, Khyber Pakhtunkhwa, Pakistan. Mr. Ghulam Jelani, a taxonomist at the department of Botany, University of Peshawar, in Pakistan, identified it. The plants were assigned voucher specimen numbers M. Adil Bot.2244 (PUP) and M. Adil Bot.2245 (PUP).

2.2 Experimental Animals:

Specific pathogen free mice (aged 8-10 weeks) were obtained from Veterinary Research Institute, Peshawar. They were adapted to laboratory condition by keeping them in 12 Hr Dark/Light cycle condition at a temperature of about 24 ± 2℃ with relative humidity of about 55± 5% with easy access to food and water [23]. Guidelines of the ethical committee of Animals and Federation of European Laboratory Animal Science Associations were strictly followed in order to mitigate stress, unbearable pain and discomfort in model animals. Protocols used for animal study were duly approved and experiments were performed following the rules and regulations recommended by the Experimentation on Animals Committee, Peshawar. Experiments were managed and controlled by well experienced researchers with the help of lab assistants. Mice were anaesthetized using 1.5%-2 % isoflurane introduced via nose cone. The mice were subjected to an overdose of anaesthesia using Xyline (7.5 mg/Kg) and Ketamine HCl (100 mg/kg) just before performing euthanasia [24] by cervical dislocation. The animal study was reviewed and approved by Ethical Committee Pharmacy Lab, University of Peshawar.

2.3 Chemicals, Reagents and Equipment used:

1.5%-2% isoflurane, Xyline, Ketamine HCl (100 mg/kg), 97% methanol, 97% chloroform, ammonium hydroxide, 1% ammonia, HCl, butanol, potassium ferrocyanide, diclofenac sodium, acetic acid, castor oil, atropine sulphate, saline, charcoal meal, indomethacin, carrageenan

Ricoh grinder (MG 601 model), spectrophotometer, plethysmometer (Ugo Basile, model 7140)

2.4 Preparation of Extract

The gathered plant components underwent a thorough cleaning and washing with tap water. For more than two weeks, the garbled plant parts were dried in a low-heat oven. The dried plant components were pulverised with the help of Ricoh grinder (MG601 model) and kept in a suitable container for a couple of days. For two weeks, the 500g of powdered plant material was steeped in 1000 cc of a mixture of 97 percent methanol and chloroform. Both extracts were filtered by Whatman filter paper No.1823. To get concentrated crude extracts, the resulting methanolic and chloroformic extracts were heated to 40 ^{o}C in a rotary evaporator.

2.2.1 Quantitative Phytochemical Screening

The quantitative chemical tests of methanol and chloroform extracts of *A. millefolium* and *Chaerophyllum villosum* were investigated for detection of alkaloids, flavonoids, saponin, phenol, terpenoids and tannins.

2.2.2 Determination of Alkaloids

The alkaloids were measured by following the methodology of Harbone 1973. A 50 ml of a 10% acetic acid solution were added to 5 g of the methanolic and chloroformic extract. After shaking, the mixture was let to stand for four hours. Whatman No. 42 filter paper was then used to filter the combination. By evaporating the filtrate in a steam bath, it was reduced to 25% of its original volume. Ammonium hydroxide (NH_4OH) was added drop by drop until the extract's alkaloid was completely precipitated. The alkaloid precipitate was recovered by filtering it through weighted filter paper, washing it in a solution of 1 percent ammonia (NH_3), and then drying it for an hour at 80 °C in the oven. Later, it was chilled in a desiccator and weighed again. The weight of alkaloid was calculated by weight difference and represented as a percentage of the sample examined using the following formula:

$$\% \text{ Alkaloid} = \frac{W2 - W1 \times 100}{W}$$

Where;

W = weight of sample W1 = weight of empty filter paper, W2 = weight of paper + alkaloid precipitate

2.2.3 Determination of Flavonoids

It was determined by applying the technique of Harborne, 1973. A 100 ml of a 2 M hydrogen chloride solution were used to boil 5 g of the methanol and chloroform extract for 30 minutes. After allowing the mixture to cool, Whatman No. 42 filter paper was used to filter the mixture. The precipitated flavonoid was weighed, then dried at 80°C in an oven, chilled in a desiccator, and reweighed. The weight of the flavonoid, expressed as a percentage of the sample weight examined, was determined by the change in weight. The following formula was used to determine the quantity of flavonoid:

$$\% \text{ Flavonoids} = \frac{W2 - W1 \times 100}{W}$$

Where;

W = weight of sample analysed

W1 = weight of the empty crucible

W2 = weight of the filter paper + flavonoid precipitate

2.2.4 Determination of Saponins

The saponin content was determined by gravimetric method as described by Harborne, 1973. The five grams of methanol and chloroform extract was added in 50 ml of a 20 percent aqueous ethanol solution. The mixture was cooked on a water bath for 90 minutes at 55 ^{o}C with intermittent stirring. It went through Whatman No. 42 filter paper to be purified. A second extraction of the residue was performed using 50 cc of 20% ethanol. The two extracts were then mixed and 40 cc of the mixed extracts were again reduced over a water bath at 90^{o}C. After transferring the concentration into a 250 ml separating funnel, 40 ml of diethyl ether was added, and the mixture was violently agitated. The aqueous layer was recovered during partition separation, but the ether layer was discarded. Up till the aqueous layer's colour changed to clear, partition re-extraction was carried out regularly. With 60 cc of regular butanol, the saponins were extracted. The mixed n-butanol extract was evaporated to dryness in a pre-weighed evaporating dish after being cleaned with a 5 percent aqueous sodium chloride solution. It was then weighed

after being further oven-dried at 60°C. The following formula was used to calculate the saponin content;

$$\% \text{ Saponin} = \frac{W2 - W1 \times 100}{W}$$

Where;

W = weight of sample W1 = weight of empty evaporating dish

W2 = weight of dish + saponin extract

2.2.5 Determination of Phenol

The phenol content was determined by the Folin-Ciocalteu colorimetric method by following the protocol of Pearson 1956. A test tube containing 0.2 g of the sample was filled with 10 ml of methanol and thoroughly agitated. After 15 minutes, it was filtered using Whatman filter paper (No. 42). A test tube containing 1 ml of the extract and 5 ml of distilled water was filled with 1 ml Folin-Ciocalteu reagent. At room temperature, the colour was given one to two hours to develop. At a wavelength of 760 nm, the absorbance of the produced colour was measured. After two phases, the average was calculated.

2.2.6 Total Terpenoids Determination

The total terpenoids were calculated by following the protocol of Fergusan,1956. Two grams of plant powder was added to 50 millilitres of 95 percent ethanol and left for 24 hours. After 24 hours it was filtered by using Whatman No. 42. The filtrate was heated from 60 to 80°C, extracted with petroleum ether and dried in a water bath (Memmert, Germany) at 65°C. The total terpenoids was calculated as;

$$\text{Percentage of Total Terpenoids (\%)} = \text{weight of residue} \div \text{weight of sample taken} \times 100$$

2.2.7 Quantification of Tannins

The content of tannin was measured by the methodology of Buren and Robinson 1969. In 500 mg extract 50 mL distilled water was added with continuous stirring for one hour. After one hour it was filtered and volume was raised up to 50 ml. In a test tube 5 ml of filtrate was taken and

2 mL $FeCl_3$ (0.1 M) in HCl (0.1 N) and potassium ferrocyanide (0.008 M) was added. In comparison to standard curve of gallic acid 120 nm absorbance was measured with the help of spectrophotometer. Results were depicted in the form of GAE (mg of Gallic Acid Equivalent) per gram of dried extracts.

