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Full Length Research Paper

Pharmacognostic, larvicidal and phytotoxic profile of *Coleus forskohlii* and *Rosmarinus officinalis*

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This study was performed to conduct the pharmacognostic study of *Coleus forskohlii* Linn. and *Rosmarinus officinalis* and find larvicidal and phytotoxic effects of these plants. The pharmacognostic parameters for both plants were set out using macroscopic and microscopic methods and the larvicidal and phytotoxic potential was determined using recommended methods. Various pharmacognostic parameters such as stomatal index, palisade ratio, vein islet number and vein termination number were determined for both plants. In the powder drug of both plants, various fragments were detected such as epidermal cells, non-glandular trichomes, and glandular sessile and stalked trichomes, epidermal cells with stomata. Biological activity of ethanolic extract of *C. forskohlii* and *R. officinalis* showed that *R. officinalis* extract is more effective than *C. forskohlii* against *Culex quinquefasciantus* larvae. In case of phytotoxic effect, the effect of *R. officinalis* was better than *C. forskohlii.* Both tested plants were found to be phytotoxic and larvicidal.

Key words: Pharmacognostic, *Coleus forskohlii* Linn., *Rosmarinus officinalis* Benth.

INTRODUCTION

The pharmacognostic study of crude drug is very essential for the identification of medicinal plants and prevention of adulteration (Muhammad et al., 2012; Ismail et al., 2011). Medicinal plants have acquired increasing significance in development of cooperation among various organizations in the recent years. The medicinal use of the plants is well established even in the modern world, which is indicated by the fact that more than 30% of the allopathic drugs are of plant origin and about 80% of the world population relies chiefly on traditional medicines (Saeed et al., 2010; Shinwari, 2010). *Rosmarinus officinalis* (Lamiaceae), locally know as rosemary is a long lived semi woody perennial shrub, with evergreen leaves, stem of 1 to 2 m long, calyx densely white tomentose, corolla bilabiate, two prominent stamens with simple filament and carpal consisting of long style. *Coleus forskohlii* (Lamiaceae) is a perennial shrub, with fleshy leaves, stem of 18 to 24 inch long, calyx and corolla bilabiate, stamens didynamous, and carpal consist of bifid style (Qaiser and Omer, 1985).

In the traditional system of medicines, *R. officinalis* is used as anti-asthematic, bronchodilator, antiplasmodial, antioxidant (Inatani et al., 1983), anti-inflammatory, anticancer and as hepatoprotective agent (CA R, 1999). Antimicrobial activities of this plant have also been reported by Oluwatuyi et al. (2004). *C. forskohlii* (local names are Coleus, mainmul, and karpuravali) is also a well know medicinal plant and has been reported with various pharmacological activities like ant-obesity and anti-asthematic. Its use in glaucoma and heart disease has been reported by Kavitha et al. (2010).

This study was conducted with aim to documents the

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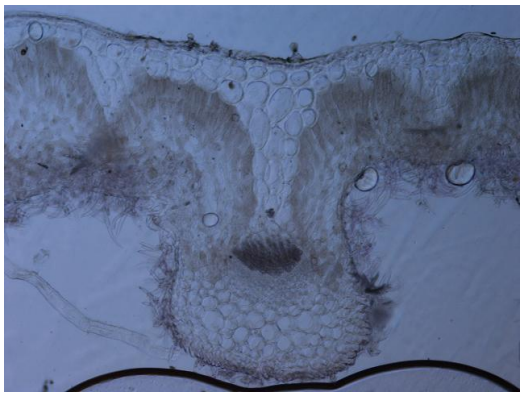

Figure 1. Transverse section of *Rosumrinus* leaf.

Figure 2. Lower epidermis of *Rosmarinus* with gland and trichomes.

pharmacognostic profile of both plants and the ethanolic crude extract of both plants was screened for their larvicidal activity.

MATERIALS AND METHODS

Collection of plants and larvae

R. officinalis and *C. forskohlii* were collected from plant growing at Department of Botany University of Peshawar, Peshawar. Each of the collected plant samples was cleaned washed and dried at room temperature.

