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Phytochemical analysis of methanolic extracts of *Elymus repens*, *Typha angustifolia* and *Caralluma edulis*

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Abstract

Introduction: Medicinal plants have been the source of medicine to all civilizations for hundreds of years. In classical medicine, *Elymus repens* (Poaceae) is consumed as a diuretic, emollient and tonic. It also soothes the pain and spasm in the urinary tract as well as treats the condition of urolithiasis (formation of kidney stones) and urinary tract infections (UTI). In Chinese folk medicine, *T. angustifolia* is employed to improve the microcirculation, improve body's defense mechanism, activate contractions of uterus, heal atherosclerosis, treat wounds and promote the differentiation and trigger the division of keratinocytes in humans. The traditional healers recommend *C. edulis* to cure hypertension, rheumatism, leprosy, diabetes, infections, gastric issues and Alzheimer disease.

Objectives: This study was aimed to investigate different phytochemical constituents in methanolic extracts of *E. repens* (plant body and roots), *T. angustifolia* (stem and fruiting body) and *C. edulis*.

Methodology: Phytochemical potentials of methanolic extracts of plants were investigated using standard procedures.

Results: Phytochemical analysis of *E. repens* crude methanol extract confirmed the existence of tannins, saponins, flavonoids and anthraquinones. However, phenols were absent in methanol extract of roots. *T. angustifolia* stem contained anthraquinones. Tannins, saponins, phenols and flavonoids were absent from *T. angustifolia* stem. *C. edulis* contains saponins, tannins, flavonoids and anthraquinones except phenols.

Conclusion: Results of this study shows that *E. repens*, *T. angustifolia* and *C. edulis* are enriched with different phytochemical constituents hence validating their uses for the treatment of various diseases.

Keywords: *Elymus repens*; *Typha angustifolia*; *Caralluma edulis*; Phytochemicals

1. Introduction

Ethnobotany includes the analysis and determination of plant-human interactions in every episode of time and the interaction of plant ambiance with human civilization. It can be defined as “the subject of connection which exists between people of primitive civilization and their plant surroundings” [1]. Some ancient traditional medical practices are Ayurveda, Unani, Siddha and Chinese. Natural medicines are largely consumed in India and China [2]. The Ayurveda and Chinese medicine are teaming up with the pharmaceutical companies for scientific investigation of herbal medicines [3]. These modern allopathic medicines work on the principle of “one drug- one target- one disease” and they focus on

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selectively curing the disease. This approach is a failure in multifactorial complicated diseases. Herbal medicines are capable of targeting more than one component. That is why they are a best choice to be utilized as an alternative medicine [4]. These properties earn medicinal plants a prominent place in the treatment of various ailments to maintain a proper health [5].

Formerly, medicinal plants have been emphasized as a treatment for variety of disorders and also used as food supplements. Medicinal plants are the ones which have some healing properties. These plants have abundant natural compounds which have therapeutic effect and can be employed in the discovery of new drugs. The different parts of the plants or sometimes the whole plant can be used for extracting and formulating new medicines. Many active components are composed and reserved in the body of these medicinal plants and have many physiological impacts on the body of the living organisms. The medicinal plants have some potent compounds which directly or indirectly act as healing agents [6]. The advancement in pharmacology and chemistry has disclosed the existence of diverse chemical compounds in the medicinal plants which make these plants bioactive against pathogens. The presence of hundreds and thousands of such compounds in medicinal plants make them propitious resources for the discovery of new drugs [7].

Elymus repens is an invasive grass. Its common names are quack grass and wild rye. It is a wriggling perennial grass member of family Poaceae that can cause heavy losses to the crops [8]. In classical medicine, it is consumed as a diuretic and also soothes the pain and spasm in the urinary tract. It is also used as emollient and tonic [9]. It is a popular grass used to treat the condition of urolithiasis (formation of kidney stones). In Ayurveda, kidney stones are considered a very exasperating disease which has been running from ages in the mankind. Despite the progress in the modern medicine regarding urolithiasis, people prefer to manage and cure it with herbal medicines [10]. *E. repens* has been thrivingly used to cure UTI without comprehensive scientific investigations [11]. It is amidst the plants whose potency has been approved by clinical testing [12].