2.3 Gas Chromatography-Mass Spectrometry (GC/MS)

A gas chromatography-mass spectrometry of the crude extract of *C. Villosum* and *A. Millefolium* was carried out by coupling of Thermo GC Trace Ultra version 5.0 with Thermo MS DSQ II following the protocols proposed. To purify the samples, the mixtures were divided on a ZB 5-MS capillary regular non-polar column (30 m 0.25 mm ID 0.25 l m FILM). The temperature of the column was kept at 70°C with increasing rate of 2°C/minute. Finally, the increase in temperature was raised up to 260°C at 6°C/minute with holding time of about ten minutes. The split less mode was used to introduce the particle-free, diluted sample (10 mL/min split flow, 1 min splitless period). Helium was used as the carrier gas at a constant flow rate of 1 mL/min, and 1 L of sample was injected. Peak area normalisation was used to quantify the relative percentages of crude extract elements. In full scan mode, the mass spectral scan range was adjusted to 50 to 650 (m/z). By comparing the retention indices of the compounds with those of real samples stored on the Wiley and main lab computer library search software, the compounds were identified.

2.4 Analgesic Activity

2.4.1 Acetic acid Induced Writhing Method

In the current study, albino mice of either sex weighing 18–22 g were employed. The approach of [25] Koster *et al.,* 1959 was followed for evaluating the analgesic effect of the methanolic and chloroformic extracts of *C. villosum* and *A. millefolium.* Four groups consisting of five mice were made. Group 1 acted as the control and received intraperitoneal administration simply of normal saline (ip). Groups 3-4 received a single oral dosage of methanolic and chloroformic extracts (100 mg/kg, 200 mg/kg, and 300 mg/kg, respectively) while Group 2 received the normal dose of diclofenac sodium (25 mg/kg). These extracts were given orally an hour before receiving a 0.6 percent v/v acetic acid injection intraperitoneally. After five minutes the number of writhing was counted. The following formula was used to compute the % analgesic results for all of the aforementioned groups.

$$\% \text{ Analgesic effect } = 100 - \frac{\text{No of writhing in tested animals}}{\text{No of writhing in control animals}} \text{ X } 100$$

This method was approved by Ethical Committee Pharmacy Lab, University of Peshawar.

2.4.2 Hot Plate Method

The procedure of Eddy and Leimbach 1953 [26] was followed for studying the central analgesic effect of methanolic and chloroformic extracts of *C. villosum* and *A. millefolium* by the hot plate method. In the current study, albino mice of either sex weighing 18–22 g were employed. Four groups of five mice each were created by randomly dividing the mice. Group 1 received normal saline as a control, Group 2 received the conventional medication diclofenac sodium (25 mg/kg), and Groups 3-4 had various dosages of methanol and chloroform extracts (100, 200, and 300 mg/kg body weight, respectively) given orally. The control group and test group animals were placed on hot plate (55±0.5°C) and jumping of animals was observed. The reaction time of each animal was observed from 30 to 90 minutes after administration of plant extracts. Eddy's Hot Plate Apparatus was the equipment employed. Percent in increase of the response time, before and after administration of drug was noted.

This method was also approved by Ethical Committee Pharmacy Lab, University of Peshawar.

2.5 Antidiarrheal Activity

The antidiarrheal potential of *A. millefoium* and *C. villosum* was studied by charcoal meal test by following the protocol of [27]. In this study, albino mice of either sex weighing 18–22 g were employed. Four groups of five albino mice were formed for this test. Each rat in each group received 1 mL of castor oil orally initially to induce diarrhoea. Saline (2mL/kg) was administered orally to Group I (the control group) after 1h. Atropine sulphate, a conventional medication, was given to Group II (10 mg/kg), while Groups III–IV received methanol and chloroform extracts (100, 200, and 300 mg/kg) of plants. All animals were given 1mL of charcoal meal (10% suspension of charcoal in 5% gum acacia) orally after 1hr. All animals were slaughtered one hour after the charcoal meal was administered, and the distance travelled by the charcoal meal in the intestine from the pylorus to the caecum was measured and expressed as a percentage of distance moved by the formula below;

Intestinal transit (%) = (D/L) x 100

Where;

D = distance covered by charcoal (in meters) and L = intestinal length (in meters).

The above-mentioned procedure was approved by Ethical Committee Pharmacy Lab, University of Peshawar.

2.6 Anti-inflammatory Activity

The carrageenan-induced paw oedema method described by [28] was used to examine the anti-inflammatory effect of the chloroform and methanol extract of *C. villosum* and *A. millefolium*. In the current investigation, albino mice of either sex weighing 18–22 g were used. The experimental animals were divided into 4 groups containing 5 animals in each group. Groups 3 and 4 were given various doses of medicinal plant extracts i.e., 100mg/kg, 200mg/kg, and 300 mg/kg, whereas Groups 1 served as the negative control and Groups 2 as the positive control, treated with 10 mg/kg indomethacin, respectively. In the right hind paw of the rats of all groups acute inflammation was induced by injecting 0.1 ml 1% suspension of carrageenan. Then with the help of plethysmometer (Ugo Basile, model 7140) the paw volume was measured at 1hr to 5hr. Percentage inhibition of paw volume was calculated by the following formula;

$$\% \text{ inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where;

Vt- means increase in paw volume in rats treated with test compounds.

Vc- means increase in paw volume in control group of rats.

The protocol used above was duly approved by Ethical Committee Pharmacy Lab, University of Peshawar.

2.7 Statistical Analysis

The data present in tables is expressed as mean ± SD**.** The Dunnet's t-test is used for analysis of results.

3. Results

3.1 Quantitative Phytochemical Analysis

The results of phytochemical screening of methanol and chloroform extract of *Chaerophyllum villosum* and *Achellia millefolium* revealed that alkaloids (27.6%), phenols (34.5%), tannins (26.5) and saponin (31.6%) were greater in methanolic extract while flavonoids (32.6%) and terpenoids (35.5%) were greater in chloroformic extract of *A. millefolium* (Table 1). However, phenols (35.5%), tannins (30.0) and saponin (28.6%) were greater in methanolic extract while alkaloids (31.4%), flavonoids (20.5%) and terpenoids (33.3%) were greater in chloroformic extract of *C. villosum* (Table 1).

3.2 Gas Chromatography-Mass Spectrometry

Gas chromatography-mass spectrometry profiling identified the probable phytochemicals in the methanolic and chloroformic extract of *C. villosum* and *A. millefolium* by analysing their molecular structure and retention time (Tables 2-5; Figure 1-4). In methanolic and chloroformic extract of *C. Villosum* twenty-one phytoconstituents were detected by GC–MS (Tables 2-3; Figure 1-2). Similarly, eleven phytoconstituents were detected in the methanolic extract of *A. Millefolium* and eleven phytoconstituents were detected in its chloroformic extract (Tables 4-5; Figure 3-4). The results showed that phytoconstituents found in maximum concentration in methanolic extract of *C. villosum* was tritetracontane (7.52%) followed by myristic acid (3.52%) and E-9-tetradecenoic acid (2.41%) (Tables 2; Figure 1). In the chloroformic extract of *C. Villosum* methyl stearate (8.53%) was detected in highest concentration followed by stigmasterol (6.34%) and ethyl iso-allocholate (4.50%) (Tables 3; Figure 2). In methanolic extract of *A. millefolium* bioactive constituents detected in maximum amount was octadecanal (6.21%) followed by ethanone, 1-(4-hydroxy-3, 5-dimethoxyphenyl) (5.35%) and ergost-5-en-3-ol, (3. beta.) (3.85%) (Tables 4; Figure 3). The phytoconstituents detected with maximum concentration in chloroformic extract of *A. millefolium* include n-hexadecanoic acid (10.6) %) followed by Squalene (5.43%) and 9,12,15 octadecatrienoic acid (3.67%) (Table5; Figure 4).

