Determination of Essential Oil Content of Calamus Rhizomes Collected from Paghman district of Kabul Province, Afghanistan

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Abstract

Acorus calamus commonly known as sweet flag and calamus, is a semi-aquatic rhizomatous aromatic plant of Acoraceae family, and is distributed in eastern parts of Afghanistan. Different parts of the plant have been used in traditional medicine of Asian and European countries. Rhizome of sweet flag has medicinal value due to presence of different phyto constituents including essential oil (EO). The EO of calamus is responsible for its various physiological as well as toxic effects. Both geo climatic and environmental factors could affect EO content of *A. calamus*. *A. calamus* rhizomes were collected from selected regions of Kabul, Afghanistan, in month of September, 2019 that is the senescence stage of the plant development. The EO of shade-dried rhizomes was extracted by hydro-distillation using Clevenger type apparatus. The yield (percent) of calamus rhizomes EO was measured on the basis of volume (ml) of the EO obtained from 100 grams of plant biomass. The EO content was 2.61± 0.19 percent (v/w) for the *calamus* rhizomes collected from selected accession area.

Keywords

Acorus calamus; Essential oil; Hydro-distillation; Afghanistan

1. INTRODUCTION

Acorus calamus Linn., commonly known as sweet flag or calamus in English and Egair or Acseer-e-turkey in Persian or Dari, is a semi-aquatic perennial rhizomatous aromatic plant, which belongs to Acoraceae family (Figure 1) (Prasad *et al*., 2017 ; Oommen *et al*., 2000). Taxonomic classification of *A. calamus* is described below. *A. calamus* is widely distributed in the northern hemisphere (Pattanaik *et al*., 2013 ; Umamaheshwari *et al*., 2018). In Afghanistan, calamus is found as a wild plant in central parts including Kabul, Kapsa and Parwan provinces (Breckle *et al*., 2013).

Afghanistan as a mountainous country and by virtue of its unique geo climatic condition hosts

a diverse array of medicinal aromatic plants including *A. calamus*. This monocot species is widely distributed in central parts of Afghanistan (Breckle, S. W *et al*., 2010).

The medicinal usages of calamus is well documented in both Asian and European traditional medicines (Subha *et al*., 2011 ; Kumar Amit *et al*., 2013 ; Das Sangram Keshari *et al*, 2019) and it is one of the most known prescribed herb in Ayurvedic formulations (Rupali Singh *et al*., 2011) In India, calamus is used to cure fever, asthma, bronchitis and as a sedative. In ancient Chinese medicines, sweet flag is used to lessen swelling and for constipation. In the Arabic, Roman and European folk medicines, calamus is reported to use as an aphrodisiac (Kaushik Rahul *et al*., 2012).

Use of medicinal plants by Afghans have been practiced since long back. The Afghan people use sweet flag for digestive problems, as anthelmintic, skin ailments etc, "personal communication". Rhizome of sweet flag is used medicinally for treatment of many mental problems such as memory impairment and epilepsy, digestive disorders like bloating, colic, flatulence and poor digestive functions (R. Balakumbahan *et al*., 2010). However, leaves of the plant also possess some beneficial therapeutic properties such as; an insect repellent, antihyperlipidemic, antidiabetic, antipsychotic, antiinflammatory and analgesic (MythiliAvadhani MN *et al*., 2019).

Figure 1: A. calamus plant collected from Kabul province

Taxonomical classification of *Acoruscalamus* L.
Kingdom: Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliophyta
Class: Liliopsida
Subclass: Arecida
Order: Arales
Family: Acoraceae
Genus: *Acorus* L.
Species: *Calamus*

Phytochemical screening of calamus rhizome revealed that the rhizomes contain different primary and secondary metabolites like inorganic compounds, sugars, amino acids, terpenoids/ essential oil (EO), alkaloids, steroids, phenols, flavonoids, phenyl propanoids, xanthone glycosides, flavones, lignans, etc. (Bisht, A *et al*., 2013 ; Nadir Shahzad *et al*., 2019), which contribute to its various pharmacological properties. Among all these phyto constituents, the EO obtained from rhizome of *A. calamus* attracts much attention, recently.

