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Research Article

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Morphological and Phytochemical Study of Cirsium Arvense from District Mardan Pakistan

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ABSTRACT

In the present study local information, morphology, medicinally uses, collection, stomata studies and phytochemical screening analysis of cirsium arvense has been done. C.arvense is an herbaceous wild plants belong to family asteraceae. The local information about this plant were collected from the local people of that area (Mardan). In the March to April it emerges with flower and stem from the soil. The ideal conditions for growth are high moisture content, high humidity and a temperature of around about 25 C0. This plants are used as antifungal, antiamoebic, antifeedant, antiviral, antibacterial, antimalarial, allergies, sores, anemia, fever, skin diseases and as tonic blood purifier,vermifuge,aborative and insecticide etc. The phytochemical screening analysis of tannins, flavonoids, alkaloids, steroids, phenols has been analyzed which show the presence or absence of these chemical compounds. The carbohydrate in leaf extract, the methanolic extract have carbohydrate present while in the distilled water extract and ethanolic extract was in not present. The carbohydrate in root extract, the methanolic extract shows presence and ethanolic extract shows more presence while the distilled water extract shows absence. The alkaloid in leaf extract, in the ethanolic extract is not present while methanolic extract is present and in distilled water in more amount present. The Alkaloid in root extract, the methanolic extract shows most presence and ethanolic extract shows presence while the distilled water extract shows more presence. The saponins in leaf extract, in the methanolic and ethanolic extract shows presence while in the distilled water have most presence.

Keywords: Morphological, Phytochemical Cirsium Arvense Mardan Pakistan

Introduction

The word 'pharmacognosy' had its debut in the early 19th century to designate the discipline related to medicinal plants; it is derived from the Greek pharmakon, 'a drug', and gignosco, 'to acquire a knowledge of' and, as recorded by Dr K. Ganzinger [1]. Pharmacognosy for the first time was defined as a pharmaceutical discipline in 1815 by Seidler [2]. Pharmacognosy, a term coined about 200 years ago, is derived from the Greek, meaning knowledge of drugs. Although the term strictly should apply to all drugs, the fact was that most of the drugs used in the early nineteenth century consisted of plant material, and the term evolved to become restricted to the analysis of drugs and medicines from natural sources, especially as a means to check their authenticity and purity [3]. Its scope includes the identification or authentication of crude drugs (using microscopically, microscopically, radiological or chemical methods), and their bio pharmacological and clinical evaluations. During earlier investigations studies have been conducted on ethnobotanical and pharmacognostical characterization of medicinal plants [4]. The pharmacological treatment of disease began long ago with the use of herbs [1]. Drug discovery from medicinal plants led to the isolation of early drugs such as cocaine, codeine, Digi toxin and quinine, in addition to morphine, of which some are still in use [5]. Chemical constituents have played a role of ever-increasing importance in the analysis of crude drugs, both qualitatively and quantitatively. The absence or presence of a particular constituent, or group of constituents, may be a very useful marker as to the identity of a species and full use has been made of chromatography, especially TLC, in this respect [6]. Plants have been utilized as medicines for thousands of years. These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders and other herbal formulations [7]. Medicinal plants and human beings have unique relationship since time immemorial. Man's vital interest in plants, primarily as a source of food, shelter and clothing, dates back to the very origin of human civilization. Plants are nature's "Chemical factories" providing the richest source of organic chemicals on earth. The world is blessed with a great variety of natural vegetation's, some of which are used as traditional medicine to cure various sicknesses and diseases [8].

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Phytochemistry deals with the analysis of plant chemicals called natural products and with changes occurring in such chemicals due to alterations in environmental conditions. Ecology nowadays includes an increased amount of chemistry because communication between a plant and its environment depends to a large extent on secondary metabolites of which phenolics make up ca. 100,000 different structures [9].

