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RESEARCH ARTICLE

Pharmacognostic Studies on *Christia obcordata* (Poir.) Bakh. f. and *Christia vespertilionis* (L.f.) Bakh. f

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ABSTRACT:

Knowledge of pharmacognostic standardisation is crucial in the field of herbal research. *Christia obcordata (Poir.) Bakh.f.* and *Christia vespertilionis (L.f.) Bakh.f*., commonly known as 'butterfly wing' are ornamental plants belonging to the family Fabacae. *Christia vespertilionis* is believed as a promising anti-cancer agent and is consumed by the old folks of Malaysia in the form of decoction. Moreover, these plants have also been used to treat bronchitis, urinary blockage, and improve blood circulation. Despite the listed medicinal properties, there is no evidence in literature of the pharmacognostic standardization on the aerial parts of these plants.Hence, the current study will evaluate the pharmacognostic standardization parameters on the aerial parts of *Christia obcordata (Poir.) Bakh.f.* and *Christia vespertilionis (L.f.) Bakh.f.* Standardisation of these plants was carried out using macroscopic studies, microscopic studies, physicochemical parameters, and phytochemical investigation. Macroscopic showed both plants have similar characteristics in the followings, shape, phyllo taxis, margin-entire, leaf type, and powder microscopy results showed the upper cuticle, upper epidermis, palisade cells, vascular bundle, spongy mesophyll, phloem fibers, sclerenchyma, xylem vessels, collenchyma, sclerenchyma, lower epidermis, lower cuticle, parenchyma, stomatal index, vein number, vein islet, and palisade cells are studied as important key differentiating features on the selected plants. Phytochemical screening on the aerial part extracts presence of steroid, flavonoid, tannin, alkaloid, and carbohydrate on both plants. Fluorescence analysis was carried out individually on the four different extracts and was examined under daylight and U.V light at a wavelength of 254nm and 365nm. To conclude, based on this research finding, the study can be a standardization tool for these plants, providing ease in identifying and determining the purity and quality of the investigated plant.

KEYWORDS: *Christia obcordata, Christia vespertilionis,* Macroscopic, Microscopic, Physicochemical parameters, Fluorescence analysis, Preliminary phytochemical investigation.

INTRODUCTION:

In recent years, the usage of herbal plants and their parts gained major attention among natural product researchers as a vital source of medicinal agents and cosmetics due to easy accessibility, friendly, economic, and no or fewer side effects.

According to World Health Organization 2014, it was stated that in most developing nations, plants-based drug plays an essential role in meeting the major healthcare needs of the people and highlights specific types of its treatment[1]. According to Chanda et al., adulteration occurs when an original plant is replaced or added with another plant material as well as any foreign substance for the purpose of increasing the potency of the product or reducing its cost[2]. Standardization is an initial stage and essential to ensure a predefined amount of quantity, quality and therapeutic effect of ingredients in each dose[3]. To establish the standardization and authentication parameters of crude drugs of natural origin, pharmacognostic studies of the medicinal plant

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are considered vital. In such consideration, *Christia obcordata* (*C.obcordata*) and *Christia vespertilionis (C. vespertilionis*) have gained great attention among researchers as these plants show to have potential health benefits. Christia is a legume belongs to the Fabaceae family comprising of 13 varieties that is found in Malaysia, Australia and tropical Southeast Asia. In these countries it is initially observed as an ornamental plant[4]. Every species of Christia has unique butterfly-shaped and heart shaped leaves. *Christia obcordata* (Poir.) Bakh.f. (syn. Ploca humilis, Hedysarum obcordatum, Desmodium obcordatum, or Lourea obcordata) is also known as green butterfly plant with kidney- shaped and flutter like butterfly wings and can grow up to 1 metre. The plant has trifoliate leaves and slender stems. The juvenile background leaves were light green and became bright green with dark burgundy stripes when matured[5]. *Christia vespertilionis* (L. f.) Bakh. F., (syn. Hedysarum vespertilionis, Lourea vespertilionis), is known as red butterfly wing, mariposa (butterfly in Spanish) or "rerama" (butterfly in Malaysia)[6]. It can grow up to 1.2 metre in height and has trifoliate leaves and slender stems. The leaves of the plant are purple in colour and dark green colour with pale green stripes along prominent veins when the leaf is matured. Traditionally *C. vespertilionis* has been used to treat cold muscle weakness, poor blood circulation, bronchitis, and inflamed tonsils. *C.obcordata* is also reported to be used to treat acute, chronic nephritis and urinary blockage[5]. The picture of the plants was shown in figure 1.