Typha is a genus which belongs to family Typhaceae found worldwide which contains monocotyledon plants. These plants are found in marshy or wetlands [13]. These plants are commonly known as reed mace, bulrush or cattails [14]. *T. angustifolia* is an upright rhizomatous enduring plant that grows 1–2 m in height and has the potential to withstand brackish environments [15]. It is utilized in folk medicine to treat several health issues. Leaves and rhizome of *T. angustifolia* are diuretic, astringent, desiccant, hemostatic agents, vulnerary and anti-mycobacterial. Pollen of *T. angustifolia* is applied to treat abdominal cramps and abscesses. The small grains of fluff and rootstocks are availed to avert kidney stones, diarrhea, abrasion, bruises and swellings [16]. In Chinese folk medicine, *T. angustifolia* is employed to improve the microcirculation, improve body's defense mechanism, activate contractions of uterus, heal atherosclerosis, treat wounds, promote the differentiation and trigger the division of keratinocytes in humans [17].

Family Apocynaceae is the most salient and substantial group of angiosperms. The plant members grouped in this family are an ample source of glycosides, steroids, alkaloids, terpenoids, flavonoids, hydrocarbons, lactones and simple phenols [18]. *Caralluma edulis* is a part of the family Apocynaceae [19]. It is known as choong in urdu and Edgew in English [20]. It is a food-cum-medicinal plant [21]. The traditional healers recommend *C. edulis* to cure hypertension, rheumatism, leprosy, diabetes, infections, gastric issues and Alzheimer disease [22]. It has a great property to suppress appetite [23] and can be used as an effective tool against fat buildup in the body. Unfortunately, it is a threatened succulent plant in Pakistan [24].

2. Material and methods

2.1. Plant Collection and Identification

Elymus repens was taken from Dolat pur village near Pasrur, Sialkot (Fig 1 a, b and c). *Typha angustifolia* was taken from Chitti Sheikhan village, Sialkot and *Caralluma edulis* was bought from a local vegetable shop in Sialkot (Table 1). The plants were recognized by Dr. Saleem Ahmed, Pakistan Museum of Natural History, Islamabad and voucher specimens were deposited in the museum.

The roots and plant body of *E. repens*, stem and fruiting body of *T. angustifolia* and whole plant of *C. edulis* were collected and shade dried at 21-30 °C temperature range. The dried samples were grinded into powder using a blender and stored in polythene bags at room temperature (Fig. 2).

Table 1 Scientific and common names of the selected plants

No.	Scientific Name	Common Name in Urdu
1.	<i>Typha angustifolia</i>	Era
2.	<i>Elymus repens</i>	Dub
3.	<i>Caralluma edulis</i>	Choong or Choongan

2.2. Preparation of extract

2.2.1. Methanol extract

Dried powdered samples were soaked in methanol (95%) at room temperature for one week with random stirring and shaking. This process was repeated thrice. The extracts were filtered using whatman filter paper no. 42 (125 mm) (Fig. 3). The resulting filtrates were combined and concentrated to get a solid mass. The extract was stored at -4 °C in airtight vials till further use.

2.3. Determination of phytochemical constituents

The extracts were assessed for the presence of flavonoids, saponins, tannins, phenols and anthraquinones using qualitative methods of Rizwan et al., [25], Rashid et al., [26] and Munazir et al., [27].

2.3.1. Flavonoids

The aqueous plant extract (3 ml) was poured in a test tube and some drops of 1% NH₃ were added in it. After that 1 ml concentrated H₂SO₄ was added. Appearance of yellow color indicated the existence of flavonoids [27].

2.3.2. Saponins

Frothing test – 3 ml of extract was combined with 20 ml of distilled water. It was then shaken robustly for about 15 minutes. Formation of foam displayed the presence of saponins [27].

2.3.3. Tannins

Extract (0.5 g) was mixed in 20 ml of distilled water and filtered through filter paper. Some drops of 0.1% ferric chloride was then added in it. Tannins were indicated by the blue black or brownish green color [26].

2.3.4. Phenols

Extract (0.5 g) was mixed well in distilled water. Five drops of 5% FeCl₃ solution were added and presence of dark green color indicated the existence of phenols in it [27].

