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PHARMACOGNOSTIC AND ETHNOMEDICINAL STUDIES ON *TRILLIUM GOVANIANUM*

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Abstract

Trillium govanianum belongs to the family Trilliaceae and is mainly distributed in Asia, from Pakistan to Bhutan. In traditional system of medicine, *T. govanianum* rhizome is used in conditions like dysentery, wound healing, menstrual and sexual disorders. In this research work the pharmacognostic evaluation i.e macroscopic, microscopic and physicochemical characteristics of the *T. govanianum* rhizome is presented. Moreover ethnomedicinal study through questionnaire was carried out in four districts of Khyber Pakhtunkhwa in order to explore the availability and potential traditional uses of this valuable medicinal plant. The microscopic examination confirmed the presence of cortex cells, carinal canal, starch grains, trichomes, sclerids and abundant calcium oxalate crystals. Total ash and acid soluble ash determined was 12.5% and 2.4 % w/w respectively. Extractive values decreased in percent (*w/w*) from polar to non-polar solvents. Ethnomedicinal studies showed that the rhizome was abundantly available in District Dir and Swat Kohistan as compared to Shangla and Buner. Furthermore majority of the informants of these districts were unaware of the medicinal use, though a small percent of the informants (Hakims and local elderly people) confirmed the use of rhizome in cancer, sexual disorder, infectious diseases, and kidney problems. These pharmacognostic findings are particularly helpful in establishing parameters for standardization, recognition of adulteration and identification of *T. govanianum* rhizome. To best of our knowledge, the pharmacognostic profile and some of the ethnomedicinal uses of this plant specie is herein reported for the first time.

Keywords: Pharmacognostic, *T. govanianum,* Microscopy, Ethnomedicinal

Introduction

The use of medicinal plants as therapeutic agents has a very ancient history. Ayurveda, Unani, Kampo, and traditional Chinese Medicine have developed successfully as a system of medicine in use for thousands of years (Okigbo *et al*., 2009). Many drugs have entered the international pharmacopoeias through the study of ethno pharmacology and traditional medicines (Patwardhan *et al*., 2005). According to a survey of World Health Organization, in developing countries, more than 80% of the people depend in use of traditional system of medicines for their basic health needs (Abbasi *et al*., 2010). This means that about 3.5 to 4 billion people in the world are dependent on plants as source of drug (Patwardhan *et al*., 2004). In contrast to the synthetics products, today the herbal products are considered safe to human beings (Ehsan *et al*., 2009). Research on plants, which are used traditionally as remedy for systemic and topical infections, has shown that they contain anti parasitic, antifungal, antibacterial, antihistaminic etc compounds (Ficker *et al*., 2003a; Da Silva *et al*., 2006; Ficker *et al*., 2003b). To study in detail, the safety profile and to determine the efficacy of the medicinal plants, correct identification and standardization is important, which can be achieved through pharmacognostic studies (Thomas *et al*., 2008).

Trillium govanianum Wall. ex Royle (Fig. 1) belong to family Trilliaceae, and is used in traditional system of medicine in Indo-Pak. The plant is up to 30cm tall herb, having stout rhizome with numerous adventitious roots. In folk medicine *T. govanianum* rhizome is used to cure dysentery, backache, healing of wounds, inflammation, Skin boils, menstrual and sexual disorders (Rani *et al*., 2013; Mahmood *et al*., 2012; Sharma & Samant, 2014). It has also been reported that the powdered plant is also used as anthelmentic (Lone *et al*., 2013). The plant is recently explored and got a high sell value for its folkloric use (Sher *et al*., 2014).

Moreover there is a great deal of traditional knowledge available in rural people of Khyber Pakhtunkhwa, especially in case of the populations living in small villages in remote areas and valleys. Therefore, ethnomedicinal study is the most effective way to record the natural resource and their management by indigenous people. Keeping in view these facts the present ethno medicinal and pharmacognostic study was carried out to explore the availability, potential traditional uses and pharmacognostic parameters to facilitate proper identification of crude drug.

Material and Methods

Plant Collection: The plant material i.e rhizome was collected from Dir Upper, Kohistan Valley (34° 54' and 35° 52' North latitudes and 72° 43' and 73° 57' East longitudes, at the altitudinal ranges of 2700 - 3800m) in August, 2013. A voucher specimen [No.Bot.20092 (PUP)] has been deposited in the herbarium of the Department of Botany, University of Peshawar, Pakistan, as reference. The rhizome was washed with distilled water and dried under shade at ambient temperature and then crushed to powder for analysis.