Calamus EO is mainly composed of calamen, clamenol, calameon, asarone and other volatile hydrocarbons that are responsible for its important physiological as well as toxic effects (Hashmat Imam *et al*., 2012 ; Deepak Chandra *et al*., 2019). The a- and a-asarone as the main components of calamus EO (Yaremenko *et al* ., 2018), are the key factors for many pharmacological (sedative, CNS depressant, anticonvulsant, antiinflammatory, anti-oxidant, antispasmodic, hypolipidemic, immune suppressant, cytoprotective, antimicrobial, fungicidal, anthelmintic, analgesic and toxicological (genotoxic, mutagenic and carcinogenic) activities of calamus rhizome (Umamaheshwari *et al*., 2018 ; K. Dušek, *et al*., 207).

Studies show that, there are many influencing factors such as: geographical zone (25, 26), climatic conditions, altitude, soil pH and soil texture, photoperiod (Farooqi *et al*., 1999 ; Fahlén *et al*., 1999), etc. that affect both quantity and quality of EO produced by aromatic plants. However, from quality control point of view, quantification of plants' EO in accordance to standards, is very important for standardization and qualification of aromatic plants, and also to increase their agricultural and economical values. Into extent of our knowledge, the EO yield of *A. calamus* growing wildly in Afghanistan, especially in Kabul province, is not studied so far. Therefore, the aim of the present study was to investigate the EO yield of calamus rhizomes collected from selected region of Kabul province, Afghanistan.

2.MATERIALS AND METHODS

2.1 Plant materials

The rhizomes of calamus were collected from the wild habitats in Kabul province (Paghman district), Afghanistan, in September, 2019.

Collected plant material was identified as *A. calamus* rhizome by Prof. Mohammad Nasim, faculty staff of Pharmacy Faculty, Kabul, Afghanistan. Herbarium sheets of the collected plant were prepared and the voucher specimens (No. 2232-2235) were deposited as references in herbarium of faculty of pharmacy, KU. Collected rhizomes were thoroughly washed, cleaned from remains of leaves, chopped into small pieces, and shade dried for one week.

2.2 Pharmacognostic Evaluation

Pharmacognostical studies are very important to ensure plant identity and to prevent adulteration. *Acoruscalamus* is a rhizomatous aromatic herb with sword shaped leaves and spadix inflorescence, which grows up to 1.5 meters in marshy and wetland areas (Figure 1). Rhizomes were yellowish-brown curve-shaped, aromatic with sweet odor and bitter taste (Figure 2). Histological studies of calamus rhizome was characterized by presence of aerenchyma, central stele, scattered vascular bundle and oil-containing cells (Figure 2).

The total ash-, water- soluble ash and acid-insoluble ash values, moisture content, water soluble- and alcohol soluble extractive values of calamus rhizomes collected from selected Eco zone are summarized in Table 1.

Table 1. The EO yield percentage and numerical indices of *A. calamus* rhizomes collected from *Paghman* district of Kabul province.

Essential oil % (v/dw)	Total ash % (w/dw)	Acid insoluble ash % (w/dw)	Moisture content % (w/dw)	Water soluble extractive % (w/dw)	Alcohol soluble extractive % (w/dw)
2.61± 0.19	7.1± 0.51	0.75± 0.05	6.26± 0.35	8.96± 0.25	2.33± 0.23