Figure 1: *Christia obcordate* (left) and *Christia vespertilionis* (right)

In a study, it was reported that these plants are rich in triterpenes, alkaloids, fatty acids, phenols, alkanes[7]. A Study report from Smitha et al., showed the phytochemical analysis of methanolic leaves of *C. vespertilionis* confirms the presence of alkaloids, flavonoids, glycoside, tannin, diterpenes, coumarin, and quinine[8]. Furthermore, a study conducted by Harish et al., reported thirteen compounds were identified in this plant which are two of flavonoid glycosides (quercetin-3-O-glucoside and catechin-3-O-β-D glucopyranoside), three of isoflavonoids (2'-hydroxy genestin, orobol and 2,3-dihydro-2'-hydroxy-genestin), and three of pentacyclic triterpenes (D:C- friedoolean-8-en-29α-ol), ursolic acid methyl ester, oleanolic acid methyl ester, and erythrodiol). In addition, three steroids (stigmasterol, β-sitosterol acetate and β-sitosterol) and a monoterpene called geraniol were identified. Furthermore, 7-isopropylidene-1-methyl-1,2,6,7,8,9-hexahydronapthalene (called as christene) and 2-hydroxydecanylpentadec-5, 8,10, 12- tetraenoate (called as christanoate) were also reported in the plant[9]. The compound christene has shown significantly active (IC_{50} = 9.0µg/ml) against Plasmodium falciparum[4].

In Malaysia, *C. vespertilionis* leaves decoction was believed to treat cancer. Furthermore, the study reported that rubbing the leaves of this plant on the affected area helps in healing the scabies[10]. The Whole plant was believed to treat tuberculosis and snake bite while the leaves of this plant can be used as a topical treatment to heal bone fractures[11]. The plant is also reported for its potency in increasing blood circulation, healing muscle weakness, treat bronchitis and colds and the study on, different parts of *C. vespertilionis* decoction (methanol and aqueous-methanol) of the roots, leaves and stems have been evaluated in vitro for its antiplasmodial potential against *Plasmodium falciparum* NF-54[12]. Based on the reported benefits this herbal plant was in high focus among the researchers. However, currently there is no published informations regarding the standardization and authentication of *C.obcordata* and *C. vespertilionis*. Thus, this study will focus on pharmacognostic characteristics of the selected plants and will increase its visibility to study more on their effectiveness in the field of herbal medicine. Moreover, there is less degree research has been done regarding standard parameters for these plants.

MATERIALS AND METHODS:

Collection and preparation of plant material:

The fresh plant materials of *C.obcordata* was collected from Hock Loke Siew Nursery, Ipoh, Perak and *C. vespertilionis* was collected from Asow Crafts and Landscaping, Ipoh Perak. The specimen was authenticated by Madam Fadhilah binti Abdul Rani, Deputy director of Kompleks Pertanian Telok Chengai, Kedah. Upon confirmation, the aerial parts of the plants were cleaned, shade dried, and grounded using a mechanical blender. The powdered materials of the plants were sieved seperately and stored individually in an airtight container until further use.

Macroscopic evaluation:

Macroscopic studies like shape, colour, size, texture, margin, odour, taste, and phyllo taxis were studied using fresh plant materials like leaves and stems[1].

Microscopy evaluation:

a) Transverse section of plant leaves:

The thinnest section of fresh leaves and stems of both plants were separately stained with a mixture of 1:1 phloroglucinol and concentrated hydrochloric acid. After that, the stained samples were placed onto the slide followed by a few drops of glycerin added to give a

moisture effect and covered with coverslip respectively. The slide was then observed under a binocular optical microscope, a clear picture was captured using (Leica DM750, Germany) microscope fitted with a digital camera[13].

b) Powder study:
Dried leaves powder was taken instead of a fresh section of the plant similar to microscopic study. Before staining, the powders were heated in a mixture of 5g chloral hydrate and 100ml of distilled water. Staining agents such as concentrated hydrochloric acid, phloroglucinol N/20 iodine solution at the ratio of 1:1 was used to study the powder. Distilled water was used to identify the presence of cellular components.

