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FLUORESCENCE ANALYSIS OF MEDICINAL PLANTS IN THE FAMILY RUBIACEAE TRIBE *SPERMACOCEAE*

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ABSTARCT

Natural products have been a significant source of commercial medicines and bioactive compounds. The ethanolic extracts of s*permacocea pusilla and Spermacocea ocymoides* were screened for Florescence analysis. The present study revealed the significance of the plant as a drug. The quality control of herbal drug is important in justifying their acceptability in modern system of medicines. Modern system of medicine offers no problem with very well defined parameters of analysis and based on experimental data, toxicity studies and human clinical studies. It is not uncommon to have as many as five or more different herbal ingredients in one single product. The batch to batch variation starts from the collection of the raw materials itself in absence of any reference standard for identification. These variations multiply during dry, storage and further processing. Complete pharmacopoeial guidelines of standards on herbal drugs are not available. cGMP for the herbal industry are not well defined quality control parameters of herbal drugs are maintained or regulated. The lack of quality standards has result serious adverse effect. The pharmaceutical industry has shown interest to development of standardized plant drugs with proven safety, efficacy and quality. World Health Organization (WHO) set specific guideline for the quality control of medicinal plants products by using modern techniques and by applying suitable standards and parameters. Standardization brings important benefits to business including a solid foundation upon which to develop new technologies and an opportunity to share and enhance existing practices. Standardization also plays an essential role in assisting governments, administrations, regulators and the legal profession as legislation, regulation and policy initiatives are all supported by standardization of herbal drugs (Anonymous, 1998, 2004). Internationally, several pharmacopeias have provided monographs stating quality parameters and standards of many herbs and their herbal products (Kumar, 2013).

KEYWORDS: *Spermacocea pusilla*, *Spermacocea ocymoides*, Ethanol extract, Florescence analysis.

INTRODUCTION

Nature has a treasure of medicines to treat all kinds of ailments. Our prehistoric ancestors, roamed the earth in search of food, they perhaps earned better information about herbs. The importance of this information and experience was vital to the health of tribes, was delivered from generation to generation for thousands of years for human existence. Out of this fundamental knowledge came written and spoken knowledge's on herbs, which was grown continuously to the present day. Natural products from plants have played major, sustaining roles in the lives of humans, especially for food sources and medicinal products. As of now majority of the drugs introduced to Western medicine are derived mostly from natural products and about 25% of commonly used prescription drugs are derived from traditionally used medicinal plants. In addition, there are myriad plant extracts and plant materials that are employed commercially in various parts of the world. For approximately 85% of the world's population, these plant materials are the primary source of health care. The Rubiaceae family comprises one of the largest angiosperm families, with 650 genera and approximately 13,000 species, distributed mainly in tropical and subtropical regions also reaching the temperate and cold regions of Europe and Northern Canada as well. The genera *Borreria* and *Spermacoce,* are the largest of the tribe *Spermacocea*, comprising about 280 species distributed in tropical and subtropical America, Africa, Asia, and Europe. In Brazil, 36 *Borreria* species were recorded, of which 22 are endemics. Both *Borreria* and *Spermacocea* species are used medicinally in various manners and are reputed in the traditional medicine of Latin America, Asia, Africa, and the West Indies. ***B. pusilla*** (Wall.) DC. [Syn.: *B. stricta* (Linn. f.) K. Schum., *S. pusilla* Wall.] is an annual erect herb native to tropical Africa and Asia. In India, the fresh buds associated with flowers are used for cuts and wounds and crushed leaves are applied to the affected areas for bone fracture and scabies, and for snake and also for scorpion bites. ***B. ocymoides*** (Burm. f.) DC. (Syn.: *S. ocymoides* Burm. f.) is common in all of America a, also occurs in eastern Africa and East India. In Nigeria, *the* juice of the leaves is applied for ringworm and eczema and the sap is squeezed onto the wound or lesion. Rubiaceae is well known for its medicinal value, used in the treatment of malaria, diarrhea, digestive problems, skin diseases, fever, hemorrhage, urinary and respiratory infections, headache, and inflammation of eyes and gums. Traditional medicinal systems like Ayurvedic, Chinese Medicine, and Unani developed from plant resources have been used to treat various diseases. The secondary metabolites such as alkaloids, glycosides, resins, volatile oils, gums, and tannins of medicinal plants are the chemical compounds that are active pharmacologically and utilized to develop drugs. So, the objective of the present study is to evaluate Florescence analysis of *Spermacocea ocymoides* (Burm. F.) DC and *Spermacocea pusilla* root.