Data are recorded as mean ±SD (n=3), w; weight in mg, dw; dry weight

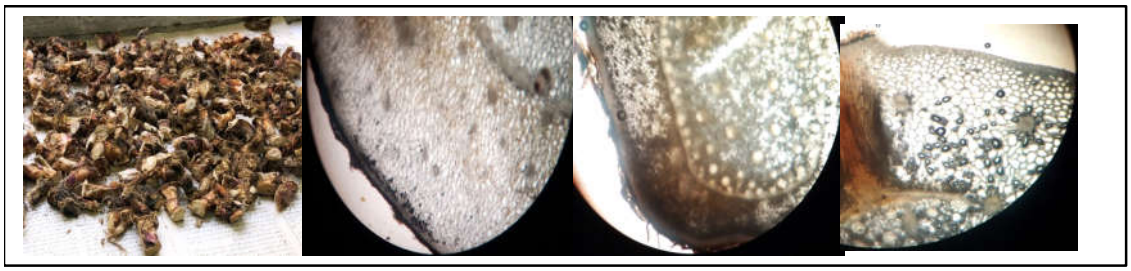

Fig-2: *A. calamus* rhizomes collected from Phagman, Kabul province and its microscopic illustration in 10X magnification.

2.3. Essential oil Extraction

Dried calamus rhizome was powdered using an electric blender. For extraction of EO, accurately weight amount (30 g) of the powdered material was subjected to hydro-distillation for 4 hours, using a Clevenger type apparatus, as previously described (38). After completion of the distillation process the apparatus was allowed for a while to be cool and meanwhile for formation of clear yellow EO phase above the water inside the graded tube of the distillation apparatus. The volume of extracted EO was directly read and recorded from the graded tube of Clevenger apparatus which facilitated the volume measurement of collected EO therein. The yield percentage (v/w) for the EO of collected calamus samples was calculated on the basis of volume (v) of EO obtained from dry weight (dw) of plant biomass.

3. Statistical analysis

The experiments were repeated in triplicates and the results were recorded as mean ± SD. Microsoft excel 2010 was used in data analysis. Microsoft excel 2010 tool-pack was used for the calculations of mean and standard deviations.

3. RESULTS AND DISCUSSION

The EO yield and numerical indices of A. calamus rhizomes collected from Paghman district of Kabul province is summarized in Table1. The EO content was 2.61± 0.19 %for the samples collected from the selected accession area. The obtained EO was pale yellow with a characteristic odor, and produced an irritating sensation in the eyes.

Our result was in agreement with the findings of the investigation done by Hema Lohani *et al* 2012) on variation of EO composition of *A. calamus* collected from Uttarakhand Himalaya, India. Liu and colleagues) reported the yield of *A. calamus* rhizome's EO 1.31% (v/w). According to Parki *et al*., 2017) the yields of EOs in different seasons were 1.2–4.8% w/v for rhizomes. (Koranga *et al*., 2019) reported the EO yield of *A. calamus* rhizomes from 2.40±0.18 to9.74 ± 0.25% in 33 accessions collected across the India, in which the positive correlation between the yield percentage and altitude revealed. Lal and colleagues documented a new variety of *A. calamus* with the average EO yield of 1.20 %. Besides other geo climatic conditions like altitude is an important influencing factor that could affect EO content of aromatic plants.The high altitude (around 2307 m) and specific climatic condition of Paghman district may affect the EO yield of *A. calamus* growing there.

Essential oils are tepen-derived secondary metabolites synthesized in different parts of aromatic pants. These volatile compounds enable plants to adapt the harsh environmental and ecological conditions and survive. High / low temperature, UV radiation, herbivory, etc. are some well-known examples of abiotic and biotic stress factors that influence plant growth and development and could induce EO production. The high content of EO in plants grown in specific geography and high altitudes could be justified by protective role of these secondary metabolism products against the mentioned stressors.

4. CONCLUSION

The aromatic plants growing wildly in different parts of Afghanistan are considered to be rich sources of EO of pharma- ceutical, cosmeceutical and nutraceutical values. Since the dry climate, hot days and cool nights, higher altitude areas are all from the unique characteristics of this mountainous country which may potentiate its aromatic plants EO yields. As far as ascertained, publications regarding Afghanistan medicinal plants are very scarce. Taking into account the increased demand for natural drug resources and increased importance of herbal medicines worldwide, researches on medicinal plants growing in Afghanistan are highly emphasized.

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