c) Determination of Plant constant:
The midrib of the leaf was removed by boiling with a 10% chloral hydrate solution. Using the forceps, the upper and lower epidermis were peeled out separately, kept on the slide, and mounted in glycerine water. To determine the plant constant following tests like palisade ratio, vein termination number and vein islet number, and stomatal index were studied[14].Stomatal index present in 1 sq. were counted using the following formula:

S. I = S/E + S x 100
Where
S. I = Stomatal Index,
S = No. of stomata per unit area,
E = No. of epidermal cells in the same unit area

d) Palisade ratio: It is the mean number of palisade cells underneath each epidermal cell. The test results will give subjective information to distinguish the characteristics of a closely related species[15].

e) Vein termination number and vein islet number: Veinlet termination number was calculated using average number of vein-islets per square mm of a leaf surface. It was determined by counting the number of vein-islets in an area of 4 square mm of the central part of the leaf between the midrib and the margin[16].

f) Extraction: About 2g of each dried plant material was extracted using petroleum ether, chloroform, methanol, and water (increasing in the order of polarity) in an ultrasonicator for 30 minutes. Obtained extracts were used to perform preliminary phytochemical screening to identify the phyto constituent of the plant material[17].

Qualitative Preliminary Phytochemical Screening: Preliminary phytochemical screening was performed on various extracts on both plants and the aerial parts. The secondary metabolites like alkaloid, carbohydrates, proteins, tannins, steroid saponins, and flavonoids were tested[18,19].

Fluorescence Analysis:
The fluorescence characteristics of all extracts were studied under ultraviolet light at short wavelength (254nm) and long wavelength (365nm)[20,21].

RESULTS AND DISCUSSION:

Macroscopic evaluation:
The macroscopic features of the leaves of *C.obcordata* and *C.Vespertilionis* were examined and recorded as shown in figure 2.

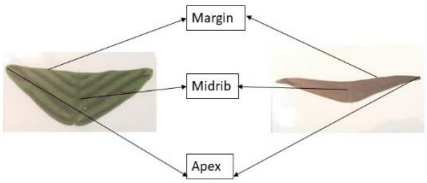

Figure 2: Macroscopic characteristics of *C. obcordata* and *C. vespertilionis*

Macroscopic evaluation is a method that is used to study the morphological characteristics of the plant part which can be observed by our naked eye or through a magnifying lens. Macroscopic evaluation is considered an important aspect of herbal analysis for the correct identification of crude drugs. Based on the observation made, both leaves show similar characteristics in the followings, shape-obcordate, phyllo taxis – alternate, margin-entire, leaf type- compound, leaf veination – recticulate, odour- no fragrance. However, colour of the leaf for *C.obcordata* was found to be green and permanently veined with deep maroon stripes on the other hand *C.vespertlionis* showed purple red tones, prominently veined with shades of pink.

Microscopic evaluation:
Transverse section of the leaf:
A Transverse section on the leaves of *C. obcordata* and *C. vespertilionis* was examined and shown in Figure 3 and 3.1. The transverse section of the *C.obcordata* leaf shows single layered barrel shaped epidermal cells, arranged compactly with numerous unicellular covering trichomes and a thin cuticle. The leaf structure demonstrates the dorsiventral nature of the leaves that represents the presence of palisade cells below the upper epidermis only. Crystal sheaths and starch grains are dispersed in the collenchyma and parenchyma cells. Vascular bindle is leptocentric where the layer of xylem vessels surrounds the phloem fibres. The transverse section of the leaf shows single layered barrel shaped epidermal cells on both surfaces covered with thin cuticles. The cells are arranged closely without any intercellular spaces in between them. These cells are devoid of chloroplasts. The presence of uniseriate multicellular covering trichomes were seen. Paracytic stomata are seen on the epidermis. The mesophyll is a ground green tissue present in between upper epidermis

and lower epidermis. The leaf structure demonstrates dorsiventral nature that represents presence of the palisade cells below the upper epidermis only. Palisade cells are elongated, arranged in 3-4 layers, compact, and contain greener chloroplasts. Crystal sheaths and starch grains are dispersed in the collenchyma and parenchyma cells. The mid rib portion consists of a vascular bundle, collenchyma, and parenchyma. There are discontinuous palisade cells in the mi rib region. Vascular bindle is leptocentric where the layer of xylem vessels surrounds the phloem fibers. Xylem vessels are arranged in groups and a layer of collenchyma is seen below the layer of xylem vessels. A layer of collenchymatous cells is seen below the vascular bundle.