Cirsium arvense (L.) Scop. C.arvense is a perennial plant with deep and extensive horizontal roots that can form new shoots. Stems usually grow 30½ to 122 cm tall and branch above. Leaves are alternate, sessile, and shallowly to deeply pinnatifid or lobed with spiny margins. The lower surfaces of leaves are often covered with soft, woolly hairs. Male and female flower heads appear on separate plants. Flower heads measure 13 to 19 cm in diameter. Flowers are purple and almost exclusively insect-pollinated. Seeds are brownish with a tuft of hairs at the top [10]. Its diuretic, anti-inflammatory and hemostatic, astringent, anti-phlogerstic and hepatic medicinal values have been documented to be associated with the presence of flavonoid and coumarin [11]. Creeping and other thistles are food plants for several insect pests including bean aphis, mangold fly, celery fly and larvae of swift moths. Likewise, antioxidant and antimicrobial aspects have also been previously reported as positive properties of C. arvense [12]. C.arvense seed is an important constituent in the diet of many farmland birds including linnets (Carduelis cannabina). Allopathic effects of C. arvense plant extracts and residues on agronomic crops and weeds have been reported in many studies. American Indians purportedly used an infusion of C. arvense roots for mouth diseases, worms and poison-ivy (Toxicodendron radicans) and in treatment for tuberculosis the nectar of Cirsium arvense flowers purportedly makes good honey [13].

Material and Methods

Collection of Plants and Their Parts

Rhizome and whole plants of Cirsium arvense Collected in October 2018 from district Mardan of Khyber Pakhtunkhwa

Botanical Identification

Plant samples are collected throughout the field work were taxonomically identified by using Flora of Pakistan, and placed in the Herbarium of Abdul Wali khan university Mardan. The voucher specimens were kept after broad documents for future references. From Medicinal Plant Names Services (mpns.kew.org/mpns) the correct name of plant were confirmed.

Solvent System used

The solvent like methanol ethanol and water were used. For the preparation of crude extract of the Cirsium arvense.

Crude Extract Preparation

Plants and their parts were collected in the field and then transfer in lab and cleaned with the help of tap water to take away the unwanted constituents and silicate material and then were placed for 30 days for dryness purposes in shade at room temperature 20-30°C [14].

Crashing and Filtration of the Plants

After the dryness of the whole plants and their parts then with the help of electric grinder selected plant and their parts were grinded. 10 g of plant powdered was taken than retained in distinct conical flask and 90 ml of solvent i.e. (Methanol, Ethanol and aqueous) was added to the plants powdered separately. Than with the help of aluminum file the Flask were covered and retained in shaker for 72 hrs. For the shaking purposes. After 72hrs shaking the extracts were filter with the help of what man filter paper and then through filtration process plants husk were removed.

Rotary Evaporation of the Solvents

The extract enclosed organic solvent such as Ethanol, Methanol and water which were basically the filtrate of the particular plant and their parts. Beneath the control temperature 30°C - 35°C They were evaporated with the help of rotary evaporator [15].

Crud Extract

After the process of rotary evaporation certain of liquids leftovers were further dried at regulator temperature of 30°C- 35°C through water bath. And then the plants paste were obtained known as crude extract and then the extract were place in air tight bottles [15].

Phytochemical Detection

The plants extract of Cirsium arvense were taken in different types of solvents i.e. Methanol, Ethanol and aqueous and then tasted for the absence or presence of phytochemical constituents like Alkaloids, Tannins, Phlobatannins, Flavonoids, Carbohydrates, Phenols, Saponin and Glycosides [15].

Carbohydrates

The 0.5 ml of filtrate of Cirsium arvense was treated with 0.5 ml of Benedict's regent and the solution were heating for 2 minutes on a boiling water both. Than the presence of carbohydrate was confirmed by the formation of reddish brown precipitate [15].

Flavonoids

The alkali substitute test were useful when extract solution the selected plants was treated with sodium hydro oxide solution. formation of red precipitation indicate the presence of flavonoids [15].

Phenols

2 ml of ferric chloride ($FeCl_3$) solution were added to 2 ml of Cirsium arvense extracts solution in a test tube. Formations of deep bluish green solution shown the presence of phenol [15].

Tannins

Ferric chloride test was done for the detection tannins The Ferric chloride ($FeCl_3$) was assorted with an extract solution. Formation of blue green coloration indicate the presence of tannins. [16].

Saponins

In the test tube Five milliliter of Cirsium arvense plants extract were shaken dynamically. When the formation of froth occurred shown the existence of Saponins [17].

Alkaloids

Few drops of Wagner's reagent (Potassium iodine) are add to the two gram of plants extracts. when the formation of reddish brown precipitate occurred shown the presence of alkaloids [18].

Tests for Terpenoids

Salkowski test: 1ml of Cirsium arvense plant extract was assorted with 2ml of chloroform and carefully added concentrated sulphuric acid along the sides of tube for the formation of a layer. The formation of reddish brown coloration indicate the presence of terpenoids [19].

