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Phytochemicals and GC–MS analysis of bioactive compounds present in ethanolic leaves extract of *Taraxacum officinale* (L).

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ABSTRACT

Objective: To identify the phytochemical constituents present in ethanolic leaves extract of *Taraxacum officinale* (*T. o)* and to elucidate the bioactive compounds.
Methods: The fresh leaves of *T. officinale* (1000g) were shade dried at room temperature for 30 days and the dried leaves were made into a fine powder. The ethanolic leaves extract obtained was dried with help of desiccator and rotatory evaporator. The dried samples tested for phytochemical analysis and bioactive compounds profiling through GC-MS analysis.
Results: The phytochemical screening studies showed presence of alkaloids, flavonoids, phenol, cardiac glycosides and anthraquinones. in the leaves extract of *T. officinale.* GC-MS analysis showed 10 essential bioactive compounds and it revealed the presence of significant anticancer, Antimicrobial and antidiabetic compounds.
Conclusions: The ethanol extract of *T. o* possess the most important phytochemical compounds and it has various medicinal properties to cure dangerous diseases and disorders.

Keywords: *Taraxacum officinale***,** Phytochemicals analysis, GC–MS analysis, Biological compounds.

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INTRODUCTION

India is the chief producer of medicinal herbs and is appropriately called the botanical garden of the world[1]. Plant use in the treatment of diseases is as old as civilization [2, 3] and complementary medicine is still a major part of habitual treatments of different maladies [4]. The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents [5]. Medicinal plants are a precious heritage for humanity; our ancestors used these plants to ensure their health and transmitted their knowledge and their experiences generation to generation. About 80% of the world population uses this mode of therapy (Azaizeh *et al.,* 2003) especially in undeveloped countries when the modern medical system was absent (Tabuti *et al.,* 2003) [6, 7].

Medicinal plants constitute the main source of new pharmaceuticals and healthcare products [8]. Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are a significant source with various structural arrangements, functions and properties [9]. Plants are capable of synthesizing an overwhelming variety of low-molecular-weight organic compounds called secondary metabolites, usually with unique and complex structures. Many metabolites have been found to possess interesting biological activities and find applications, such as pharmaceuticals, insecticides, dyes, flavours and fragrances. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [10]. Phytochemicals are naturally occurring in the medicinal plants like leaves, stem bark, fruits and roots that have defence mechanism and protect from various diseases. Natural products from plants are called secondary metabolites are the end products of primary metabolites such as carbohydrates, amino acid and chlorophyll lipid so on. They are synthesis large variety of chemical substances known as secondary metabolites which include alkaloids, steroids, flavonoids, terpenoids, glycoside, saponin, tannins, phenolic compounds etc [11]. Phytochemicals were the chemicals that present naturally in plants.

Nowadays these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play the main role in the number of diseases such as asthma, arthritis, cancer etc. Extraction and characterization of many active phytocompounds from these green factories have given birth to some high activity profile drugs [12]. The active principle of many drugs found in plants is secondary metabolites. Therefore basic phytochemical investigation is vital [13]. The identification and isolation of such active compounds make it a more effective therapeutic application. It present consumes from taking certain plants that have no medicinal value or poisonous to them. It will lead to better understanding of diseases.

According to the World Health Organization, medicinal plants would be the greatest source to obtain an array of drugs. Thus, such plants should be investigated to a better understanding of their properties, safety practices in addition to usefulness [14]. Gas chromatography-mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify various substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. In the last few years, gas chromatography-mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species [15, 16, 17]. Gas- Chromatography-Mass Spectrometry (GC-MS) is a helpful technique for reliable profiling of secondary metabolites [18-19]. GC-MS can also be used in airport security to detect substances in luggage or on human beings. However, fewer reports are available with respect to the pharmacological properties of the plant [20]. GC-MS is one of the best techniques to identify the bioactive constituents of long-chain branched chain hydrocarbons, ester, acids, alcohols etc.