3.3 Analgesic Activity

3.3.1 Acetic Acid Induced Method

Cyclooxygenase (COX) enzymes: COX enzymes, particularly COX-1 and COX-2, are involved in the production of prostaglandins, which are lipid molecules that play a role in inflammation and pain signaling. Nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen and aspirin work by inhibiting COX enzymes, thereby reducing pain and inflammation. The phytochemical of *Chaerophyllum villosum* and *Achellia millefolium* such as alkaloids, tannins, flavonoids, terpenoids, saponins and some other chemical compounds of *C. villosum* and *A. millefolium* such as myristic acid, E-9-tetradecenoic acid, methyl stearate, stigmasterol and ethyl iso-allocholate, octadecanal, have exhibited beneficial properties as analgesic agents. The methanolic and chloroformic extract of *C. villosum* and *A. millefolium* showed significant ($p< 0.001$) anodyne activity against acetic acid induced writhing response in mice which is less than diclofenac sodium (25 mg/kg) which showed highly significant ($p< 0.001$) analgesic activity. The percent inhibition of methanolic and chloroformic extract of *C. villosum* at 300mg/kg was 59.0%, 52.5% while percent inhibition of methanolic and chloroformic extract of *A. millefolium* at 300mg/kg was 58.0%, 62.0% whereas percent inhibition of diclofenac sodium was 65.0%. (Table 6,7).

3.3.2 Hot plate Model

Cyclooxygenase (COX), which is an enzyme responsible for the synthesis of prostaglandins. Prostaglandins are lipid compounds involved in inflammation and pain. COX has two isoforms: COX-1, which is constitutively expressed in various tissues, and COX-2, which is induced during inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and aspirin work by inhibiting COX, thereby reducing the production of prostaglandins and decreasing pain and inflammation. The phytochemical of *Chaerophyllum villosum* and *Achellia millefolium* such as, tannins, flavonoids, alkaloids, terpenoids, saponins and some other chemical compounds of *C. villosum* and *A. millefolium* such as, linoleic acid ethyl ester, 9,12,15 octadecatreinoic acid, E-9-tetradecenoic acid, methyl stearate, stigmasterol and ethyl iso-allocholate, octadecanal, have exhibited beneficial properties as analgesic agents.The methanol and chloroform extract of *C. villosum* and *A. millefolium* significantly ($p< 0.001$) increased the latency time at 300mg/kg after 90 min which was comparable with diclofenac sodium which showed highly significant ($p< 0.001$)

analgesic activity. The percent inhibition of methanolic and chloroformic extract of *C. villosum* and *A. millefolium* at 300mg/kg after 90 min was 61.0 % and 64.0% while the percent inhibition of *A. millefolium* at 300mg/kg was 59.0%, 63.0% after 90 min whereas the percent inhibition of diclofenac sodium was 65.0%. (Table 8,9).

3.4 Antidiarrheal Activity

3.4.1 Charcoal Meal Test

Some antidiarrheal agents, like kaolin and pectin, have adsorptive properties. They bind to toxins, bacteria, or excess water in the intestines, forming a bulkier stool that is less watery. This helps reduce the frequency and fluidity of diarrhea. The phytochemical of *Chaerophyllum villosum* and *Achellia millefolium* such as terpenoids, tannins, flavonoids, alkaloids, saponins and some other chemical compounds of *C. villosum* and *A. millefolium* such as, methanon (1-hydroxyly clohexyll) Phenyl, Stigmast-5-en-3-ol, (3. beta.), Tetracontane, 9,12,15 octadecatreinoic acid, E-9-tetradecenoic acid, methyl stearate, stigmasterol and ethyl iso-allocholate, octadecanal, have exhibited beneficial properties. These compounds can help reduce intestinal contractions and relieve spasms associated with diarrhea. The methanolic and chloroformic extracts of *C. villosum* showed significant ($p<0.001$) reduction in charcoal meal transit time at 200mg/kg and 300mg/kg while the methanolic and chloroformic extracts of *A. millefolium* showed significant ($p<0.001$) reduction in charcoal meal transit time at 300 mg/kg. At 300 mg/kg, the methanolic and chloroformic extracts of *C. villosum* inhibited charcoal movement by 67 and 58 percent, respectively, while methanol and chloroform extract of *A. millefolium* inhibited charcoal movement by 56 and 60 percent, respectively. The percent inhibition of Atropine sulphate was 64%. (Table 10, 11).

3.5 Anti-inflammatory Activity

3.5.1 Carrageenan Induced Paw Oedema

Cyclooxygenase (COX): COX is an enzyme that plays a central role in the synthesis of prostaglandins, which are lipid molecules involved in inflammation. There are two isoforms of COX: COX-1 and COX-2. COX-2, in particular, is induced during inflammation and is associated with the production of prostaglandins that promote pain, fever, and swelling. The phytochemical

of *Chaerophyllum villosum* and *Achellia millefolium* such as saponins, terpenoids, tannins, flavonoids, alkaloids, and some other chemical compounds of *C. villosum* and *A. millefolium* such as, n-hexadecanoic acid, 2,4-bis (1,1-dimethylethyl, Tetracontane, 9,12,15 octadecatreinoic acid, E-9-tetradecenoic acid, methyl stearate, stigmasterol and ethyl iso-allocholate, octadecanal, have exhibited beneficial properties these compounds have shown effective inhibition of COX-2 and demonstrate mild inhibitory effects on COX-1. The methanolic and chloroformic extract of *C. villosum* and *A. millefolium* at 300 mg/kg showed a significant ($p<0.001$) reduction in paw volume in a dose dependent manner at 5th h when compared to diclofenac sodium which also showed a significant ($p<0.001$) decrease in paw volume. The percent inhibition of methanolic and chloroformic extract of *C. villosum* and *A. millefolium* at 300 mg/kg was 58.3%, 61.3% and 59.0%, 57.0%. The chloroformic extract of *C. villosum* at 300 mg/kg showed maximum percent inhibition 61.3% in paw volume than *A. millefolium* (57.0%) at 300mk/kg but less than diclofenac sodium which showed 63.0% inhibition in paw volume at 5th h. (Table 12,13).

4. Discussion

Chaerophyllum villosum (Ganjari) and *Achillea millefolium* L. (Yarrow) are highly medicinal plants widely used in folk medicine from many years. In the present study, the phytochemical and GCMS analysis of methanol and chloroform extracts of *C. villosum* and *A. millefolium* revealed the presence of various bioactive constituents (Table 1-Table 5, Figure 1-4). These bioactive phytoconstituents might be responsible for the therapeutic ability of various extracts of *C. villosum* and *A. millefolium*.