Results

Morphology

Appearance

Cirsium arvense a perennial herb grows from 1-5 ft. (0.3-1.5 m) tall. Roots can grow deep into the ground. Stems do not have conspicuous spines.

Leaves

Leaves are dark green and lanceolate to oblong-lanceolate. They are glabrous above, but their undersides have short, white hairs. They may be pinnatifid and very prickly. Basal leaves are 5-8 inches (12-20 cm) long. Leaves are usually sessile to slightly clasping. Leaves alternate on the stem with their base sessile and clasping or shortly decurrently. The leaves are very spiny, lobed, up to 15-20 cm long and 2-3 cm broad.

Stem

Stems 30-150 cm, slender green and freely branched, smooth and glabrous (having no trichrome or glaucousness), mostly without spiny wings.

Root

Consists of four types of structures, 1) long thick horizontal roots, 2) long thick vertical roots, 3) short fine shoots, and 3) vertical underground stems. Though asserted in some literature, creeping thistle does not form rhizomes. Root buds form adventitiously on the thickened roots of creeping thistle, and give rise to new shoots. Shoots can also arise from the lateral buds on the underground portion of regular shoots; particularly if the shoots are cut off through e.g. mowing or when stem segments are buried.

Histology

The fresh leaves, stem and roots were collected for histology .A transverse sections of the leaves, stem and roots was observed under the electrical microscope by 30X(objective lens) and 10X(objective lens).From these observation the following cells were recorded

Stem

The outline of transverse section of c.arvense is nearly circular. In transverse section stem was found nearly round in shape and no ridges were found with naked eyes as well as microscope

Epidermis

The epidermis consists of round cells and forming the outer most layer was covered by cuticle. The next cells were hypodermis. Then next layer cortex region starts.

Cortex

The next cells were cortex region starts. Cortical cells consist of 3 to 5 layers of parenchymatous cells, collenchymatous patches and chlorenchyma cells. There is present a continuous layer of collenchyma and collenchyma cells above the cortical region. Then single layer endodermis starts. Its cells contain large starch grains and hence referred as the starch sheath. Multilayered pericycle is irregular and made up of large distinct sclerenchymatous cells.

Vascular Bundles

The pericyclic encloses the vascular bundles. Vascular bundles are arranged in a ring. They are conjoint (xylem and phloem are lying on the same radius), collateral (xylem lies inwards and phloem outwards), closed (absence of cambial strip between the xylem and the phloem) and exarch (metaxylem towards center and protoxylem faces the periphery) type. Xylem is further differentiated into protoxylem and metaxylem. The parenchymatous tissues constitute the conjunctive tissue between the two vascular bundles. The well-developed parenchymatous pith is located in the center.