Taraxacum is a large genus of flowering plants in the Asteraceae family. *Taraxacum officinale* (L) is a very well known medicinal herb in Ayurvedic medicine since times immoral. *Taraxacum* is the largest genus of Asteraceae family, is a herbaceous perennial herb commonly called dandelion, found especially in lawns and along roadsides, and it is used as a medicinal herb and in food preparations. Dandelion has been extensively used as traditional folk medicine, and as a diuretic in modern phytotherapy. It is used to treat a variety of diseases including cancer [21, 22] in China, Arab and Native America. In Chinese, Arabian and Native American traditional medicine it is used to treat a variety of diseases including cancer [23, 24]. To explore the medicinal importance the rhizomes of *Taraxacum officinale* L were screened primarily for the phytochemicals present in it and was analyzed using GC-MS.

MATERIALS AND METHODS

Plant Collection and Preparation of plant extracts

Fresh, healthy, and young leaves of *Taraxacum officinale* were collected from the Himalayan region, India. The leaves were cleaned and dried in shade for 7 days and then ground well to a fine powder. About 500 g of dry powder was extracted with ethanol (80%) at 70°C by continuous hot percolation using Soxhlet apparatus. The extraction was continued for 24 hrs, and the ethanolic extract was then filtered and kept in a hot air oven at 40°C for 24 hrs to evaporate the ethanol from it. A dark brown residue was obtained. The residue was kept separately in airtight containers and stored in a deep freezer.

Phytochemical analysis tests

Phytochemical analysis of an ethanolic extract of *Taraxacum officinale* leaves for secondary metabolites such as alkaloids, flavonoids, carbohydrates, proteins, phenols, saponins, tannins, terpenoids, phytosterols, and phlobatannins was done using standard methods [25].

Gas Chromatography-Mass spectrometry (GC-MS) analysis

The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm df capillary column. The instrument was set to an initial temperature of 110°C and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised up to 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (MHz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from the relative peak area of each component in the chromatogram.

Identification of Compounds

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the known component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

Phytochemical analysis

Table 1: Phytochemical Analysis of *Taraxacum officinale* (L)

S.No	Test	Result
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	-
4	Tannins	-
5	Terpenoids	-
6	Phenol	+
7	Cardiac glycosides	+
8	Anthroquinones	+

- = Absence; + = Presence

The results of phytochemical characterization ethanolic extracts of *T. officinale* are shown in Table 1. Phytochemical analysis of an ethanolic extract of the plant also revealed the presence of alkaloids, flavonoids, phenol, cardiac glycosides, anthraquinones. Phytochemical analysis of an ethanolic extract of the plant also revealed the absence of saponins, tannins, terpenoids.

Gas Chromatography-Mass spectrometry (GC-MS) analysis

Phytochemical components in ethanolic extract of T. officinale by GC-MS report. The GC-MS analysis revealed the presence of 10 compounds (Table 2 and 3) from the

ethanolic leaves extract of *T. officinale* (Figure 1). From the results, it was observed that presence of 3-Cyclopentene-1-ethanol, 2,2,4-trimethyl-, (+)-2-Bornanone, Isobornyl thiocyanoacetate, 1,2-cis-1,5-trans-2,5-dihydroxy-4-methyl-1-(1-hydroxy-1-isopropyl)cyclohex-3-ene, 2-Trifluoro acetoxytridecane, Tridecane, Dodecane, 2,6,11-trimethyl-, Phenol, 2,6-bis(1,1-dimethylethyl)-, Decane, 2,4,6-trimethyl-, 2,2-bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane. Above these compounds were identified based on the RT value, molecular weight, molecular formula, etc (Fig 1) and table 2, 3.