Phytochemicals, which are often bioactive substances found in medicinal plants, have demonstrated therapeutic qualities such as antioxidant, antibacterial, anticancer, analgesic and anti-inflammatory effects etc. The methanol and chloroform extract of *A. millefolium* and *C. villosum* showed the presence of various phytochemicals (Table 1). According to Njoya *et al*. 2018 tannins are used to cure urinary tract infection and dysentery fahal 2018 reported that flavonoids have anticancer, immunomodulatory, anti-inflammatory and antioxidant potential. Phenols function as an antioxidant and prevent the development of cancer, heart disease, and osteoporosis. It is antiseptic, which decreases inflammation [29]. reported that the saponins possess antimicrobial and anticancer potential. According to Yang 2020 terpenoids possess antitumor, anti-

inflammatory, analgesic, antibacterial, antiviral, antimalarial effects, promote transdermal absorption, prevent and treat cardiovascular diseases, and have hypoglycemic activities.

A widely accepted and effective pain model for evaluating peripherally acting analgesics is acetic acid induced writhing reflex model [30]. Acetic acid induces pain by cyclooxygenase pathway of the arachidonate cascade from local release of arachidonic acid and prostaglandins [31]. In the current research *C. villosum* and *A. millefolium* methanol and chloroform extracts at higher doses (300 mg/kg) considerably decreased the number of writhing in mice. This decrease suggested that the plant extracts exerted its analgesic effects via a peripheral route by inhibiting prostaglandin formation by binding to acetic acid-sensitive visceral receptors [32]. The peripheral analgesic effect of the plant extracts might be attributed to the presence of several phytoconstituents such as flavonoids, alkaloids, phenols, saponins detected in phytochemical screening and GCMS analysis (Table 1-5, Figure 1-4).

An appropriate assay for evaluation of centrally acting analgesic drugs is the hot plate method [33]. Centrally acting analgesic drugs e.g opioids prolonged the reaction time in hot plate model by acting on supra spinal and spinal receptors [34]. Diclofenac sodium, is used as the standard drug in this test. This is a widely used nonsteroidal anti-inflammatory drug (NSAID) against pain and inflammation [35]. The present study showed that methanol and chloroform extract of *C. villosum* and *A. millefolium* at higher dose of 300mg/kg significantly prolonged the response to heat stimulus in mice (Table 8,9). The methanol and chloroform extracts of *C. villosum* and *A. millefolium* showed a mild central analgesic activity as compared to the standard drug diclofenac sodium. The methanol and chloroform extracts of *C. villosum* and *A. millefolium* revealed positive dose-dependent effects in both bioassays, demonstrating that they have both peripheral and central analgesic efficacy. These results are in accordance with [36] who reported significant peripheral and central analgesic potential from *Achillea fragrantissima* at higher dose. The presence of phytochemicals such as flavonoids, terpenoids, steroids, alkaloids, tannins, etc. that were found in the methanolic and chloroformic extract may be responsible for the analgesic effect of both extracts of *C. villosum* and *A. millefolium* (Table 1). These findings support the claims made by [37] that terpenoids, saponins, flavonoids, tannins, phenols, among other compounds, are accountable for analgesic efficacy [38]. reported that phenolic compounds may regulate eicosanoid synthesis, suppress the binding of pro-inflammatory mediators by inhibiting

the activity of COX-2 and NO synthase [39] reported that flavonoids make interaction with the 5-HT3 and 5-HT2A receptors or enhance endogenous serotonin, which inhibits prostaglandins and lessens discomfort. A number of compounds in the methanol and chloroform extracts of *C. villosum* and *A. millefolium* were also identified through GC-MS that might be responsible for their analgesic activities (Table 2-Table 5, Figure 1-4) [40] revealed that tetracontane possess anti-inflammatory and analgesic activity [41] reported that ethyl iso-allocholate exhibit analgesic, antimicrobial, anti-inflammatory and diuretic properties.

A widely used method for assessing the antidiarrheal activity of plant extracts is castor oil-induced diarrheal model. In mice the castor oil changes the permeability of electrolytes through the intestinal mucosa and thus induces diarrhea [42]. Castor oil contain, ricinoleic acid, which increases production of prostaglandins and causes irritation and inflammation of intestinal mucosa and results in increased gastrointestinal motility and secretion [43]. In the current study, methanol and chloroform extracts of *C. villosum* and *A. millefolium* at high dose i.e. *300mg/kg* significantly decreased gastrointestinal motility in a dose dependent manner by inhibiting prostaglandin synthesis induced by castor oil. According to [44] the plant extracts exhibited their antidiarrheal effect by inhibition of ricinoleic acid secretion, which enhances absorption of electrolytes in the intestinal mucosa by stimulating Na^+,K^+ ATPase activity [45]. claimed that decrease in gastrointestinal motility facilitate intestinal water and electrolyte absorption by lengthening the time spend by gastrointestinal contents in the intestine. The antidiarrheal effect of *C. villosum* and *A. millefolium* could probably be associated with the occurrence of phytochemicals such as tannins, alkaloids, terpenoids, flavonoids, and saponins (Table 1). According to [46] flavonoids are capable of limiting prostaglandin E2 driven intestinal secretion by decreasing the release of prostaglandins and autocoids [47] reported that the tannins convert to protein tannates in the intestinal mucosa, reducing peristaltic motion and intestinal output [48] claimed that terpenoids prevent the release of prostaglandins and autacoids; phenols strengthen the intestinal mucosa, decrease secretion, and have a strong impact. A common animal paradigm for evaluating acute inflammation is carrageenan-induced paw edoema. The researchers widely use this model for assessing the anti-inflammatory potential of natural compounds [49]. Typically, it is a biphasic curve model. First-phase inflammation that develops within the first hour may be brought on by injury at the injection site or by the secretion of serotonin and histamine while release of bradykinin, protease,

prostaglandin, and lysosomes triggers the second phase. In present study methanol and chloroform extract of *C. villosum* and *A. millefolium* extract at a dose of 300mg/kg significantly inhibited both the phases of carrageenan induced rat paw edema. The methanol and chloroform extracts of both plants were more effective from 3rd hour, which indicated that extracts might had inhibited synthesis of prostaglandins. The effects of NSAIDS are usually investigated by acute carrageenan induced inflammation method, as it works on the principle of inhibiting cyclooxygenase involved in prostaglandin secretion [50]. Therefore, it was concluded that methanol and chloroform extract of *C. villosum* and *A. millefolium* at higher dose of 300mg/kg showed its anti-inflammatory effect by decreasing secretion of prostaglandin by inhibition of cyclooxygenase activity [51] reported similar higher anti-inflammatory effect from *Achillea nobilis* at higher dose. Alkaloids, flavonoids, saponins, tannins, and other compounds found in both plants might be responsible for their anti-inflammatory properties (Table 1). According to [52] flavonoids reduce inflammation by inhibiting the secretion of prostaglandins and other associated enzymes [53]. claimed that tannins possess its anti-inflammatory potential by blocking cyclooxygenase-1 activity [54] documented that alkaloids diminish the severity of the oedema caused by carrageenan by reducing the histamine-induced vascular permeability. Additionally, anti-inflammatory effects of both plant extracts might be due to the presence of fatty acids such as hexadecanoic acid, linoleic acid, oleic acid etc which were identified by GCMS analysis (Table 2-Table 5, Figure 1-4). It is in accordance with [55] who claimed that linoleic acid and hexadecanoic acid might be used as anti-inflammatory agents.

