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GC PROFILING OF *URTICA DIOICA* FOR THE EFFICACY OF TRADITIONAL URTICATION THERAPY

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Urtication is an age-old homeopathic therapy used to eradicate or improve the condition of joints in patients suffering from Osteoarthritis and in paralysis. The present study aims in determining the phytochemical composition of the aerial part of the plant as well as the characterization of chemical present that imparts such anti-arthritic properties to the plant. The phytochemical analysis was done for characterization of compounds responsible for exhibiting the anti-arthritic effect in urtication therapy, using appropriate standard established protocols for GC-MS Gas Chromatography and Mass Spectrometry analysis of aerial plant parts. The result of analysis showed presence of 58 compounds, of which 7 compounds were found to exhibit antiarthritic, anti-inflammatory and antipyretic properties. These compounds were phenolisc, flavonoids, alcohols and fatty acids in nature. 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one was reported in highest concentration among the 7 compounds imparting potential properties for use in the traditional urtication process.

Keywords: Anti-arthritic, Anti-inflammatory, Nettle, Osteoarthritis, Urtication

In the past decade there has been a massive increase in people's interest in resorting to herbal remedies for disease treatment due to the acceptance of natural therapies and its minimalistic side effects. This is also due to the major preference of people towards natural therapeutics as an alternative medicine and the dissatisfactory results from the orthodox pharmaceutical. Now-a-days availability of herbal medicines are not only limited to the drug stores but to retail super markets as well. Almost 80% of the world's developing country's total population relies on natural herbs as a primary source of healthcare. (Mukherjee 2002, Bodeker *et al.* 2005; Bandaranayake 2006, Martins Ekor 2014).

Urtication is one such ancient traditional natural medicinal practice which involves flogging or rubbing viz., deliberate pricking of skin of the affected joints with nettle stings.
(Silver *et al.* 2019). The literature on application of plants being used in medicinal practices dated back in the first century AD by Galen (ca. 130–200 AD) and Dioscorides. The earliest reference of the application of *Urtica* in paralytic conditions dates back to (ca. 23–79 AD) by a roman naturalist Pliny the Elder. Urtication therapy was reported to be effective in curing patients suffering from partial paralysis by John Piper (1821), an assistant of a surgeon in Birmingham. The single recent report on Urtication to be used in musculoskeletal pain is given by Randal C. (2000) via a trial investigation of double blind crossovers on 27 patients of which 17 patients claimed Urtica to be effective and was ready for its use in future as well. Another report on use of nettle in musculoskeletal pain remedy was reported by Alford L, 2008 on a 38 year old woman with prompt relief in recurring muscular pain.

Although being used for centuries in various medicinal practices the mode of action of nettle on skin is still unknown due to scientific investigation lacking. The present study aimed at profiling the phytochemical constituents found in stinging nettle viz., *Urtica dioica* L. of prospective anti-arthritic, anti-inflammatory and pain relieving potential to provide evidence in support of the application of urtication therapy in musculoskeletal pain.

U. dioica or most commonly known name stinging nettle is a perennial, herbaceous plant growing up to the height of 1.5-2m. This

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plant is famous for the presence of stinging hairs. These stings are specialized hairs having a bulbous tip which upon coming in contact breaks off and leaves a needle like tube that pricks the skin injecting acetylcholine and histamine that cause painful burning sensations and itching may prolong from 12-24 hours. The plant is found distributed profoundly in the temperate regions near the roadsides, drainage areas or the wastelands. In India, the species have been reported along the north eastern Himalaya range area majorly concentrated around Srinagar, Himachal and Uttrakhand.

MATERIAL AND METHODS

Plant Material Collection: The *U. dioica* plant was collected from the drainage areas, roadsides and barren land areas of Palampur District of Himachal Pradesh, India. Aerial part of the plant, that is the plant parts from above ground level only (root absent) is plucked up and collected. The collected plant part is washed with distilled water for removal of any soil or microbiota from the collected sample of plants and then allowed to dry completely until all moisture is evacuated from the plant material. The dried plant was at regular intervals mixed, and cut into smaller pieces. The plant samples using a grinder were powdered which was then kept stored in an airtight container at 4ºC for future extraction.

Extraction: About 50 g of powdered sample was weighed. The sample is extracted with 250 ml of methanol as solvent in Soxhlet extraction method until the plant material is completely exhausted. Soxhlet extraction for methanol is carried out at 64ºC. The solvent extract of the aerial part of the plant was then completely dried in a hot air oven at 40ºC. After evaporating the solvent the concentrated solvent extract was transferred to vials of 15 ml sizes and the remaining solvent was further evaporated in the laboratory at room temperature. The dried extract was properly stored in a refrigerator at 4ºC until further analysis.

Table 1: GC- MS Instrumentation Parameters

Instrument	GC-MS Shimadzu QP 2010 Plus
GC Conditions	
Carrier Gas	Helium; Constant flow
Column Volume	Rxi-5Sil MS column (30m length, 0.25 mm ID, 0.25 µm thickness)
Column Oven temperature	60 ºC
Injection Temperature	260 ºC
Injection Mode	Split
Split Ratio	1: 10
Flow Control Mode	Linear Velocity
Linear Velocity	40.1 cm/sec
Flow Pressure	73.3 kPa
Total Flow	16.3 ml/min
Column Flow	1.21 ml/min
Purge Flow	3.0 ml/min
Wash Volume	6 µl
Rate	60 ºC (hold for 5 min) with a hold time of 2 min. raised to 300 ºC at the rate of 10 ºC/min; ending with an isothermal temperature of 300 ºC for 16 minutes run time
MS-parameter	
Ion Source Temperature	230 ºC
Interface Temperature	270 ºC
Solvent Cut time	3.50 min
Ionization mode	Electron energy
Electron Energy	70 eV
Scan Range	40-650 m/z

GC-MS Analysis: Gas Chromatography-Mass Spectrometry analysis of the methanolic extract of aerial plant part of *U. dioica* was carried out on a Shimadzu QP-2010 Plus with criteria given below in Table 1.