Macroscopic and Microscopic features: Macroscopic appearances of the fresh rhizome and the Color, shape, size,

surface, odor and taste of the crude drug were determined. Thin transverse section of the rhizome was prepared. The material was mounted in center of potato pith and a large number of transverse cuts were made across the material with the help of a sharp razor and was kept moist in water. The thin section was selected and staining was done on glass slide. The staining was carried out by putting the section in safranin for 3-4 minutes. The section was then gradually dehydrated in 10 %, 30 %, 50 %, and 90 % of alcohols. The dehydrated section was then put into a drop of methylene green and then washed with absolute alcohol for 2-3 minutes. Finally the section was mounted with Canada balsam to make them permanent and was examined under Olympus Digital microscope (MIC-D). The powder drug was also treated on glass slide, mounted with Canada balsam and was subjected to microscopic examinations (Akcin *et al*., 2010; Evans, 2002).

Physicochemical parameters: Total ash, water soluble ash, acid insoluble ash, acid soluble ash, loss on drying and extractive values were determined following established methods (Shome *et al.,* 1984; Jain & Argal, 2013).

Loss on drying: One gram of dried powder was placed in a previously dried weighing beaker. The sample was dried in an oven at 100-105^{o}C. The loss of weight in mg per air dried material was calculated (Shome *et al*., 1984).

Extractive values

Methanol soluble extractive value: Powder drug (2.0 g) was macerated with 100 ml of methanol in a closed flask for 24 hours, shaken frequently during the first 6 hours and allowed to stand for 18 hours. The mixture was then filtered and the methanol was evaporated and allowed the filtrate to dryness in a tarred shallow dish, and weighed. The percentage of methanol soluble extractive value was calculated with reference to the air dried drug (Jain & Argal, 2013).

Water and other soluble extractive values: The procedure for the determination of extractive values of water, ethanol, butanol, ethyl acetate, chloroform and hexanes was similar to the methanol soluble extractive value, using these solvents instead of methanol.

Ethnomedicinal study

Site selection: Four main districts of Khyber Pakhtunkhwa were selected for the study i.e Buner, Swat, Shangla and Dir, keeping in view the fact that the plant under study is found in these areas.

Sampling informants and ethnomedicinal data collection: The ethnomedicinal survey was carried out from March, 2013 to November, 2013. In addition to local people who had practical knowledge on medicinal plants, traditional healers / Hakims and pansaries (crude drug and general items sellers) were interviewed according to method described by Bruni *et al*., 1997 with slight modifications.

Results and Discussion

Macroscopic features: The macroscopic findings of rhizome can serve as diagnostic parameters. In this study the rhizome observed was grayish to brown (Fig. 2) while the internal matrix was slightly whitish in color. The external surface was rough having striation and fractures. The pieces were 3 to 5 cm long and up to 0.8 to 1.5cm thick slightly curved and twisted. The dried powder was slightly whitish in color having bitter taste and pungent odor.

Microscopic features: In the current scientific era, although modern research techniques for evaluation of the plant drugs are available but still microscopic examination method is one of the simplest and economic method used for correct identification of the source materials (Kumar *et al*., 2011).The transverse section of rhizome (Figs. 3 a & b) showed presence of cortex cells, trichomes, carinal canal, sclereids, vascular bundles (xylem and phloem), fibers, cambium, calcium oxalate crystals and starch grains. Calcium oxalate crystals were abundant in rhizome. These histological and morphological studies of the rhizome are key in rapid identification of *T. govanianum* rhizome.