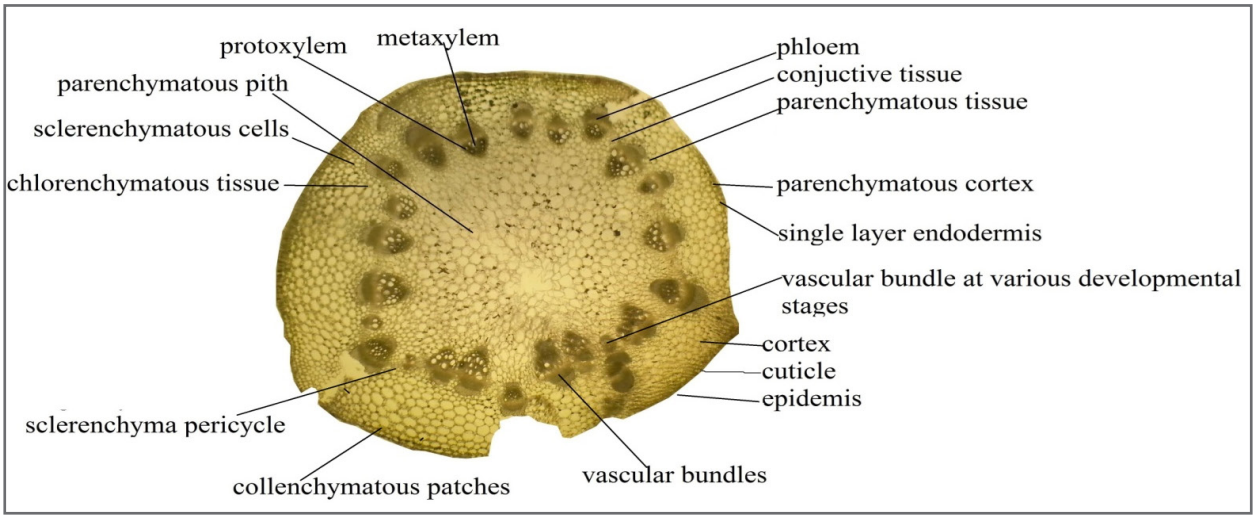

Figure 1: Transverse Section of Cirsium Arvense Stem

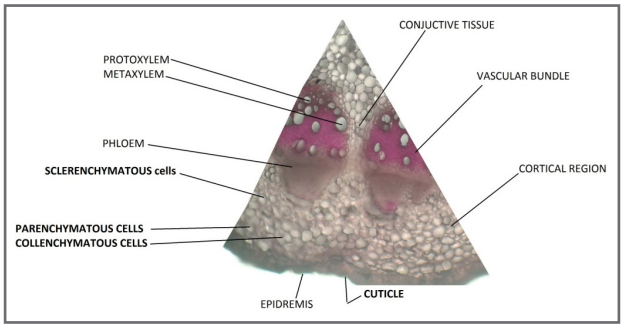

Figure 2: Transverse Section (Slice) of Cirsium Arvense Stem

Leaf

In transverse section of leaf polygonal and irregular shaped epidermal cells and non-glandular trichrome with pointed ends. Outline of midrib had irregular ridges and was not smooth.

Epidermis

The Transverse Section shows the presence of single layered upper and lower epidermis which is made up of compactly arranged barrel shaped cells. Both the surfaces are covered up with a thick and wavy cuticle. It is more on the upper epidermis and lesser on the lower epidermis. Inner to the epidermis present mesophyll cells which is differentiated into two to three layered parenchymatous palisade cells. It consists of green colored columnar cells which are arranged in compact rows. Each palisade cell contains several chloroplasts positioned around its walls.

Vascular Bundles

A prominent and large vascular bundle (collateral and closed type) is present in the region of midrib. A layer of parenchymatous cells separates the vascular bundles from the epidermis. The vascular bundles are encircled by a parenchymatous bundle sheath cells. The upper and lower sides of the vascular bundle are covered by bundle sheath extensions and sclerenchymatous cells. In addition, there are present two to four layers of collenchyma cells near the upper and lower epidermis. Each vascular bundle consists of xylem present towards the upper epidermis and phloem close to the lower epidermis. Xylem is differentiated into metaxylem and protoxylem. Protoxylem vessels are smaller in size and facing towards the upper epidermis. Phloem has various components like sieve tubes, companion cells and phloem parenchyma cells. Along with the largest vascular bundle present in midrib, there are present certain accessory bundles which are smaller in size and they are present towards the upper epidermis near the wing of the leaf