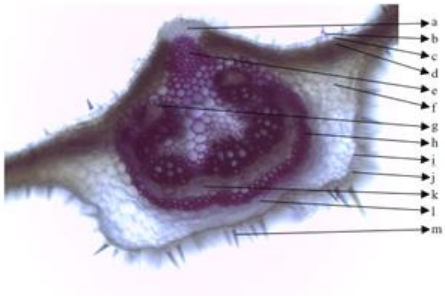

Figure 3: Transverse section of *C.obcordata* leaf.
a- Collenchyma, b- Upper cuticle, c- Upper epidermis, d- Palisade cell, e- Xylem vessel, f- Parenchyma, g- Phloem fibre, h- Pericyclic fibre, i- Lower epidermis, j- Lower cuticle, k- Phloem fibre, l- Collenchyma, m- covering trichome.

The transverse section of *C. vespertilionis* leaf consists of an upper and lower epidermis with single layered closely arranged almost isodiametric and rectangular cells with no inter cellular spaces in between them. Chloroplasts cannot be seen in these cells. Both upper epidermis on their outer surface is covered by a thin cuticle. The lower epidermis appeared wavy in outline and after epidermis, chlolenchyma cells were present.

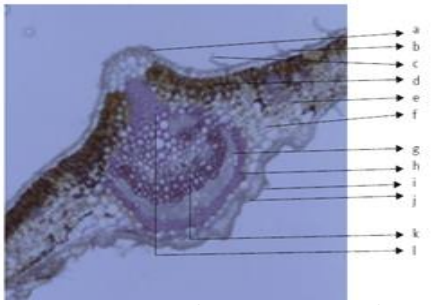

Figure 3.1. Transverse section of *C.vespertilionis* leaf.
a- Upper cuticle, b- Upper epidermis,c-Trichomes, d- Palisade cell, e- Spongy mesophyll, f- Parenchyma, g- Collenchyma, h- Sclerenchyma, i- Lower epidermis, j- Lower cuticle, k- Xylem vessels, l-Phloem.

Paracytic (Rubiaceous) stomata have two subsidiary cells, of which the long axes parallel to the axis of the stoma and are seen on both surfaces. However, the distribution of stomata on the epidermal surface is hypostomatic indicating the presence of a greater number of stomata on the lower surface as compared to the upper surface. Numerous uniseriate multicellular covering trichomes were seen on both epidermal layers. The mesophyll is a ground tissue present between the upper epidermis and lower epidermis. The mesophyll tissue consists of palisade cells and spongy parenchyma which are well differentiated. The palisade cells are present only below the upper epidermis and reveal the dorsiventral nature of the leaf. The palisade parenchyma is discontinued at the midrib region and shows 2-3 layers of palisade cells closely packed without any intercellular space between them. The palisade cells are filled with many chloroplasts. The spongy parenchyma region consists of 4-5 layered parenchyma cells which are spherical, and isodiametric with the presence of inter cellular space between them.The midrib consists of a vascular bundle, collenchyma and sclerencyma tissue. The vascular bundle consists of xylem vessels and phloem fibers and is embedded at the center of the mid rib. Xylem tissue of the vascular bundle is present towards the upper epidermis and the phloem tissue towards the lower epidermal surface of the leaf. Bundle sheath is absent. A few patches of collenchyma cells are present towards both the dorsal and ventral surfaces close to the upper and lower epidermal ends.

Transverse section of stem:

The transverse section of *C.obcordata* stem shows single layered barrel shaped epidermal cells, arranged compactly with numerous unicellular covering trichomes and a thick cuticle. The vascular bundle has lignified vessels with outer phloem. The hypodermis is composed of 3 to 4 layers of collenchymatous cells. The endodermis is distinct and embedded composed of collenchymatous tissue with numerous pericyclic fibers arranged in groups at regular intervals. The vascular bundle is hadrocentric (amphicribral) where the xylem vessels are surrounded by phloem fibres. Phloem is broad and consists of phloem fibers in groups, sieve tubes, and companion cells. Xylem consists of vessels, tracheids, fibers, and xylem parenchyma. Medullary rays are 3 to 4 cells broad and radiating. The central portion is occupied by pith consisting of isodiametric parenchymous cells and contain starch granules. Figure 4 and 4.1 show the transverse section of the stem, and Iodine stain for starch identification respectively.