Fig. 1. *Trillium govanianum* plant

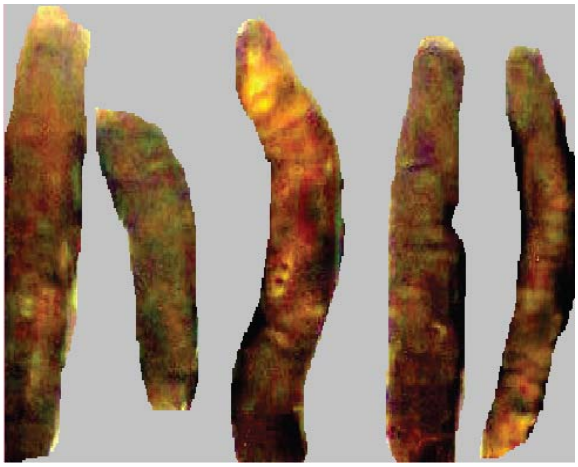

Fig. 2. *T. govanianum* rhizome

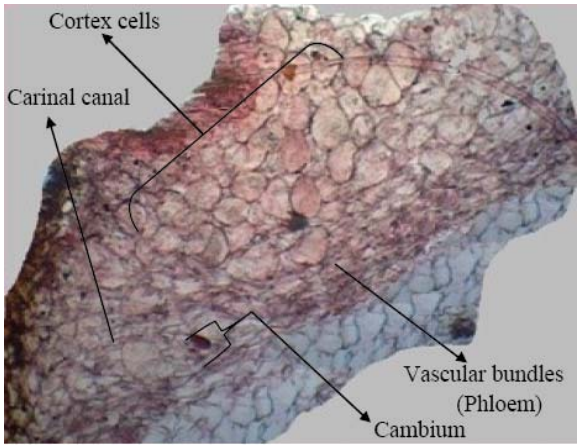

Fig. 3a. Transverse section of rhizome

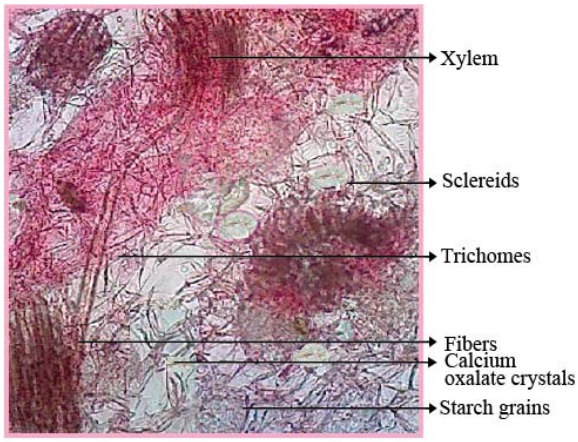

Fig. 3a. Transverse section of rhizome

Physicochemical parameters: The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent (Thomas *et al*., 2008; Kumar *et al*., 2011). Ash values of a drug provide an insight into the earthy matter, inorganic composition and other impurities present along with the crude drug (Fatima *et al*., 2014). With respect to physicochemical parameters obtained from this study, total ash value was found to be 12.5%, water soluble ash 4.0%, acid soluble ash 2.4% and acid insoluble ash 0.8% w/w (Fig. 4). Loss on drying of powder rhizome was 14.8 %. Ultimate dryness is not necessary for the drug, and majority of the drugs contain some percent of moisture contents, but higher moisture can result in spoilage by microorganisms especially the fungi and chemical reactions such as hydrolysis and oxidation (Evans, 2002). Thus it is key element in drug preparation to know the rate and condition at which moisture is removed. The loss on drying observed was 14.8% w/w, which shows high proportion of moisture and thus it can be assumed that the powder drug has high moisture content, and it is also likely, that it is highly hygroscopic. Extractive values (Fig. 4) were high for solvents like water (21.5%) and methanol (18.75%) as compare to non-polar solvents, which is an indicative of abundance of sugars, and other polar compounds like glycosides, saponins, flavonoids and steroids.

Ethnomedicinal study: In this study, regarding the medicinal uses of *T. govanianum* rhizome, information was collected from people of four districts of Khyber Pakhtunkhwa. Informants included Plant collectors, local drug sellers, Hakims and local elders having drug knowledge (Fig. 5). From ethnomedicinal survey it was found that this plant is abundantly available in District Upper Dir (Kohistan) and District Swat (Kohistan and mountainous areas) of Khyber Pakhtunkhwa in comparison to District Shangla and Buner. Furthermore, during field survey it was observed that a large number of local people were involved in digging and collection of this plant species for commercial sale and earning purposes. The local people of these areas particularly in Dir and Swat Kohistan sold the collected rhizome at a rate of Rs. 1000 per Kg (≈ $11/ Kg).