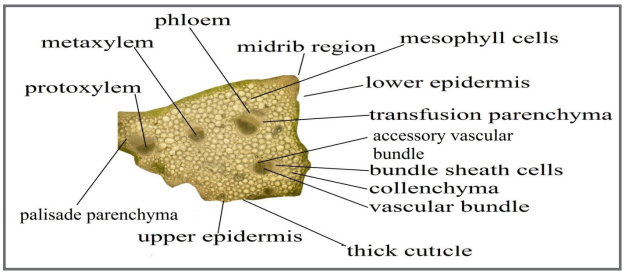

Figure 3: Transverse section of cirsium arvense leaf

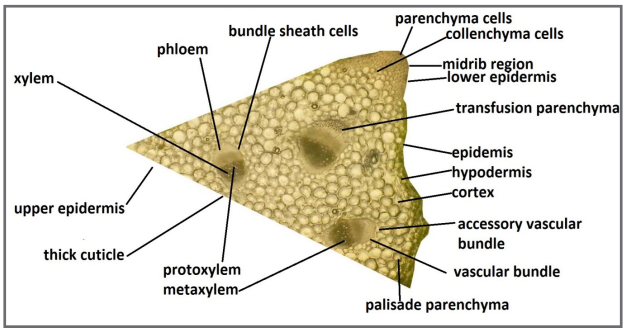

Figure 4: Transverse Section (Slice) of Cirsium Arvense Leaf

Root

In Transverse Section of the root, the outer most layer is epidermis which is single layered and consists of thin walled parenchymatous cells. Some of these cells get elongated to form the new roots which are later designated as the lateral roots. Cortex is being depressed and reduced due to the occurrence of secondary growth in roots. There is present a discontinuous epidermal layer which later on differentiated into phellem and the phellogen. Only two to three layers of cortical cells are visible in reduced form. There are present distinct layers of cambial cells (2 layers) which are responsible for the secondary growth. The major portion of root is occupied by the secondary xylem which is distinguished by its vessels or xylem tracheid's. There are present distinct layers of thin walled ray parenchyma cells. Both of these components (vessels and ray parenchyma) form the secondary xylem while the primary xylem is present towards the centre in a crushed miniature form. In the centre there is present a small sized parenchymatous pith. It is surrounded by certain sclerenchymatous cells which forms a portion of the xylem tissue.

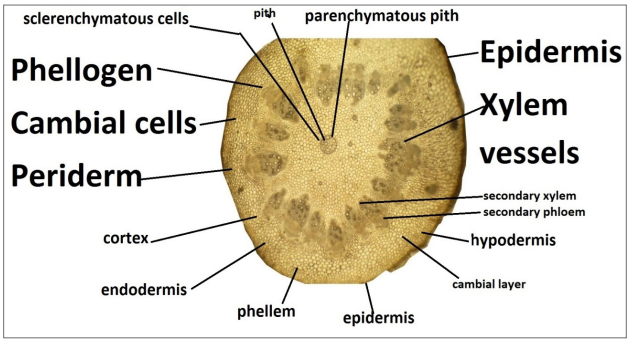

Figure 5: Transverse Section of Cirsium Arvense Root

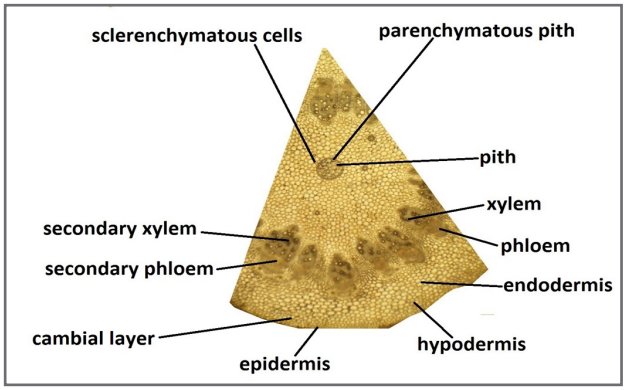

Figure 6: Transverse section (slice) of cirsium arvense root

Phytochemical screening of c.arvense leaf extract (Qualitative analysis)

Detection of carbohydrate in leaf

One milliliter ml of the plant leaf extract solution was treated with few drops of Benedict's reagent and then boiled on water bath. The Presence of carbohydrate was confirmed by appearance of reddish brown precipitate, but in the methanolic extract was present while in the distilled water and ethanolic extract was in not present (Table 1).

Table 1: Phytochemical Analysis of C Arvense Leaf Extract

S.No	Phytochemicals	Part	Carbohydrate	Alkaloid	Saponins	Flavonoid	Phenol	Quinine
1	Ethanol	root	++	+	+	-	-	+++
2	Methanol	root	+	+++	++	-	-	++
3	Aqueous/Distilled Water	root	-	++	+	+++	-	-

Key: +++ indicates most presence, ++ indicates more presence, + indicates presence, - indicates absence

Detection of Alkaloid in Leaf

To 2 gram plant powder sample addition of a few drops of Wagner's reagent (potassium iodide and iodine) exhibit the presence of alkaloid by production of reddish brown precipitate, but in the ethanolic extract is not present while methanolic extract is present and in distilled water in more amount present (Table 1).

Detection of Saponins in Leaf

3ml leaf extract mixed with distilled water and then shake and standing for 10 minutes. Formation of froth indicates the presence of saponins, but in the methanolic and ethanolic extract shows presence while in the distilled water have most presence (Table 1).

Detection of Flavonoid in Leaf

Alkali reagent test was applied when extract solution was treated with sodium hydroxide solution. Presence of flavonoids was detected by appearance of red precipitation, but in the methanolic and ethanolic extract have more amount presence while the distilled water show most presence (Table 1).

Detection of Quinine in Leaf

Concentrated Hcl mixed with extract yellow precipitate shows the presence of quinine, but the methanolic and ethanolic extract shows absence while the distilled water shows presence (Table 1).