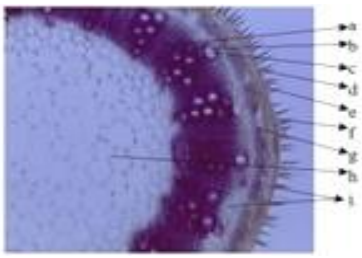

Figure 4: Transverse section of *C.obcordata* stem where, a:Medullary rays, b: Phloem, c: Xylem, d: Covering trichome, e: Epidermis, f: Cuticle, g: Pericyclic fibre, h: Pith, i: Collenchyme.

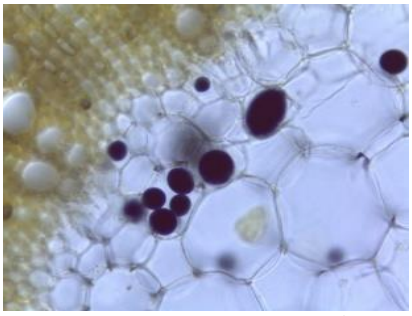

Figure 4. 1. Iodine stained transverse section of *C.obcordata* stem showing the presence of Starch.

The transverse section of *C.vespertilionis* stem reveals the presence of epidermis with a single layer of cells coated with a thin and striated layer of cuticle. Numerous uniseriate covering trichomes emerge from the epidermal layer and are found to be unicellular. The cortical region is formed by a few layers of collenchyma followed by 4 – 5 layers of sclerenchyma cells. Below the layer of sclerenchyma cells, bundles of phloem fibers are arranged with a few layers of sclerenchyma cells. Starch grains are seen in the endodermis. The vascular bundle is conjoint, consisting of the xylem and phloem that lie opposite to each other on the same radius. Isolated sclerenchyma fibers are located adjacent to the phloem differentiating multiple vascular bundles from each other. The medullar region consists of parenchyma and inclusions are found in some. The outer part consists of a single layer of epidermal cells made of parenchyma cells and covered with a thick cuticle. The cells are barrel shaped and compactly arranged with no intercellular spaces and chloroplasts. Several uniseriate covering trichomes emerge from the surface of the epidermis. The cortex is present below the epidermis in several layers and can be differentiated into the hypodermis and general cortex. The hypodermis is made up of a few layers of collenhyma cells. Underneath the epidermis, there is a ring of sclerenchymatous cells which surrounds the central structure. Cortex has collenchymatous hypodermis regularly or as discontinuous patches. The pericycle is composed of sclerenchyma and intervening masses of parenchyma as irregular patches (heterogeneous). The vascular bundles are wedge shaped, collateral, and arranged in a ring surrounding the pith. Cambium is absent. Each bundle is composed of an outer phloem and inner xylem on the same radius. Pith is extensively developed and occupies the central portion of the ground tissue. It is made from rounded or oval, thin-walled parenchymatous cells with distinct intercellular spaces. Figure 5 represents the transverse section of *C.vespertilionis* stem.

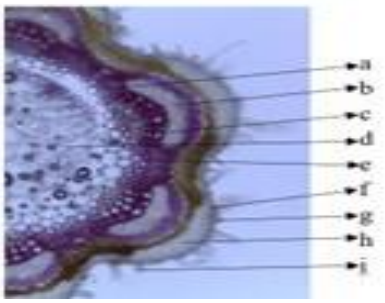

Figure 5. Transverse section of *C.vespertilionis* stem where, a:Xylem vessels, b: Sclerenchyma, c: Pholem, d: Parenchyma, e: Endodermis, f: Epidermis, g: Epidermis with cuticle, h: Collenchyma, i: Covering trichomes.

Powder microscopy of the levaes of C.obcordata and C. vespertilionis:

Powder microscopy of both plant leaves was observed individually and recorded in Figure 6 and 7. Powder microscopy of *C.obcordata* and *C.vespertilionis* revealed the presence of uniseriate multicellular covering trichomes, calcium oxalate crystals, xylem vessels, phloem fibres. However, in addition powder microscopy of *C.vespertilionis* leaves showed the presence of stomata, covering trichomes with epidermal cells, parenchyma cells, and sieve tubes.