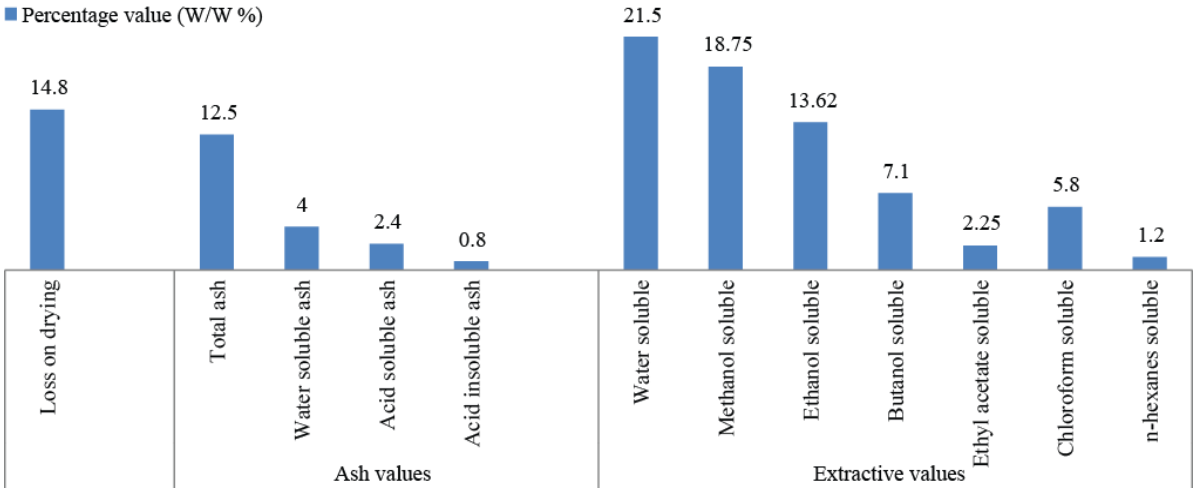

Fig. 4. Physicochemical parameters of *T. govanianum* rhizome.

Majority of the informants in these areas were unaware of the uses of rhizome. They were engaged only in the collection and marketing of the rhizome as their earning source. Only a limited number (<17% in any category) of informants knew about the uses of rhizome (Table 1). The hakims and local elderly people of District Dir and Swat confirmed the medicinal uses of the rhizome in the treatment of cancer, GI disorders, sexual disorders, backache, kidney problems and as vermicide. The percent information of informants regarding the uses of rhizome were higher in district Dir followed by district Swat in comparison to Shangla and Buner districts.

The ethnomedicinal uses of this plant as reported by the informants from the four districts indicate that highest presumed indication is inflammatory disorders including backache, headache, general inflammation, joint pains, kidney problems (with highest 21.6% and 14.7% informants from Dir and Swat having a consensus at this use) followed by anti-cancer use (15% and 12.8% from Swat and Dir respectively at this use). In case of other indications, applications in infections (16.8% from Swat and 13.4% from Dir); GI disorders (14.7% from Swat and 10.4% from Dir); and sexual disorders (9.2% from Dir and 7.3% from Swat) came to picture. From this survey an interesting finding was the response from people of Swat who appeared to have more information regarding the uses of this plant followed by the people of Dir district. This probably is due to the higher educational level in these two districts in comparison to Shangla and Buner districts. Moreover, highest numbers of informants (124) were from Swat followed by Dir (81), Shangla (39) and Buner (9) that shows the level of understanding at these districts (Fig. 5). It was also evident from this survey that local elders were having appreciable information on the plant use and that is shared and transferred to other people. These presumed uses are in confirmation to some recent reports of plants of *Trillium* genus that have reported impact in sexual disorders (Rani *et al*., 2013), skin infections (Lone *et al*., 2014), infections other than skin infections (Mahmood *et al.,* 2012; Sharma & Samant, 2014), as anthelmintic (Bhardwaj *et al*., 2013; Lone *et al*., 2013), and other inflammatory disorders (Shah *et al*., 2015). However, the use in cancer needs to be sifted scientifically and if found to have an impact will be of great significance since it will not only serve the humanity but will also be source of great earning for the people associated with the collection and processing of this plant as well as will generate revenue for our country.

The ethnomedicinal study enables the researcher to work with local population, so that to investigate knowledge based on experiences of ages (Martin, 1995). Moreover the indigenous plants particularly medicinal species even in this modern era, play a key role in the socioeconomic strengthening of the rural areas and a variety of locally produced medicines are still commonly used as household remedies for treating different aliments (Qureshi & Ghufran, 2005). If this medicinal herb is processed, commercialized and sold in such a way that no conservation strategy is adopted, there is chance of extinction of this herb from these areas. Therefore, it is necessary for the concerned authorities and the government to prepare a conservation strategy to safeguard this valuable asset of this region. There is also need for creating awareness among the local people regarding the propagation and cultivation methods in order to conserve this valuable medicinal herb.