5. Conclusion

The results of phytochemical and gas chromatography-mass spectrometry analysis of *Chaerophyllum villosum* and *Achillea millefolium* confirmed that both plants are rich sources of natural compounds. According to the results of the analgesic activity both methanol and chloroform extracts of *C. villosum* and *A. millefolium* have strong, dose-dependent peripheral and central analgesic activity, which supports the use of these plants in the treatment of pain. The anti-diarrheal activity showed that *C. villosum and A. millefolium* methanol and chloroform extracts protected mice from castor-oil induced diarrhea in a dose dependent manner validating the possibility that these plants are rich sources of antidiarrheal agents and could be used to treat gastrointestinal disorders. Following carrageenan injection, the methanol and chloroform extracts

of *C. villosum* and *A. millefolium* dramatically decreased paw edema in rats in a time- and dose-dependent manner, indicating the potential use of carrageenan as a potent anti-inflammatory agent. The analgesic, anti-diarrheal, and anti-inflammatory properties of both plants might be attributed to the existence of several of active metabolites in their crude extracts. However, the particular secondary metabolites in *C. villosum* and *A. millefolium* extracts with analgesic, antidiarrheal, and anti-inflammatory activities need to be isolated, characterized and quantified.

Author Contributions

Muhammad Adil; Supervision, Conceptualization, Data Curation, Ghulam Dastagir: Formal Analysis, Visualization, Fatin Zubair Filimban; Investigation, Funding Acquisition. Muhammad Naseer; Resources, Original Draft Preparation, Ambrin: Writing – Review & Editing, Atifa Quddoos: Validation, Writing Ayaz Ali Sher: Project Administration.

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The data such as the source file associated with this finding are available from the corresponding author upon request.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Yasmen, N., Aziz, M., Tajmim, A., Akter, M., Hazra, A. K., & Rahman, S. M. Analgesic and anti-inflammatory activities of diethyl ether and n-hexane extract of *Polyalthiasuberosa* leaves. *Evidence-Based Comp. Alter. Med*, (2018).

2. Shanta, A. P., Perveen, F. F., Das, R., Awoal, M. A., Hoque, M. M., Panday, A. S., &Saha, S. Evaluation of analgesic and antidiarrheal activity of ethanolic extract of *Swieteniamahagoni* (Meliaceae) leaves. *Res. J. Pharm. Med. Sci*, 2, 59-63. (2018).

3. Al-Mansur, M.A., Siddiqi, M.M.A., Saha, K Analgesic, antidiarrheal and antidepressant activities of *Anethumsowa* Linn.in Swiss-Albino mice model. *Bangladesh Pharma. J*. 21, 1-6. https://www.banglajol.info/index.php/BPJ/article/view/37899/25764. (2018).

4. Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Zhao, L. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, 9, 7204. https://pubmed.ncbi.nlm.nih.gov/29467962. (2018).

5. Moura, M.D.G., Lopes, L.C., Silva, M.T., Barberato-Filho, S., Motta, R. H. L., & de Cássia Bergamaschi, C. Use of steroid and nonsteroidal anti-inflammatories in the treatment of rheumatoid arthritis: systematic review protocol. Medicine, 97(41). (2018).

6. Amo-Mensah, J., Darko, G., Borquaye, L. S. Anti-inflammatory and antioxidant activities of the root and bark extracts of *Vitexgrandifolia* (Verbanaceae). *Scientific African*, 10, e00586. https://www.sciencedirect.com/science/article/pii/S2468227620303240. (2020).

7. Zubair, K.L., Farhina, R.L., Mir, M.A.M., Sariful, I.H.. Antidiarrhoeal activity and total tannin content of ethanolic leaf extract of *Codiaeumvariegatum*. *Dhaka Univ. J. Pharm. Sci*. 14, 87-90. https://www.banglajol.info/index.php/JPharma/article/view/23740.(2015).

8. Derebe, D., Abdulwuhab, M., Wubetu, M., Mohammed, F. Investigation of the antidiarrheal and antimicrobial activities of 80% methanolic leaf extract of *Discopodiumpenninervum* (hochst.). *Evidence-Based Complement. Alter. Med.* 2018, 1-7. https://www.hindawi.com/journals/ecam/2018/1360486. (2018).

9. Teferi, M. Y., Abdulwuhab, M., Yesuf, J. S. Evaluation of in vivo antidiarrheal activity of 80% methanolic leaf extract of *Osyrisquadripartita*Decne (Santalaceae) in Swiss Albino Mice. *J. Evidence-based Integ. Med*. 2019, 1-9. https://pubmed.ncbi.nlm.nih.gov/30889972/.(2019).

10. Birru, E. M., Asrie, A. B., Adinew, G. M., Tsegaw, A. Antidiarrheal activity of crude methanolic root extract of *Idigoferaspicata*Forssk.(Fabaceae). *BMC Complement. Alter. Med*. 2016, 16, 1-7. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4974709/.(2016).

11. Muhammad, S., Hussain, M., Rahman, I. U., Shah, G. M., Ijaz, F., Ullah, K. Indigenous medicinal usage of family Asteraceae in Sadda Lower Kurram Agency: A case study. *Asian J. Sci. Tech*. 2016, *7*, 3998-4003.https://www.researchgate.net/publication/312764787. (2016).

12. Barda, C., Grafakou, M. E., Tomou, E. M., Skaltsa, H. Phytochemistry and Evidence-Based Traditional Uses of the Genus *Achillea* : An Update (2011–2021). *Sci. Pharma*. 2021, 89, 50. https://www.mdpi.com/2218-0532/89/4/50. (2021).

13. Lakshmi, T., Geetha, R. V., Roy, A., Kumar, S. A. Yarrow (*Achilleamillefolium* Linn) a herbal medicinal plant with broad therapeutic use-A review. *Int. J. Pharm. Sci. Rev. Res*. 2011, 9, 136-141. http://globalresearchonline.net/journalcontents/volume9issue2/Article-022.pdf. (2011).

14. Ali, S. I., Gopalakrishnan, B., Venkatesalu, V. Pharmacognosy, phytochemistry and pharmacological properties of *Achilleamillefolium* L.: A review. *Phytother. Res*. 31, 1140-1161. https://pubmed.ncbi.nlm.nih.gov/28618131/.(2017).

15. Daniel, P. S., Lourenço, E. L. B., Sete Da Cruz, R. M., De Souza Goncalves, C. H., Marques Das Almas, L. R., Hoscheid, J., ... and Alberton, O. Composition and antimicrobial activity of essential oil of yarrow (*Achilleamillefolium* L.). *Australian J. Crop Sci*. 14, 545-550. https://www.cropj.com/daniel. (2020).

16.Yaseen, M., Ahmad, M., Wani, T. A., Ahmad, M., Gani, B. A., Qureshi, R. Phytochemical screening and antioxidant activity of extracts of the leaf and stem of *Achilleamillefolium*. *Int.J. Sci. Res*. 2, 55-59.https://www.researchgate.net/publication/321329468. (2017).