L. minor plants were collected from the fresh running water in University of Peshawar and were transferred to the Pharmacognosy Laboratory, Department of Botany, University of Peshawar for phytotoxic assay.

Mature larvae of *Culex quinquesfasciatus* were collected and identified by Prof. Dr. Khesroon, Department of Zoology, University of Peshawar, Peshawar, Pakistan.

Preparation of extract

The leaves of both plants were soaked in pure ethanol of commercial grade and ethanolic extract was prepared following our previous published methods (Muhammad et al., 2010a; Muhammad et al., 2010b; Barkatullah et al., 2011). The powder of *R. officinalis* and *C. forskohlii* were soaked in ethanol for one week. There after each plant extract was passed through filter papers. The obtained extracts were evaporated in a rotatory evaporator at 45°C obtained concentrated extract of each plant. These concentrates of each extract were stored at 4°C prior to use. The final collected crude ethanolic extract was scrutinized for larvicidal activity.

Pharmacognostic study

The dried leaves were powdered with the help of an electric grinder and were stored in airtight bottles. Some fresh specimens of the plants were used to study morphological and anatomical characters, and for the palisade ratio, vein-islets number, vein-termination number and stomatal studies. The hands cut sections were made of fresh specimens. The material was mounted in the center of potato pith, by making a whole, cylindrical or longitudinal according to the type of material. The thin sections were selected for staining, which was carried out on a microscopic glass slide (Evans et al.,, 2002). The powdered drug was studied on electric microscope at different power of eyepieces. The powder material was macerated with chloral hydrate suspension. One drop of solution was taken on a slide and then it was heated on spirit lamp and then examined under microscope. Different tissues were observed under the microscope and were photographed (Muhammad et al., 2012).

Larvicidal activity

One gram of each crude extract was first dissolved in 10 ml of ethanol to prepare a stock solution. From the stock solution, different concentration (50, 100 and 200 ppm) were prepared with tap water. Experiments were conducted for 24 and 48 h, respectively at room temperature. The larvicidal activity was assessed following the well recommended procedure (Rahuman et al., 2000). For bioassay, 10 test larvae were taken in 3 replicates in 50, 100 and 200 ppm concentration of the desired plant extract. And the numbers of dead larvae were counted after 24 and 48 h exposure, respectively and the percentage mortality was reported from the average of 3 replicates.

Phytotoxic activity

The crude ethanolic extract of both plants concentrations of 50, 100 and 200 ppm were tested for their phytotoxic effect using our previous published method (Muhammad and Saeed, 2011).

RESULTS AND DISCUSSION

Microscopic characteristics of the leaf of *R. officinalis* show that the upper epidermis which is unicerate cuticle is thick, and palisade cell present only below the upper epidermis (Figure 1). The trichomes are of two types, glandular and non-glandular or covering trichomes. The stomatal index of *R. officinalis* is non-measurable due to the peltate hair. The stomata were present only on the lower surface. Microscopic characteristics of the leaves of *C. forskohlii* showed that the cuticle is thin, and stomata were present on upper as well as on the lower surface but more on the lower surface (Figures 2 and 3). Non-glandular as well as glandular trichomes were present on

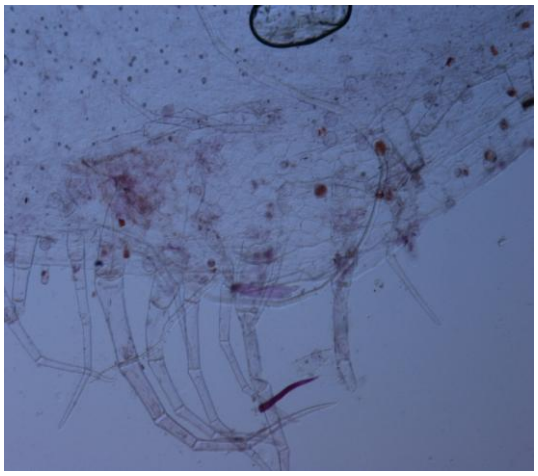

Figure 3. Transverse section of *Coleus* leaf.