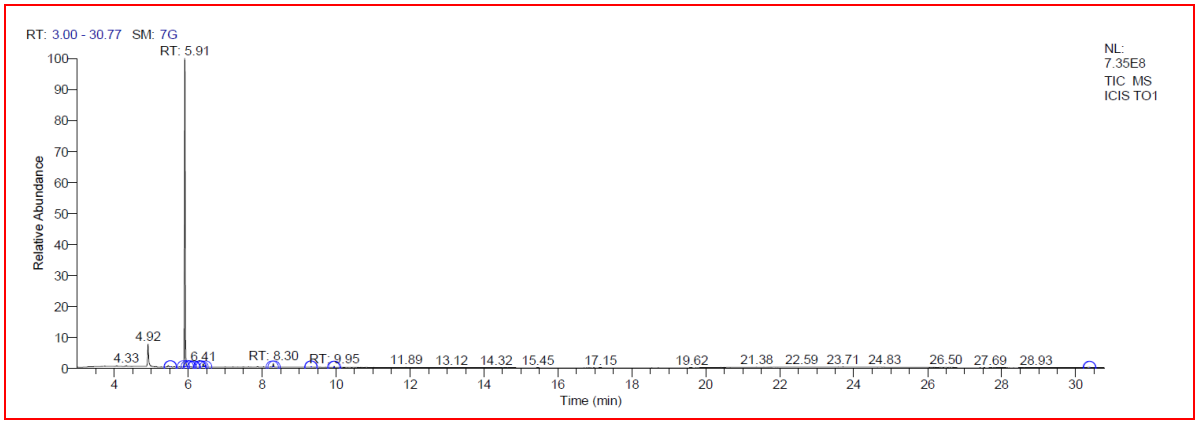


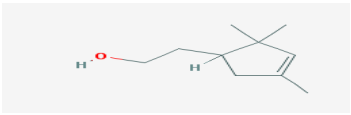
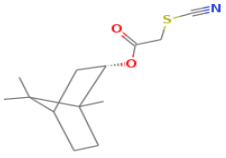
Figure 1: GC-MS CHROMATOGRAM OF *Taraxacum officinale* (L)

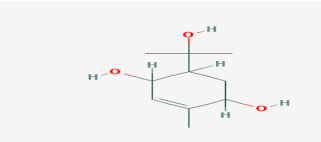
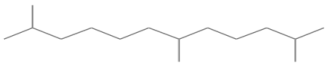
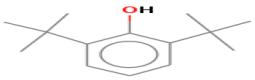
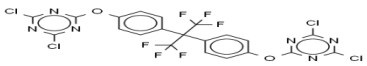
Table 2: Physical properties of bioactive compounds in *Taraxacum officinale* (L)

S.No	Name of the Compound	RT	Molecular Formula	CAS Registry Number
1	3-Cyclopentene-1-ethanol, 2,2,4-trimethyl-	5.53	$C_{10}H_{18}O$	80514-13-2
2	(+)-2-Bornanone	5.91	$C_{10}H_{16}O$	464-49-3
3	Isobornyl thiocyanoacetate	6.05	$C_{13}H_{19}NO_2S$	115-31-1
4	1,2-cis-1,5-trans-2,5-dihydroxy-4-methyl-1-(1-hydroxy-1-isopropyl)cyclohexane-3-one	6.15	$C_{10}H_{18}O_3$	87096-70-6
5	2-Trifluoroacetoxytridecane	6.32	$C_{15}H_{27}F_3O_2$	116465-18-0
6	Dodecane	6.41	$C_{12}H_{26}$	112403
7	Dodecane, 2,6,11-trimethyl-	8.30	$C_{15}H_{32}$	31295-56-4
8	Phenol, 2,6-bis(1,1-dimethylethyl)-	9.33	$C_{14}H_{22}O$	128-39-2
9	Pentadecane	9.95	$C_{15}H_{32}$	629629
10	2,2-bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane	30.38	$C_{21}H_8Cl_4F_6N_6O_2$	NA

RT= Retention Time

Table 3: GC-MS Analysis of *Taraxacum officinale* (L)

Name of the Compound	Nature of the Compound	Structure	Molecular Weight (g/mol)	Activity
3-Cyclopentene-1-ethanol, 2,2,4-trimethyl-	Alcohol		154.253	Antibacterial activity
(+)-2-Bornanone	Monoterpene oxide		152.2334	Antitumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide, Anticancer.
Isobornyl thiocyanoacetate	Ester		253.360	Antiseptic property

1,2-cis-1,5-trans-2,5-dihydroxy-4-methyl-1-(1-hydroxy-1-isopropyl)cyclohex-3-ene	Alkene		263.6	Antibacterial Activity, Antifungal Activity.
2-Trifluoroacetoxytridecane	Alkane		296.374	The antidiabetic property, urinary protection,
Dodecane	Alkane		170.34	Antibacterial activity
Dodecane, 2,6,11-trimethyl-	Alkane		212.4146	No activity
Phenol, 2,6-bis(1,1-dimethylethyl)-	Alcohol		206.3239	Anticancer property
Pentadecane	Alkane		212.421	Anti-tussive, anti-ulcer
2,2-bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane	Alkane		632.1296392	Antibacterial and Antifungal activity

3-Cyclopentene-1-ethanol, 2,2,4-trimethyl- is an aromatic alcoholic compound. It has 5.53 RT value, $C_{10}H_{18}O$ molecular formula and 154.253 molecular weight. This compound exhibited varying levels of antimicrobial activity against the investigated bacteria [26].