17. Awan, I. U. R., Mehmood, S., Awan, Z. U. R., Rahman, A., Ullah, F., Khan, H. U., & Marwat, s. U. Distribution of family apiaceae (umbelliferae) in district bannu, khyber pakhtunkhwa, pakistan. *Fuuast Journal of Biology*, *9*(1), 179-181. (2019).

18. Joshi, R. K., and Mathela, C. S. Volatile oil composition and antioxidant activity of leaf of *Chaerophyllumvillosum* Wall.ex DC from Uttrakhand, India. *Rec. Res. Sci. Technol*. 5, 25-28. https://www.researchgate.net/publication/297329561. (2013).

19. Hayta, S., and Celikezen, F. C. Evaluation of essential oil composition, antioxidant and antimicrobial properties of *Chaerophyllumcrinitum*Boiss (Apiaceae) from Turkey: A Traditional Medicinal Herb. *J. Biological. Sci.* 16, 72-76. https://scialert.net/fulltext/?doi=jbs.2016.72.76. (2016).

20. Ali, S. Biological activity and chemical compounds of the hexane extracts from *Chaerophyllum macropodum* in two different habitats. *J. Med. Plants Res*. 7, 1406-1410. https://academicjournals.org/article/article1380724698. (2013).

21. Mehta, P.S., and Bhatt KC. Traditional soap and detergent yielding plants of Uttaranchal. *Int. J. Trad. Know.* 6, 279-284. http://nopr.niscair.res.in/bitstream/123456789/920. (2007)

23. Wang, L., Feng, Y., Wang, J., Luo, T., Wang, X., Wu, M., ... & Wang, J. Arbutin Ameliorates Murine Colitis by Inhibiting JAK2 Signaling Pathway. *Frontiers in pharmacology*, *12*. (2021).

24. Sultana, N., Chung, H. J., Emon, N. U., Alam, S., Taki, M. T. I., Rudra, S., ... & Al Mamun, A. Biological Functions of Dillenia pentagyna Roxb. Against Pain, Inflammation, Fever, Diarrhea, and Thrombosis: Evidenced From in vitro, in vivo, and Molecular Docking Study. *Frontiers in Nutrition*, *9*. (2022).

25. Koster, R., Anderson, M., De Beer, E.J. Acetic acid for analgesics screening.Federation Proceeding, 18, 412-7. (1959).

26. Eddy, N.B., and Leimbach, D. Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. *J. Pharma. Exp. Therap*. 107, 385–393. https://pubmed.ncbi.nlm.nih.gov/13035677/(1953).

27. Mascolo, N., Izzo, A.A., Avtore, G., Barbato, F., Capasso, F. Nitric oxide and castor oil-induced diarrhea.*J. Pharmacol. Exp. Ther*. 268, 291-95.https://pubmed.ncbi.nlm.nih.gov/8301570/. (1994).

28. Winter, C.A., Poster, C.C. Effect of alteration in side chain up on anti-inflammatory and liver glycogen activities in hydrocortisone ester.*J. American Pharmacol. Soc*. 46, 515-519.https://www.scinapse.io/papers/1970574897. (1957).

29. Sinku, R., and Sinha, M.RPhytochemical screening of the ethanolic extract of some medicinal plants of Chotanagpur Plateau from Jharkhand, India.*Int. J. Curr. Adv. Res*. 7, 9323- 9326. https://journalijcar.org/issues/phytochemical-screening-ethanolic-extract-some-medicinal-plants-chotanagpur-plateau-jharkhand. (2018).

30. Zihad, S. N. K., Bhowmick, N., Uddin, S. J., Sifat, N., Rahman, M. S., Rouf, R., ...&Sarker, S. D. Analgesic activity, chemical profiling and computational study on *Chrysopogonaciculatus*. *Front. Pharmacol*. *9*, 1164. (2018).

31. Sarmento-Neto, J. F., Do Nascimento, L. G., Felipe, C. F. B., De Sousa, D. P. Analgesic potential of essential oils. *Molecules*, 21, 20. (2015).

32. Uka, E., Ndem, J. I., Iberi, P. A., Aluko, E. O. In-vivo analgesic activity of ethanol leaf extract of *Sphenocentrumjollyanum* in albino mice. *International. Journal. Resesarch. Pub*. 64, 16-16. (2020).

33. Rapacz, A., Rybka, S., Obniska, J., Sałat, K., Powroźnik, B., Pękala, E., &Filipek, B. Evaluation of anticonvulsant and antinociceptive properties of new N-Mannich bases derived from pyrrolidine-2, 5-dione and 3-methylpyrrolidine-2, 5-dione. *Naunyn-schmiedeberg's Arch. Pharmacol*, 389, 339-348. (2016).

34. Brijesh, S., Daswani, P., Tetali, P., Antia, N., Birdi, T. Studies on the antidiarrhoeal activity of *Aegle marmelos* unripe fruit: Validating its traditional usage. *BMC Compl.Altern.Med. 9*, 47. (2009).

35. Chen, C., Yang, Y., Yu, M. F., Shi, S., Han, S., Liu, Q. H., Shen, J. Relaxant action of diclofenac sodium on mouse airway smooth muscle. *Front. Pharmacol*. 10, 608. (2019).

36. Abdel-Rahman, R. F., Alqasoumi, S. I., El-Desoky, A. H., Soliman, G. A., Paré, P. W., &Hegazy, M. E. Evaluation of the anti-inflammatory, analgesic and anti-ulcerogenic potentials of *Achilleafragrantissima* (Forssk.). *South African J. Bot*. 98, 122-127. (2015).

37. Dev, S.K., Shukla, A., Choudhury, P.K., Singh, G.K. Analgesic and anti-nociceptive activity of hydroethanolic extract of *Capparis decidua* Linn. *Asian J. Pharm. Pharmacol.* 1, 40-44. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3081447/ (2015).

38. Rahman, M. M., Rahaman, M. S., Islam, M. R., Rahman, F., Mithi, F. M., Alqahtani, T., ...& Uddin, M. S Role of phenolic compounds in human disease: current knowledge and future prospects. *Molecules*, 27, 233. (2021).

39. Peethambar, S.K., Puttaswamya, R., Vinayaka, K.S., Padukonea, S., Achur, R.N. Pharmacological and gross behavioral studies on *Memecylonterminale*Dalz, a medicinal plant

from Western Ghats in southern India.*World J. Pharm. Sci*. 1, 81-92. https://www.researchgate.net/publication/257869919. (2013).

40. Arora, S., and Meena, S. GC-MS Profiling of *Ceropegiabulbosa*Roxb. var. *bulbosa*, an endangered plant from Thar Desert, Rajasthan. *The Pharma Inn. J*. 6, 568-573. https://www.thepharmajournal.com/archives/2017/vol6issue11/PartH/6-11-71-607.pdf. (2017).

41. Naz, R., Roberts, T. H., Bano, A., Nosheen, A., Yasmin, H., Hassan, M. N., Anwar, Z. GC-MS analysis, antimicrobial, antioxidant, antilipoxygenase and cytotoxic activities of *Jacarandamimosifolia* methanol leaf extracts and fractions. *PLoS One*, 15, e0236319. https://pubmed.ncbi.nlm.nih.gov/32726328/(2020).

42. Bulbul, I. J., Uddin, M. E., Nahar, N., Kuddus, M. R., &Haque, M. R. Antidiarrheal activity of four different species of *Litsea* available in Bangladesh. *Biomed.Pharmacol. J.* 14, 1259-1266. (2021).