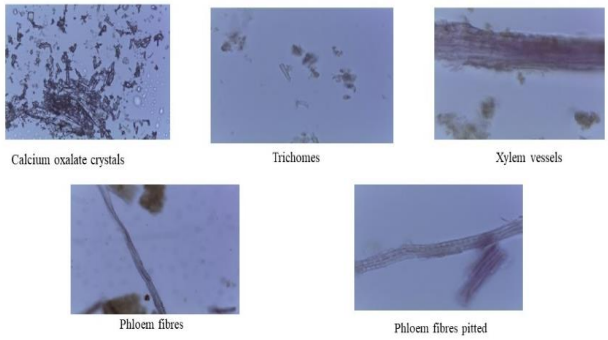

Figure 6: Powder microscopy of *C.obcordata* leaves

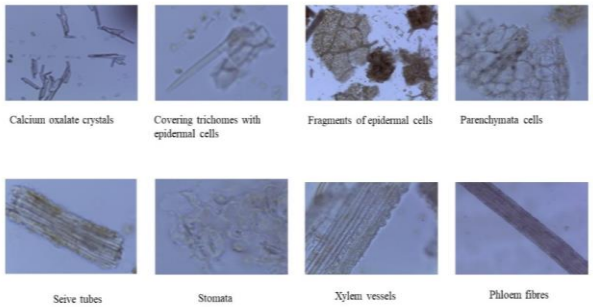

Figure 7: Powder microscopy of *C.vespertilionis* leaves

Determination of plant constant:

The plant constant was evaluated on both the plant leaves for their stomatal number and stomatal index, vein islet number, vein termination number, and palisade ratio were also observed. From the results it was found that C.vespertilionis have higher plant constant values in

most studied parameters like Stomatal number (Upper epidermis)-9.1-9.8-10.6,Stomatalnumber (Lower epidermis) -19.8-21.4 - 24.3,Stomatalindex(Upper epidermis)-12.5-14.9-16.6,Stomatal index (Lower epidermis)- 25.6-27.4-30.7, Vein islet number-5.7-7.2-10.2, Vein termination number-8.6-9.4-10.3, Palisade ratio-12. In contrast, C.obcordata has showed higher values on the stomatal number and stomatal index at their lower epidermis. Following are the plant constant values of C.obcordata, Stomatal number (Upper epidermis)-4.4-5.8-7.2,Stomatal number (Lower epidermis)-28.6-29.8-31.7,Stomatal index (Upper epidermis)-5.2-6.1-6.8, Stomatal index (Lower epidermis)- 28.6-29.8-31.7, Vein islet number-4.8-5.3-8.6, Vein termination number-7.8-8.3-9.5, Palisade ratio-8. The bolt number is the average of the plant constant values.

Preliminary Phytochemical screening:

The aerial parts of the plant were extracted and tested for their phyto constituents, and the results were presented in Table 1. The plant aqueous extract confirmed for the presence of carbohydrates and proteins, petroleum ether and chloroform extracts confirms presence of steroids, and in methanol aqueous extract tannin, saponin, and flavonoids were present. However, proteins were found to be absent in both plants.

Physiochemical parameters:

Ash values and extractive values:

The aerial parts of *C.obcordata* and *C.vespertilionis* were evaluated for their ash value and extractive values on the dry basis of the sample, total ash value, acid insoluble ash, and water-soluble ash values were observed to be 5.1% w/w, 4.9% w/w, and 3.9% w/w for *C. vespertilionis* however extractive values of water and ethanol was found to be 15% w/w and 11% w/w. In contrast with similar conditions, *C. obcordata* showed total ash value of 5.4% w/w, acid insoluble ash value of 5.3% w/w, and water-soluble ash value of 4.1% w/w, extractive values of water and ethanol were found to be 17% and 13% w/w respectively.

Fluorescence Analysis of the Plant Extracts:

To confirm the doubtful specimen, fluorescence analysis on the powdered plant sample can be used. In the current study, aerial parts of both plants were extracted with 4 solvents and evaluated in three conditions such as short ultra-violet light (254nm), long ultra-violet light (365 nm), and visible light which has shown different colors. The results of fluorescence analysis were revealed in Table 2 and the image in Figure 8 (A-F).

Observation of different chemical constituents in the crude plants can be analysed by using fluorescence analysis. Specific constituents in the plant exhibit fluorescence characteristics when tested with UV light or with the visible light. The changing in colour from visible light to ultraviolet light can be served as a marker for the existence or lack of chemical constituents as organic molecules absorb light over a specific range of wavelengths and reemit radiations. Hence, fluorescence analysis turns into one of the vital considerations in determining the purity and quality of crude drugs.