2. MATERIALS AND METHODS

2.1: Sample collection:

Spermacocea pusilla and *Spermacocea ocymoides* were collected from the local region of Karamadai, Coimbatore District, Tamil Nadu, India. The roots were removed directly from the plant to sterile polythene bags and transported to the laboratory. The roots were washed with tap water and followed a wash in distilled water and dried at room temperature.

2.2: Sample preparation:

The dried plant were grounded a fine powder using mortar and pistil followed by the preparation of different suspensions, viz., Petroleum ether, Ethyl Acetate, Ethanol and Aqueous were used to extract bioactive compounds from the sample. About 100 g of powdered material from each plant was extracted by soxhlation using various solvents such as Petroleum ether, Ethyl Acetate, Ethanol and aqueous depending on this polarity.

2.3: Fluorescence analysis

i) Fluorescence analysis of powders (Kokoshi *et al*., 1958)

Fluorescence characteristics of leaf, stem and root powders were treated with cold and hot water, different chemicals and reagents. Different colors of the samples were recorded in normal day light (visible light) and ultraviolet (UV) light conditions.

ii) Fluorescence analysis of extracts (Chase and Pratt, 1948)

Fluorescence characteristics of various extracts of leaf, stem and root were observed under normal day light (visible light) and ultraviolet (UV) light conditions. Different colors of the samples were recorded.

3. RESULTS AND DISCUSSION

A group of species that can be diagnosed microscopically using whole material but that is extremely difficult to identify when powdered. Certain drugs fluoresce when the cut surface or the powder is exposed to ultraviolet radiation, it is useful in the identification of those drugs. Some pieces of rhapontic are very difficult to distinguish in powdered form. For example, Indian and Chinese rhubarb are very difficult to identify when powdered form, but examination in ultraviolet light gives such marked differences in fluorescence that the varieties can be easily distinguished from each other (Shah and Seth, 2010). From this respect, the fluorescence analysis of various powers and extracts of *Spermacocea* was carried out under the visible and UV light conditions.

Table 1: Fluorescence analysis of Stem powder *Spermacocea pusilla*

S. No.	Reagents with Powder	Visible Light	UV - Light
1	Powder alone	Pale brown	Pale brown
2	Powder + Cold water	Colorless	Colorless
3	Powder + Hot water	Brown	Light green
4	Powder + 70% Ethanol	Pale green	Pale green
5	Powder + 70% Methanol	Pale green	Plane greenish yellow
6	Powder + 5% NaOH	Light brown	Greenish brown
7	Powder + 10% HCl	Pale brown	Pale brown
8	Powder + conc. HCl	Dark green	Dark green
9	Powder + conc. H_2SO_4	Blackish red	Black
10	Powder + conc. HNO_3	Yellowish orange	Bright yellow
11	Powder + Saturated Picric acid	Dark yellow	Bright yellow

12	Powder + Acetic acid	Pale brown	Pale green

Table 2: Fluorescence analysis of Root powder *Spermacocea pusilla*

S. No.	Reagents with Powder	Visible Light	UV - Light
1	Powder alone	Dark brown	Brown
2	Powder + Cold water	Pale brown	Pale brown
3	Powder + Hot water	Pale brown	Pale brown
4	Powder + 70% Ethanol	Pale brown	Light brown
5	Powder + 70% Methanol	Pale brown	Light greenish brown
6	Powder + 5% NaOH	Dark brown	Dark brown
7	Powder + 10% HCl	Light brown	Pale brown
8	Powder + conc. HCl	Dark brown	Dark green
9	Powder + conc. H_2SO_4	Blackish brown	Black
10	Powder + conc. HNO_3	Dark orange	Brownish yellow
11	Powder + Saturated Picric acid	Yellowish brown	Greenish yellow
12	Powder + Acetic acid	Pale brown	Pale brownish green

Table 1and 2 illustrates the fluorescence analysis of stem and root powers of *Spermacocea pusilla*. Stem power showed mostly green, and rarely brown and yellow shaded fluorescence under visible light. The powder showed mostly green and brown shaded fluorescence under UV light conditions. The root power showed mostly yellow and brown, rarely white shaded fluorescence under visible light; mostly brown and rarely black shaded fluorescence observed in UV light.