43. Rahman, M.K., Chowdhury, M.A.U., Islam, M.T., Chowdhury, M.A., Uddin, M.E., Sumi, C.D. Evaluation of antidiarrheal activity of methanolic extract of *Marantaarundinacea* Linn.leaves. *Adv. Pharmacol. Sci.* 2015, 6. (2015

44. Tadesse, E., Engidawork, E., Nedi, T., &Mengistu, G. Evaluation of the anti-diarrheal activity of the aqueous stem extract of *Lantana camara* Linn (Verbenaceae) in mice. *BMC Compl. Alter. Med*. 17, 1-8. (2017).

45. 45 Mekonnen, B., Asrie, A. B., &Wubneh, Z. B. Antidiarrheal activity of 80% methanolic leaf extract of *Justiciaschimperiana*. *Evidence-Based Compl. Alter. Medicines*. (2018).

46. Dosso, K., N'guessan, B.B., Bidie, A.P., Gnangoran, B.N., Méité, S., N'guessan, D., Ehilé, E.E. Antidiarrhoeal activity of an ethanol extract of the stem bark of *Piliostigmareticulatum* (Caesalpiniaceae) in rats. *African J. Trad.Compl.Altern. Med.* 9, 242-249. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3746628. (2012).

47. Suleiman, M. M., Oyelowo, B. B., Abubakar, A., Mamman, M., Bello, K. D. T. A controlled study to investigate anti-diarrhoeal effect of the stem-bark fractions of *Terminalia avicennioides*

in laboratory animal models. *Internationnal Journal of Veternary Science. Medicines.*5, 14-22.https://www.sciencedirect.com/science/article/pii/S2314459916300783. (2017).

48. Asrie, A. B., Abdelwuhab, M., Shewamene, Z., Gelayee, D.A., Adinew, G.M., Birru, E.M. Antidiarrheal activity of methanolic extract of the root bark of *Cordia africana*. *J. Exp. Pharma.*8, 53-59.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5074710/.(2016).

49. Ayertey, F., Ofori-Attah, E., Antwi, S., Amoa-Bosompem, M., Djameh, G., Lartey, N. L., ...& Okine, L. K. Anti-inflammatory activity and mechanism of action of ethanolic leaf extract of MorindalucidaBenth. *J. Trad. Comp. Med*. 11(3), 249-258. (2021).

50. Karim, N., Khan, I., Khan, W., Khan, I., Khan, A., Halim, S. A., ...& Al-Harrasi, A. Anti-nociceptive and anti-inflammatory activities of asparacosina involve selective cyclooxygenase 2 and inflammatory cytokines inhibition: An in-vitro, in-vivo, and in-silico approach. *Front. Immun.* 10, 581. (2019).

51. Karabay-Yavasoglu, N. U., Karamenderes, C., Baykan, S., &Apaydin, S. Antinociceptive and anti-inflammatory activities and acute toxicity of *Achilleanobilis*.subsp. neilreichii. extract in mice and rats. *Pharma.Bio*. 45, 162-168. (2007).

52. Al-Khayri, J. M., Sahana, G. R., Nagella, P., Joseph, B. V., Alessa, F. M., Al-Mssallem, M. Q. Flavonoids as potential anti-inflammatory molecules: A Review. *Molecules*, *27*, 2901.https://pubmed.ncbi.nlm.nih.gov/35566252/ (2022).

53. Tong, Z., He, W., Fan, X., Guo, A. Biological function of plant tannin and its application in animal health. *Front. Veter. Sci*. 8, 1-7. https://www.frontiersin.org/articles/10.3389/fvets.2021.803657/full. (2021).

54. Owolabi, O. O., James, D. B., Sani, I., Andongma, B. T., Fasanya, O. O., Kure, B. Phytochemical analysis, antioxidant and anti-inflammatory potential of *Feretiaapodanthera* root bark extracts. *BMC Compl. Alter. Med*. 18, 1-9. https://bmccomplementmedtherapies.biomedcentral.com/articles/10.1186/s12906-017-2070-z(2018).

55. Kumar, N.R., Reddy, J.S., Gopikrishna, G., Solomon K.A. GC-MS determination of bioactive constituents of *Cycasbeddomei* cones. *Int. J. Pharm. Bio. Sci.* 3, 344–350. (2012).