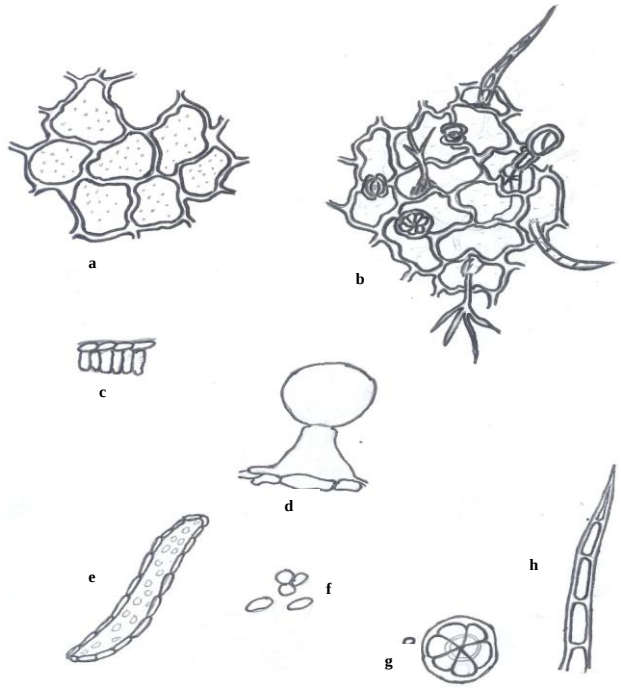

Figure 4. (a) Upper epidermis, (b) lower epidermis with stomata, glandular, non-glandular and branch trichomes, (c) palisade cells, (d) glandular trichome, (e) tracheid, (f) starch grains, (g) multicellular head of sessile gland, (h) covering trchome.

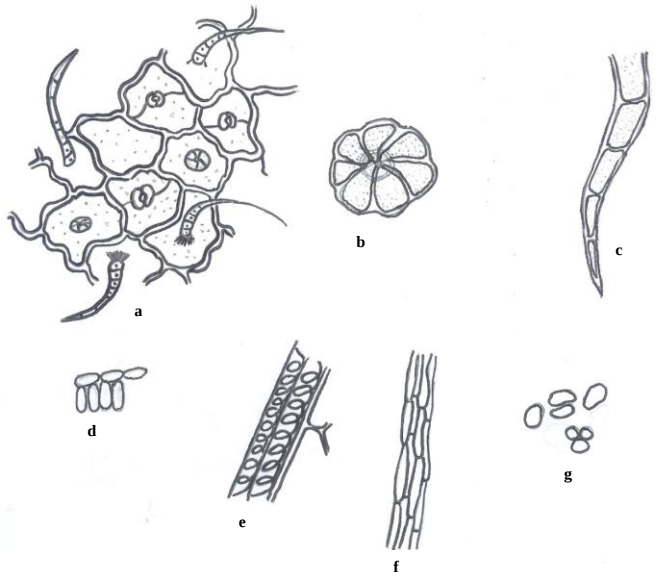

Figure 5. (a) Epidermis with stomata, sessile glandular and covering trichomes, (b) multicellular head of sessile gland, (c) covering trichome, (d) palisade cells, (e) spiral ducts, (f) vessels, (g) starch grains.

both surfaces. Palisade cells were present on both lowers as well as on upper sides.