Table 3: Fluorescence analyses of successive extracts *Spermacocea pusilla*

Extracts	**Stem**		**Root**	
	Visible light	**UV Light**	**Visible light**	**UV Light**
Petroleum ether	Pale green	Bright Green	Brown	Blackish brown
Ethyl Acetate	Green	Greenish yellow	Brownish black	Dark Blackish brown
Ethanol	Pale yellowish brown	Dark green	golden yellow	Brown
Water	Pale brown	Pale yellow	Pale whitish brown	Pale white

Table 3 illustrates the fluorescence analysis of successive extracts of stem and root of the plant under visible light and UV light conditions. Mostly green, rarely brown and yellow shaded fluorescence observed from stem extracts in both visible and UV lights. Different colors were exhibited by root extracts in visible light which are golden yellow, brown and brown under visible light. Black and brown shaded fluorescence only observed root extracts in UV light.

Table 4: Fluorescence analysis of Stem powder *Spermacocea ocymoides*

S. No.	Reagents with Powder	Visible Light	UV - Light
1	Powder alone	Brown	Pale brown
2	Powder + Cold water	Light brownish red	Brownish green
3	Powder + Hot water	Brown	Bright green
4	Powder + 70% Ethanol	Pale yellowish green	Bright green
5	Powder + 70% Methanol	Dark brownish red	Bright yellowish green
6	Powder + 5% NaOH	Pale brownish red	Blackish green
7	Powder + 10% HCl	Reddish brown	Dark brown
8	Powder + conc. H_2SO_4	Brownish black	Black
9	Powder + Saturated Picric acid	Reddish yellow	Yellowish green
10	Powder + Acetic acid	Pale brown	Bright green

Table 5: Fluorescence analysis of Root powder *Spermacocea ocymoides*

S. No.	**Reagents with Powder**	**Visible Light**	**UV - Light**
1	Powder alone	Dark brown	Brownish black
2	Powder + Cold water	Brown	Pale green
3	Powder + Hot water	Pale brown	Pale green
4	Powder + 70% Ethanol	Pale brown	Pale green
5	Powder + 70% Methanol	Brown	Pale greenish yellow
6	Powder + 5% NaOH	Dark reddish brown	Dark brown
7	Powder + 10% HCl	Brown	Pale green
8	Powder + conc. H_2SO_4	Black	Black
9	Powder + Saturated Picric acid	Dark yellow	Yellowish green
10	Powder + Acetic acid	Dark brown	Blackish brown

Table 4 and 5 illustrates the fluorescence analysis of stem and root powers of Spermacocea the plant. Stem power showed different colors in different reagents which are green, yellow, red and brown shaded fluorescence under visible and UV light. The root power showed mostly yellow and brown shaded fluorescence under visible light and yellow, brown shaded fluorescence observed in UV light.

Table 6: Fluorescence analyses of successive extracts *Spermacocea ocymoides*

Extracts	**Stem**		**Root**	
	Visible light	**UV Light**	**Visible light**	**UV Light**
Petroleum ether	Light green	Green	Light brown	Brown
Ethyl Acetate	Dark green	Pale yellow	Pale brown	Light yellowish brown

Ethanol	Pale green	Yellowish brown	Dark brownish black	Black
Water	Pale yellow	Yellowish orange	Light yellowish white	Pale yellow

Table 6 illustrates the fluorescence analysis of successive extracts of stem and root of the plant under visible light and UV light conditions. Mostly green, rarely brown and yellow shaded fluorescence observed from stem extracts in both visible and UV lights. Different colors were exhibited by root extracts in visible light which are golden yellow, brown and orange under visible light. Black and brown shaded fluorescence only observed root extracts in UV light.

In florescence analysis, the colour of plant material is mainly due to its chemical composition. Many phyto-components are fluoresce when suitably illuminated. The fluorescence colour is unique for each and every compounds. A non-fluorescent compound may fluoresce if mixed with impurities that are fluorescent. In recent years, this method invariably used as a part of quality control program by many commercial agencies. Hence, it is a useful technique in detection of adulterants or substituent's of crude drugs and is a distinctive character of plants.

4.4 Conclusion

Fluorescence analysis of the plant materials showed that mostly green and brown shaded fluorescence, red and yellow fluorescence also observed in various powders and extracts. In fluorescence analysis, different colour of *Spermacoceae* powders and extracts is mainly due to its chemical composition. It is a useful technique in detection of adulterants or substitutes of crude drugs. In the present study, Tribe *Spermacoceae* was thoroughly investigated for their pharmacognostic features to analyses the quality, safety and standardization for their safe use. The generated information of the present study will provide the data which is helpful in the correct identification and authentication of this medicinal plant and may help in preventing its adulteration.

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