For carrying out GC-MS analysis a stock solution was prepared by dissolving the dry

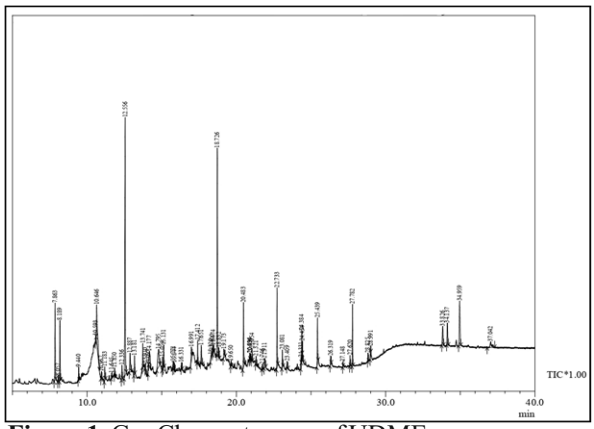

Figure 1: Gas Chromatogram of UDME

crude extract in methanol. 1 µl of the prepared stock solution was taken for GC-MS analysis.

Identification Of Compounds With Anti-arthritic Potential: The area of average peak to the total area is compared for each compound gives the relative abundance of the compound with respect to all the other constituents. The software used to handle mass spectra and chromatograms was Turbo mass. The relative percentage of every constituent of the extract was expressed as a percentage with their respective peak area.The phytoconstituents of the extract was determined by comparing their respective retention time in the solvent and the recorded mass weight from the recorded authentic samples obtained from the GC records as well as from the mass spectra of NIST (National Institute of Standards and Technology, US) database housing 62,000 patterns and from the Wiley pesticide library 3rd edition, for estimating the presence of probable compounds in the extract. The outcomes obtained from the chromatographic (GC-MS) phytochemical estimation has further been cross-checked using other research articles and availablePubChem online database and other research articles for the activities and structure of the detected compound. The gas chromatogram report of the resultant extract phytoconstituents were checked for their potential usage in arthritis, joint pain reliever and in case of inflammations.

RESULTS AND DISCUSSION

The anti-inflammatory, antipyretic and anti-arthritic role of *Urtica dioica* methanolic

Table 2: Phytochemical Compounds exhibiting properties for potential use in Urtication therapy

Peak No.	Peak Area (in %)	R_t time	Name	M. Wt.	Chemical Nature	Bio-Activity	Ref.
12	18.26	12.556	3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	144	Flavonoid fraction	Anti-inflammatory	Igwe*et al.* 2016
21	1.29	15.131	2-methoxy-4-vinylphenol	155	Phenol derivative	Anti-inflammatory, analgesic	Jeong*et al.* 2011; Dr. Duke Phytochemical database 2013
42	3.99	22.733	n-Hexadecanoic acid	256	Fatty acid	Antiarthritic,anti-inflammatory	Aparna*et al.* 2012; Uchegbu*et al.* 2015; Yamuna *et al.* 2017
43	1.05	23.080	trans-sinapyl alcohol	210	Alcohol	Anti-Inflammatory, antinociceptive	Choi *et al.* 2004
44	0.42	23.410	9-Octadecenoic acid	282	Fatty acid	Anti-inflammatory	El-Fakharany *et al*. 1999; Dr. Duke 2013
46	1.30	24.383	9,12-Octadecanoic acid	280	Fatty acid ester	Anti-inflammatory, antiarthritic	Stuyvesant *et al.* 1967; Ha *et al.* 1990; Zhao *et al.* 2005; Das *et al.* 2006; Knothe*et al.* 2013
56	1.88	34.137	Stigmasterol	412	Sterol	Anti-inflammatory	Garcia *et al.* 1999

extract (UDME) has been assessed via phytochemical profiling of the extract using GC-MS technology. Gas Chromatography is a technique which combines the features of Gas-liquid chromatography and Mass spectrometry.

GC–MS analysis of the methanolic extract of the *U.dioica* plant aerial portion showed the presence of a total 58 compounds of which 7 compounds from the stem are found to be with antipyretic, anti-inflammatory and antiarthritic potential that were mentioned in Table 2 along with their respective peaks, retention time, molecular mass, chemical formula and their respective potential activity that could contribute in beneficial aspect of plant in urtication therapy.

These compounds are fatty acyl esters, fatty acids, sterols, phenol and flavonoid derivatives in nature. These 7 compounds with antiarthritic, anti-inflammatory and antipyretic potential contribute approximately 28 % of the total phytochemical constitution percentage of methanolic plant extract. The compound that is extracted in highest concentration is 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one that makes up about 18.26% of the total phytochemical constituent and 65.2 % of the antiarthritic phytochemical compounds of the extract (Igwe*et al.* 2016); followed by a saturated fatty acid, n-Hexadecanoic acid commonly known as palmitic acid is the second highest compound with antiarthritic potential (Aparna*et al.* 2012; Uchegbu*et al.* 2015; Yamuna *et al.* 2017) taking over about 3.99% of the area of the resultant gc-ms graph (Figure 1).

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