Glandular and non-glandular trichomes were present on the lower surface, which is the characteristic feature of family Lamiaceae, but a few stalked glandular trichomes were also present on the upper epidermis. The glandular trichomes were mostly sessile and with multicellular head but few stalked glandular hairs were also present in *R. officinalis* (Figure 4). The stomatal index of *R. officinalis* was non-measurable, because the stomata present only on the lower epidermis which is covered with dense peltate trichomes which have hidden the elliptical stomata (Annalisa et al., 2003). In *C. forskohlii*, non-glandular as well as glandular trichomes were present on both surfaces. Rejdali (2008), who reported different types of trichomes which were not uniformly distribution on the leaf surface stomata of diacytic type were present on both lower and upper epidermis in *Sideritis* (Lamiaceae). In *Coleus,* the covering trichomes are more numerous, unicerate and unbranched. Palisade cells were present on both lower as well as on upper sides (Figure 5) between palisade cell, and spongy mesophylls were present. The average vein islets number of *R. officinalis* and *C. forskohlii* as observed under microscope during this work were 11 to 14 and 9 to 12, respectively and the vein termination number of the *R. officinalis* and *C. forskohlii* were worked out and tabulated as shown in Table 1. The powder drug characters especially the microscopic character have got prominent role in the identification and standardization of drugs. The organoleptic study of the powder drug of *R. officinalis* and *C. forskohlii* were also carried out.

The powder drug of *R. officinalis* was dull green in color, slightly bitter in taste with cooling effect and pungent odor. Two types of trichomes covering (trichomes and glandular trichomes), epidermal cell with elliptical stomata, trachead, parenchyma's cell and starch grain were observed. The powder of *C. forskohlii* was yellowish brown with pleasant smell and bitter taste powder shows numerous ovoid simple circular elliptical

Table 1. Leaf histological parameter of *R. officinalis* and *C. forskohlii.*

Parameter	Average ratio	
	R. officinalis	***C. forskohlii***
Palisade ratio	4.6 to 5.20	4.6 to 6.10
Stomatal index	Non measurable	27.60 (lowered) 24.40 (upper)
Vein islet number	11-14	9-12
Vein termination number	12-15	9-11

Table 2. Larvicidal activity of *Rosmarinus officinalis* and *Coleus forskohlii*.

Plant	Dose (ppm)	Mortality (%, after 24 h)	Mortality (%, after 48 h)
Control	-	**0**	**0**
	50	13	16
C. forskohlii	100	20	20
	200	33	40
	50	20	43
R. officinalis	100	46	66
	200	56	83

Table 3. Organoleptic evaluation of *R. officinalis* and *C. forskohlii*.

Parameter	Observation	Observation
Color	Dull green	Light brown
Odor	Pungent	Aromatic
Taste	Slight bitter with cooling effect	Slight bitter
Fracture	Hard	Soft
Touch	Hard	Soft

starch grain, trachied, vessels and paranchymatus cells. Two types of trichomes (Glandulalar and Non-glandular), epidermal fragment with diacytic stomata and palisade cells were also observed. Larvicidal activities of *R. officinalis* and *C. forskohlii* were carried out. The present results (Table 2) showed that the leaf ethanolic extract of *R. officinalis* was more effective than *C. forskohlii* against *Culex* larvae. The activity was observed in different concentration of the crud extracts, that is, 50, 100 and 200 ppm at 24 and 48 h exposure.

In this study, both plants (*R. officinalis* and *C. forskohlii*) at 50 ppm concentration showed low mortality rate after 24 and 48 h, respectively. But at higher concentration (100 and 200 ppm) and after 48 h, the mortality rate for *R. officinalis* was 66.67 and 83.33, respectively, whereas for *C. forskohlii* the mortality is 20.00 and 40.00, respectively as shown in Table 3. This shows that *R. officinalis* can be used effectively as a larvicide against *Culex*. Whenever there is an effective crude alternative to synthetic drugs or Larvicides, the present trend is to go for the former one not only because it is cheaper, but due to its lesser toxicities to human being and due to their biodegradability. The member of Lamnaceae family is a suitable organism to investigate physiological processes. *Lamna* (*L. minor*) plant consists of a central oral frond or mother frond with two daughter frond and filamentous root. The phytotoxic effect was tested according the well recommended methods (Atta-ur Rehman et al., 2001).

The crude ethanolic extract of the leaves of both plants were tested at concentration of 10, 100 and 1000 ppm. The percent phytotoxic effect of *R. officinalis* was 13, 20 and 67% at the tested concentrations of 10, 100 and 1000 ppm, respectively, while the percent phytotoxic effect of *C. forskohlii* was 10, 15 and 35% at the same tested concentrations, respectively.

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