2.3.5. Anthraquinones



Figure 1 (a) *E. repens* (whole plant)

Borntrager's reaction was used to identify anthraquinones in the extract. HCl (2 ml) was added to the sample and the mixture was heated on a hot water bath for 15 minutes, then cooled and filtered. The filtrate was extracted with chloroform. The chloroform layer was separated and shaken with 10% potassium hydroxide solution. The upper aqueous layer becomes pink-red [25].



Figure 1 (b) *E. repens* Stem



Figure 1 (c) *E. repens* Roots



Figure 2 Fruiting body of *T. angustifolia* (Powdered)

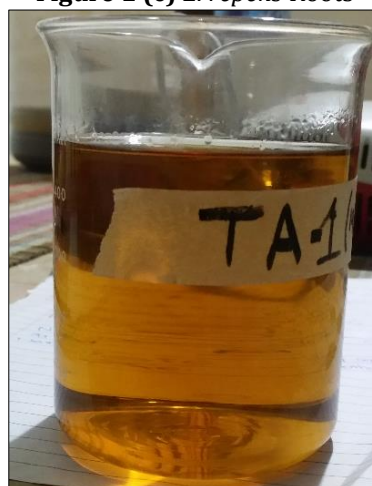


Figure 3 Methanolic filtrate of *T. angustifolia* Stem

3. Results

3.1. Qualitative studies of phytochemicals

Qualitative phytochemical screening was performed on all the extracts to identify the phytochemical classes, *i.e.* tannins, saponins, flavonoids, phenols and anthraquinones.

3.1.1. Qualitative screening of *E. repens*

The phytochemical analysis of *E. repens* roots crude methanol extract confirmed the existence of tannins, saponins, flavonoids and anthraquinones. However, phenols were absent in methanol extract. Saponins, tannins, flavonoids, phenols and anthraquinones were found in *E. repens* plant body (Table 2).

3.1.2. Qualitative screening of *T. angustifolia*

T. angustifolia stem contained anthraquinones. Tannins, saponins, phenols and flavonoids were absent from *T. angustifolia* stem. *T. angustifolia* fruiting body did not contain saponins, tannins, flavonoids, phenols and anthraquinones (Table 3).

3.1.3. Qualitative screening of *C. edulis*

C. edulis contains saponins, tannins, flavonoids and anthraquinones. Phenols were absent from the plant extract (Table 4).

Table 2 Phytochemical constituents of methanolic extracts of *E. repens* (Roots and Plant Body)

Phytochemicals	ER-1	ER-2
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Phenols	-	+
Anthraquinone	+	+

+, present; -, absent; **ER-1**: *E. repens* roots; **ER-2**: *E. repens* plant body.

Table 3 Phytochemical constituents of methanolic extracts of *T. angustifolia* (Stem and Fruiting body)

Phytochemicals	TA-1	TA-2
Tannins	-	-
Saponins	-	-
Flavonoids	-	-
Phenols	-	-
Anthraquinone	+	-

+, present; -, absent; **TA-1**: *T. angustifolia* Stem; **TA-2**: *T. angustifolia* fruiting body.

Table 4 Phytochemical constituents of methanolic extract of *C. edulis*

Phytochemicals	CE
Tannins	+
Saponins	+
Flavonoids	+
Phenols	-
Anthraquinone	+

+, present; -, absent; **CE**: *C. edulis*

4. Discussion

Ethnobotany and traditional healing systems are continuously growing and expanding. The folk medicine practices are an indispensable part of a variety of cultures. The Ayurveda, Unani and Chinese natural healing systems are the best examples. People trust nature more than the pharmacies and these natural medicines are comparatively cheap, reliable and safe. The popularity of traditional medical treatments among the masses has made the pharmaceutical companies determined to screen for active therapeutic agents [28].

This study was planned to examine different parts of *E. repens*, *T. angustifolia* and *C. edulis*. Bearing in mind their traditional usage to combat diseases, they were investigated for the active ingredients which make these plants medically valuable. Phytochemicals or phytonutrients are bioactive part of the plants. They benefit the humans for the treatment and prevention of many diseases. They have a great impact on immune system and thus regulate it to avert many maladies. They serve as antioxidants, anti-bacterial, anti-fungal, anti-aging, anti-cancer, anti-spasmodic, anti-inflammatory and anti-allergic [29].