Table 1: Preliminary phytochemical screening on the aerial parts extract of *C.obcordata and C.vespertilionis.*

Chemical constituents	Chemical tests	P.E.E C.o	C.E C.o	M.E C.o	D.W.E C.o	P.E.E C.v	C.E C.v	M.E C.v	D.W.E C.v
Steroid	Libermann Burchard test	+	+	-	-	+	+	-	-
Flavanoid	Shinoda test	-	-	+	+	-	-	+	+
Tannin	Ferric Chloride test	-	-	+	+	-	-	+	+
Alkaloid	Wagner's test	-	-	+	-	-	-	+	-
Saponin	Foam test	-	-	-	-	-	-	-	-
Carbohydrate	Molisch test	-	-	-	+	-	-	-	+
Protein	Biuret test	-	-	-	-	-	-	-	-

Where, P.E.E – Petroleum ether extract, C.E -Chloroform extract, M.E – Methanol extract, D.W.E -Distilled water extract, C.o - *C.obcordata* and *C.v - C.vespertilionis.* '+' = Present '-'=Absent.

Table 2: Results of fluorescence analysis on the powdered aerial parts of C.*vespertilionis* and *C.obcordata*.

Reagents	C.*vespertilionis*			*C.obcordata*		
	Color observed in day light	Color observed under short ultraviolet light (254 nm)	Color observed under long ultraviolet light (365 nm)	Color observed in day light	Color observed under short ultraviolet light (254 nm)	Color observed under long ultraviolet light (365 nm)
Petroleum ether Chloroform Methanol Aqueous	Pale yellow Pale yellow Colourless Colourless	Pale buff colour Pale buff colour Colourless Colourless	Orange (F) Red (F) Purple (F) Colourless	Pale green Pale green Pale green Pale yellow	Pale green Pale green Deep green Pale yellow	Light green (F) Deep red (F) Pale brown (F) Colourless

F = Fluoresence.

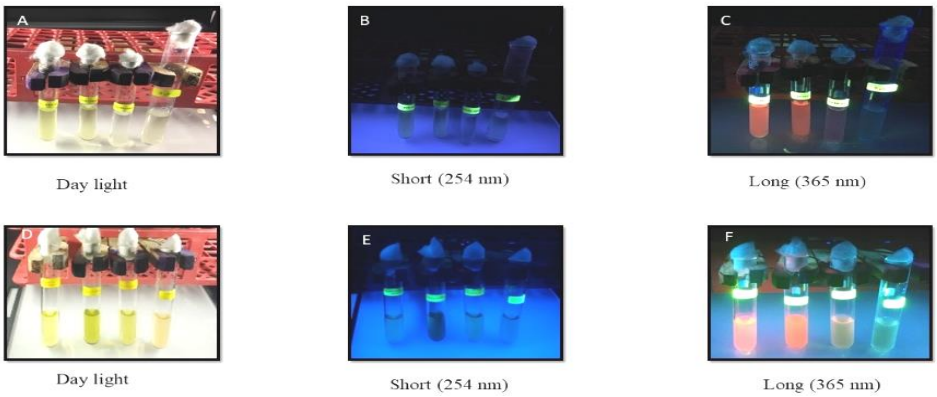

Figure 8: Fluorescence analysis of different extracts (petroleum ether, chloroform, methanol and distilled water) of C.*vespertilionis* and *C.obcordata* under day light and ultraviolet light (254 nm and 365 nm). Where A = *C.vespertilionis* at day light, B = *C.vespertilionis* at short UV light, C = *C.vespertilionis* at long UV light, D = *C.obcordata* at day light, E = *C.obcordata* at short UV light, F = *C.obcordata* at long UV light.

CONCLUSION:

To conclude, the findings of current study on the aerial parts of C.*vespertilionis* and *C.obcordata* will be considered an important tool to provide valuable information about standardization of these plants. Furthermore, the current standardization is beneficial for identifying adulterant specimens and facilitates compiling of suitable monographs for appropriate documentation purposes in the field of herbal medicine. Detailed research of the morphological and microscopical characters will be used in determining and enhancing in differentiating plants of identical or various species.

CONFLICT OF INTEREST:

There is no conflict of interest.

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