Figures

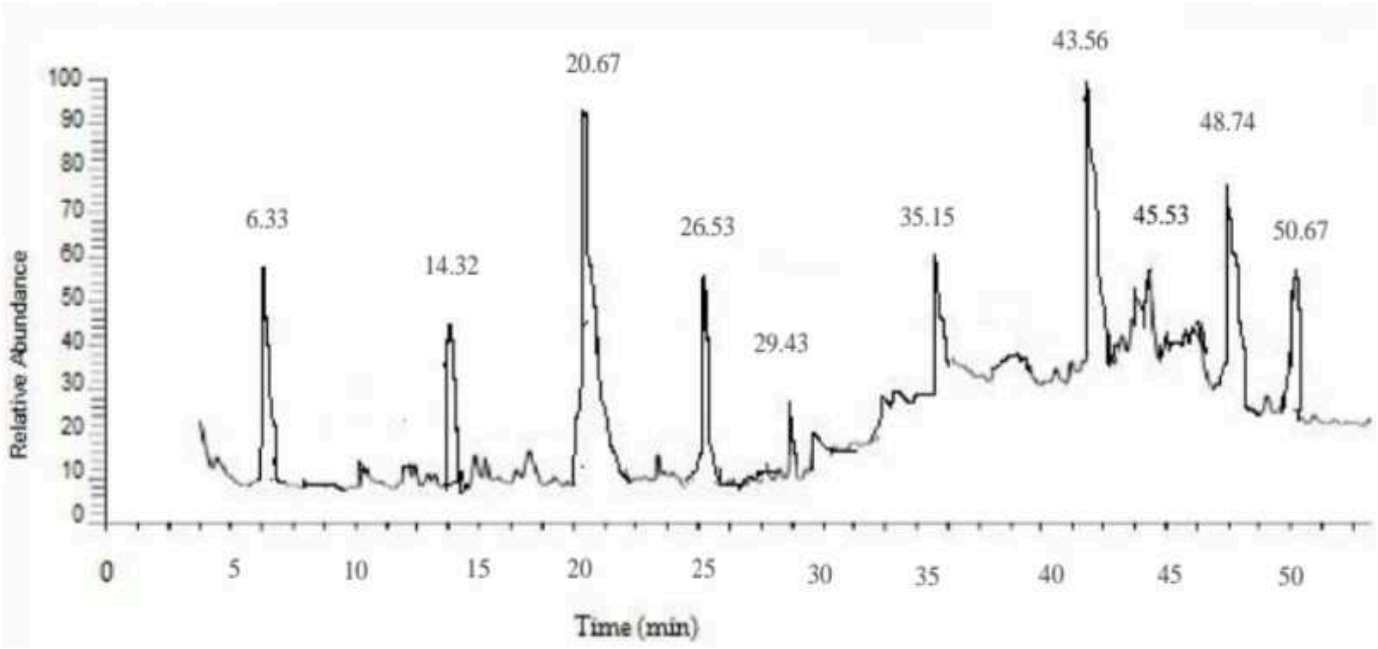

Figure 1

GC-MS Chromatogram of methanolic extract of *Chaerophyllum villosum* Wall. ex-DC

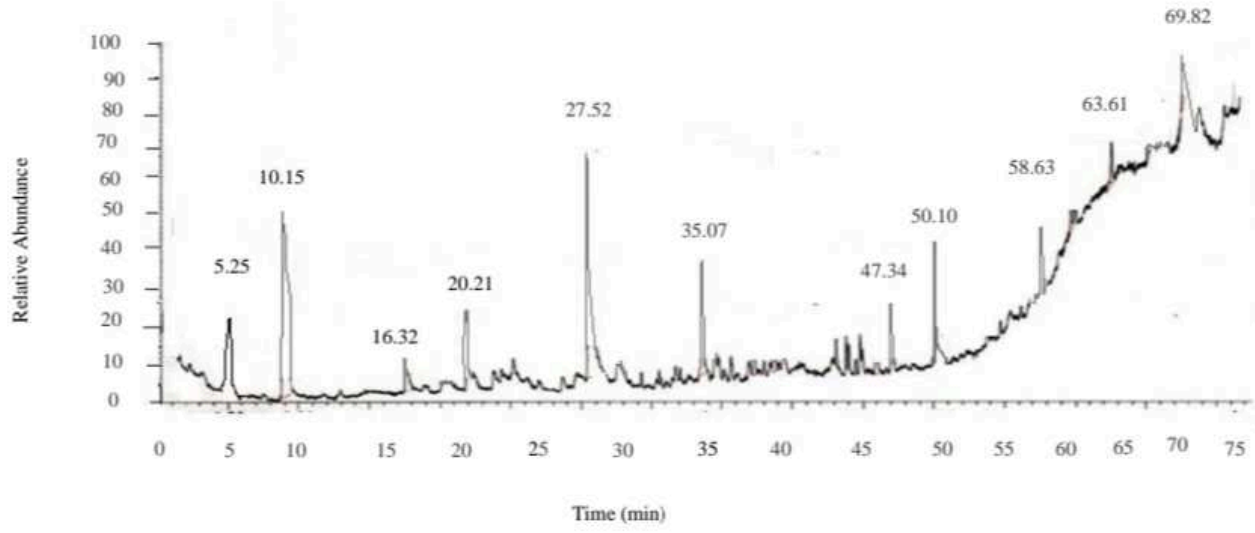

Figure 2

GC-MS Chromatogram of chloroformic extract of *Chaerophyllum villosum* Wall. ex-DC.

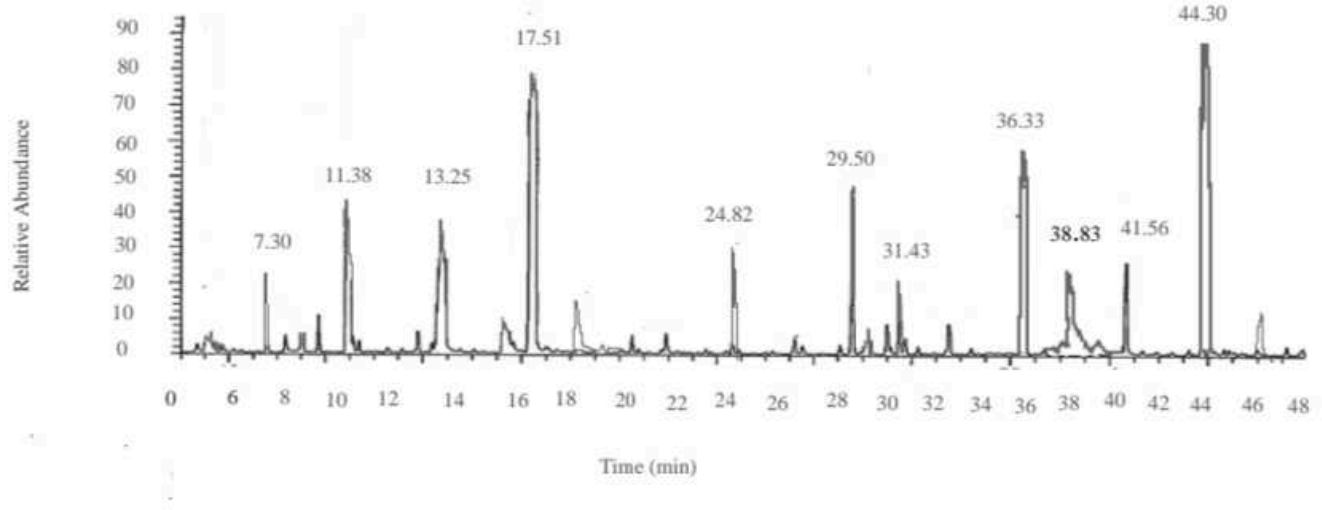

Figure 3

GC-MS Chromatogram of methanolic extract of *Achillea millefolium* L.

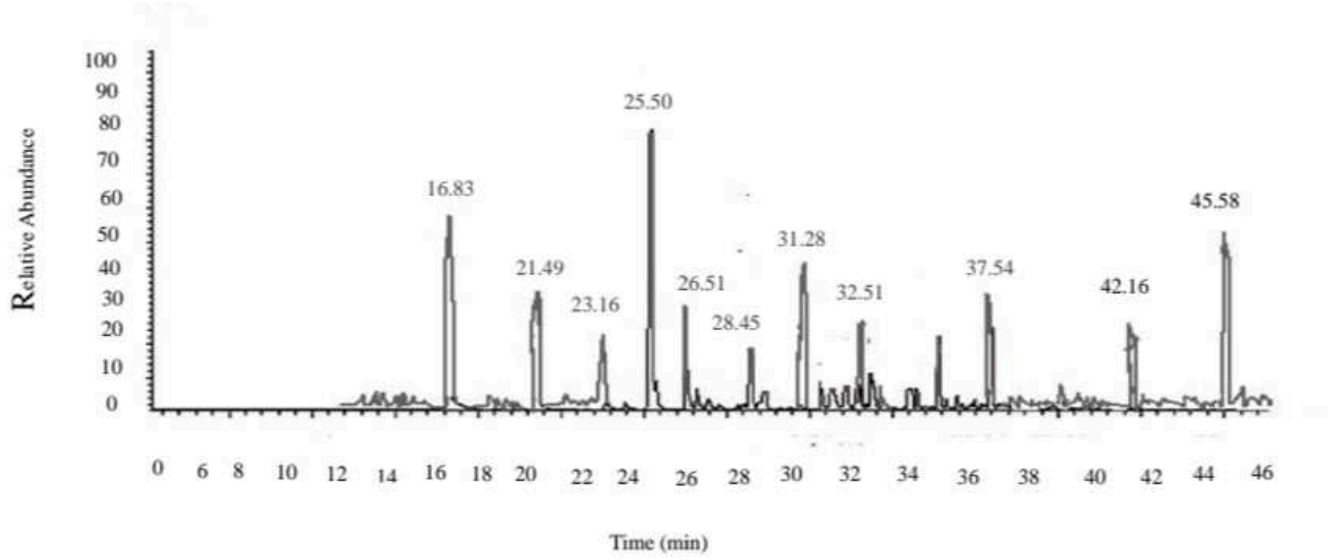

Figure 4

GC-MS Chromatogram of chloroformic extract of *achillea millefolium* L.

Supplementary Files

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- Tables.docx