Plants can endlessly manufacture aromatic compounds especially phenols and their oxygen containing derivatives. These compounds protect plants against insects, microbes and herbivores. They render plants different characteristics. Some give plant its characteristic odor, some compounds pledged to give specific pigment to the plants while some give that particular flavor to the plant. The phytochemicals studied in this research were phenols, flavonoids, saponins, anthraquinones and tannins. Phenols are a substantial class of phytochemicals. Their structure consists of a benzene ring attached to a hydroxyl (–OH) group. Their synthesis occurs in the chloroplasts in the presence of light [30]. The position and number of –OH group in phenols determine their inhibition effect on the microorganisms. More hydroxylation means more impedence to the microorganisms. Some researchers are of the view that heavily oxidized phenols are highly toxic. These phenols react with the sulfhydryl group of proteins. In this manner, microbial enzymes are inhibited thus terminating the microbes. Polyphenols and tannins are considered anti-carcinogenic. Tannins are a generic name for a body of polymeric phenolic compounds which has the ability of tanning leather. They are found in almost every part of the plant. Tannins can be hydrolysable or condensed. They have acquired an immense attention recently and it is recommended by the researchers to consume drinks which contain tannins like green tea, black tea or red wines [31]. Tannins are found to be biologically active because they restrict the growth of many bacteria, fungi, yeast and viruses. Their antimicrobial characters seemed to be related with the hydrolysis of ester linkage between gallic acid and polyols hydrolyzed after maturation of many fruits. Therefore, tannins act as an innate protection against microbial infections [32]. Tannins bind with the microbial cell wall and induce changes in its structure. The membrane is rattled by tannins and metabolism of the microbe is disrupted. Tannins chelate the cations and minimize their availability to the microorganisms thus decreasing the substrates for microbial growth [33]. The tannin molecules inhibit the mutation causing agents. Carcinogens or mutagens interfere with cellular macromolecules via free radicals. Tannins are antioxidants which makes them potent anti-carcinogen and anti-mutagen. They play their role to inhibit cellular oxidative damage.

Saponins are found in a great number of plants which are a part of human diet and medicine. They are actually steroids or triterpenoid glycosides. Saponins have many biological effects on the human health. Some effects are positive while some are negative. Saponins have some anti-carcinogenic characters. They are antioxidants and can act to inhibit several fungi and viruses [34].

Flavonoids are a class of compounds whose regular consumption can be proven as cancer preventive [35]. They are great antioxidants which have antimicrobial and anti-cancerous potential. Plants make flavonoids in response to microbial invasion. Plants have inborn characteristics to stop the dissemination of disease-causing microbes. They have different mechanisms to accomplish this task and one of them is the synthesis of flavonoids [36].

In the light of phytochemical elucidation, it is expected that the presence of biologically active phytochemicals in these plants make them bioactive. It seems that they can be good anticarcinogenic and antimicrobial plants. The phytochemical profile of *C. edulis* indicates flavonoids, tannins, saponins and anthraquinones. But the phenols are absent in it. The roots of *E. repens* are also seems to be potent. It is considered that the root exudates by the plant roots elaborate a great defense system in the rhizosphere. This effluence of phytochemicals can drive away, suppress or kill the microbes [37]. In conventional folk treatments, *E. repens* is used to cure the urinary tract pain and infections. It is used as a diuretic herbal medicine [9]. It is also an important classical treatment to cure kidney stones which are a matter of concern these days due to the static lifestyle [10]. The phytochemical screening of *T. angustifolia* stem and fruiting body shows the absence of the most important phytochemicals. The stem extract showed the presence of anthraquinones only. Anthraquinones are found to have anti-cancerous effects [38].

5. Conclusion

Hence, it can be concluded from our results that *E. repens*, *T. angustifolia* and *C. edulis* contains phytochemicals which can justify their uses for the cure of various diseases. Further investigations can be performed on these plants to isolate bioactive compounds for the formulation of drugs to treat emerging infectious diseases and the widespread cancer.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest

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