Table 1. Informants and therapeutic uses of *T. govanianum* rhizome in different districts of Khyber Pakhtunkhwa.

Therapeutic Uses	Dir (U)	Swat	Shangla	Buner	Reported References
Cancer	12.8%	15.0%	3.8%	0.5%	-
Sexual disorders (Erectile dysfunction; Sexual tonic)	9.2%	7.3%	1.9%	1.2%	Rani *et al*., 2013
GI Disorders (Abdominal spasms)	10.4%	14.7%	1.7%	-	-
Skin Infections	6.2%	11.1%	2.1%	-	Lone *et al*., 2014
Infectious diseases(Healing of wounds, antiseptic, bacterial diarrhea, dysentry)	13.4%	16.8%	-	2.1%	Mahmood *et al*., 2012. Sharma & Samant, 2014
Anthelmintic	15.3%	7.2%	3.4%	-	Bhardwaj *et al*., 2013; Lone *et al*., 2013
Others (backache; fever; inflammation; Headache; Kidney problems)	14.7%	21.6%	4.4%	-	Shah *et al*., 2015
Plant Information					-
Local name	Matarzela	Matajarra	Matajarai	Matajarra	-
Plant parts used	Rhizome	Rhizome	Rhizome	Rhizome	-
Availability	Abundant	Abundant	Rare	Rare	-

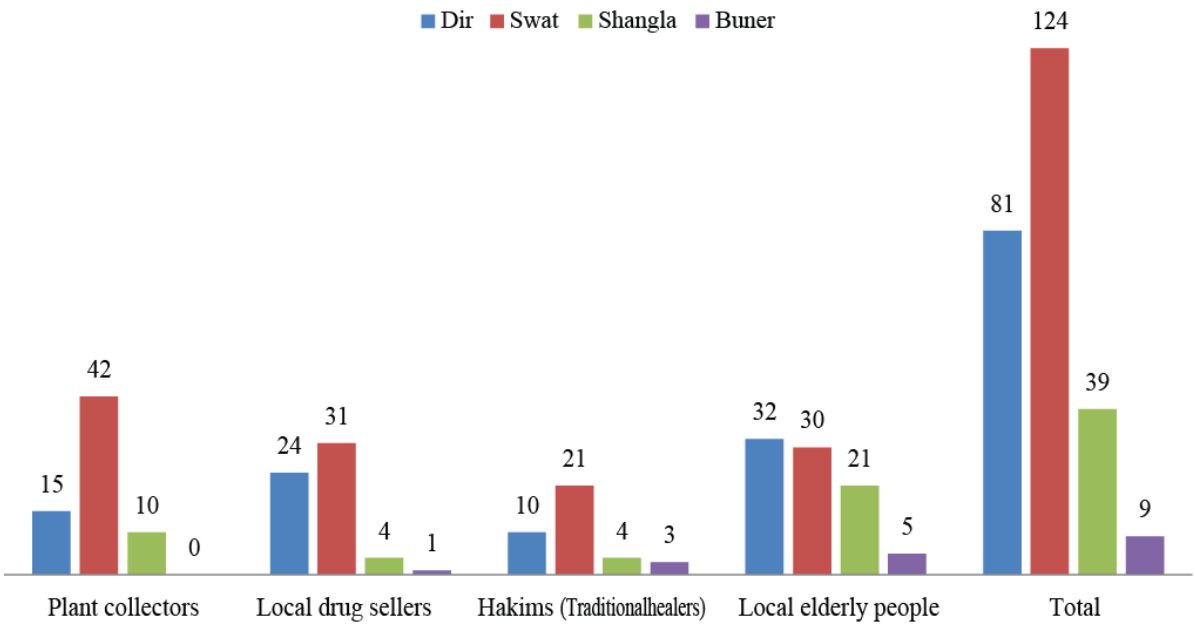

Fig. 5. Informants for the ethnomedicinal uses of *T. govanianum* rhizome from different districts of Khyber Pakhtunkhwa

Conclusion

To the best of our knowledge, this study has established for the first time, the micro and macro standards for the *T. govanianum* rhizome. These standards of the drug (rhizome) can be capitalized as useful information for identifying and authenticating the crude drug of this medicinally important plant that has a significant ethnomedicinal value and therapeutic potentials.

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