Detection of Phenol in Leaf

Extract dissolved in 7 ml distilled water and few drops of 5% ferric chloride solution, dark green colour showed the presence of phenols, but the ethanolic extract shows the most presence and methanol show more presence while distilled water shows presence (Table 1).

phytochemical screening of c.arvense root extract (Qualitative analysis)

Detection of Carbohydrate in Root

One milliliter ml of the plants extracts solution was treated with few drops of Benedict's reagent and then boiled on water bath. Presence of carbohydrate was confirmed by appearance of reddish brown precipitate, but the methanolic extract shows presence and ethanolic extract shows more presence while the distilled water extract shows absence (Table 2).

Detection of Alkaloid in Root

To 2 gram plant powder sample addition of a few drops of Wagner's reagent (potassium iodide and iodine) exhibit the presence of alkaloid by production of reddish brown precipitate, but the methanolic extract shows most presence and ethanolic extract shows presence while the distilled water extract shows more presence (Table 2).

Detection of Saponins in Root

3ml extract mixed with distilled water and then shake and standing for 10 minutes. Formation of froth indicates the presence of saponins, but the methanolic extract shows more presence and ethanolic and distilled water extract shows presence (Table 2).

Detection of Flavonoid in Root

Alkali reagent test was applied when extract solution was treated with sodium hydroxide solution. Presence of flavonoids was detected by appearance of red precipitation, but the methanolic and ethanolic extract shows absence while the distilled water extract shows most presence (Table 2).

Detection of Quinine in Root

Concentrated Hcl mixed with extract yellow precipitate shows the presence of quanin,but the methanolic extract more shows presence and ethanolic extract shows most presence while the distilled water extract shows absence(Table 2).

Detection of Phenol in Root

Extract dissolved in 7 ml distilled water and few drops of 5% ferric chloride solution, dark green colour showed the presence of phenols, but the methanolic extract shows absence and ethanolic extract shows absence while the distilled water extract shows absence (Table 2).

Table 2: Phytochemical Analysis of C.Arvense Leaf Extract

s.no	phytochemicals	Part	Carbohydrate	Alkaloid	Saponins	Flavonoid	Phenol	Quinine
1	Ethanol	Leaf	-	-	+	++	+++	-
2	Methanol	Leaf	+	+	+	++	++	-
3	Aqueous/Distilled Water	Leaf	-	++	+++	+++	+	+

Key: +++ indicates most presence, ++ indicates more presence, + indicates presence, - indicates absence

Discussion

In the present study local information, morphology, medicinally uses, collection, stomata studies and phytochemical screening analysis of cirsium arvense has been done. C.arvense is an herbaceous wild plants belong to family asteraceae. The local information about these plant were collected from the local people of that area (Mardan). In the March to April it emerges with flower and stem from the soil. The ideal conditions for growth are high moisture content, high humidity and a temperature of around about 25C0.This plants are used as antifungal, antiamoebic, antifeedant, antiviral, antibacterial, antimalarial, allergies, sores, anemia, fevr, skin diseases and as tonic blood purifier, vermifuge, aborative and insecticide etc. The phytochemical screening analysis of tannins, flavonoids, alkaloids, steroids, phenols has been analyzed which show the presence or absence of these chemical compounds. The carbohydrate in leaf extract, the methanolic extract have carbohydrate present while in the distilled water extract and ethanolic extract was in not present. The carbohydrate in root extract, the methanolic extract shows presence and ethanolic extract shows more presence while the distilled water extract shows absence. The alkaloid in leaf extract, in the ethanolic extract is not present while methanolic extract is present and in distilled water in more amount present. The Alkaloid in root extract, the methanolic extract shows most presence and ethanolic extract shows presence while the distilled water extract shows more presence. The saponin in leaf extract, in the methanolic and ethanolic extract shows presence while in the distilled water have most presence. The saponin in root extract, the methanolic extract shows more presence and ethanolic and distilled water extract shows presence. The flavonoid in leaf, in the methanolic and ethanolic extract have more amount presence while the distilled water show most presence. Robert et al., carried out the pharmacognostical study of Cirsium vulgare (asteraceae) (leaf) includes phytochemical screening, highest values were found with the extraction of water and ethanol. Phytochemical screening were confirmed by the presence of tannins, alkaloids, saponins, steroids, flavonoids, glycosides, carbohydrates [20-23]. Thier results supports our findings.

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