(+)-2-Bornanone is an aromatic monoterpene oxide compound. It has 5.91 RT value, $C_{10}H_{16}O$ molecular formula and 152.2334 molecular weight. It has antitumor, analgesic antibacterial, anti-inflammatory sedative, fungicide, anticancer activities. It was used as an anti-cancer agent reported by Mariat George *et al.,* (2015) [27].

Isobornyl thiocyanoacetate is an aromatic ester compound. It has 6.05 RT value, $C_{13}H_{19}NO_2S$ molecular formula and 253.360 molecular weight. It has antiseptic property. It was marketed as insecticides [28].

1,2-cis-1,5-trans-2,5-dihydroxy-4-methyl-1-(1-hydroxy-1-isopropyl)cyclohexane-3-ene is an aromatic alkene compound. It has 6.15 RT value, $C_{10}H_{18}O_3$ molecular formula and 263 molecular weight. This compound and their constituents are contemporary applied in food preservation and in the manufacture of medicinal antimicrobial agents and disinfectants (Voda *et al.,* 2003). [29]

2-Trifluoroacetoxytridecane is an aliphatic alkane compound. It has 6.32 RT value, $C_{15}H_{27}F_3O_2$ molecular formula and 296.374 molecular weight. This compound has an antimicrobial activity [30].

Dodecane is an aliphatic alkane compound. It has 6.41 RT value, $C_{12}H_{26}$ molecular formula and 170.34 molecular weight. Some alkanes (i.e., n-hexane, n-dodecane and n-hexadecane) were previously reported to increase metabolite production by acting as oxygen-vectors [31-33].

Dodecane, 2,6,11-trimethyl- is an aliphatic alkane compound. It has 8.30 RT value, $C_{15}H_{32}$ molecular formula and 212.4146 molecular weight. It has no activity [34].

Phenol, 2,6-bis(1,1-dimethylethyl)- is an aromatic alcoholic compound. It has 9.33 RT value, $C_{14}H_{22}O$ molecular formula and 206.3239 molecular weight. It has an anticancer property and cytotoxic property. This compound has been reported as cytotoxic compounds by inhibiting various types of neoplastic cell lines and some of the compounds are potent anticancer agents [35-36].

Pentadecane is an aliphatic alkane compound. It has 9.95 RT value, $C_{15}H_{32}$ molecular formula and 212.421 molecular weight. This compound has Antitussive and antiulcer activity [37].

2,2-bis[4-[(4,6-dichloro-1,3,5-triazine-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane is an aromatic alkane compound. It has 30.38 RT value, $C_{21}H_8Cl_4F_6N_6O_2$ molecular formula and 632.1296392 molecular weight. The TPC could be used as an important indicator of the antioxidant capacity due to a high redox [38].

CONCLUSION

The phytochemical analysis of the plants is also important and pharmaceuticals companies for the novel drugs for the treatment of various diseases. In the present investigation 10 compounds from the ethanolic leaves extract of *Taraxacum officinale* (L). were identified by Gas-chromatography– Mass spectrometry (GC-MS) analysis. The biological activities of

each of the identified phytocomponents used for antimicrobial and anti-cancer activities. Biochemical compound identification of the plant constituents was conducted depending upon their retention time (RT), molecular formula, molecular weight and mass spectral data, as well as by computer search mass spectral databases. Gas chromatography and mass spectroscopy analysis showed the existence of various compounds with different chemical structures. Thus, improving the methods for qualitative and quantitative determination of medicinal plants are very important for quality assessment in the medicinal plant industry. In addition, the phytochemical analysis gives a good monitoring method of the seasonal changes of the active constituents and during cultivations and harvesting which assists in collecting the largest amounts of the active constituents. We report the presence of some of the important components resolved by GC-MS analysis and their biological activities. Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study.

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