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PHYSICOCHEMICAL AND PHYTOCHEMICAL ANALYSIS OF *OSBECKIA MURALIS* NAUDIN

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ABSTRACT

Osbeckia muralis Naudin belongs to Melastomaceae family, is an endemic herb of Western Ghats. It is an erect herb which is termed as 'Nela Nakkarika' in Kannada, 'Cherkulathi' in Malayalam. The folklore practitioners are using this drug in respiratory disorder especially in cough. So, the scientific exploration for the proper identification has been carried out by using Physico chemical and Phytochemical analysis including HPTLC. The study helped to get the moisture content, Ash values, extractive values in different solvents, and HPTLC etc. The different peaks of Rf value help to identify various components. The study reports the presence of Protein, Carbohydrates, Tannins, Flavanoids, Alkaloids, Chloride, Sulphate, Potassium, Sodium. This study will help the identification of the genuine drug for the future.

Keywords – Osbeckia muralis Naudin., Phytochemical study, Physicochemical study, HPTLC

1. INTRODUCTION

According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants[1]. However, such plants should be investigated to better understand their properties, safety, and efficacy[2]. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids[3,4]. For this purpose scientific approach is needed.

In this study *Osbeckia muralis* Naudin, belongs to Melastomaceae family[5], a herb which is endemic to Western Ghats[6] is taken. The Physicochemical and Phytochemical analysis of the plant leaves has been carried out. The air-dried leaves of *Osbeckia muralis Naudin* was powdered finely and subjected to various analyses, such as determination of moisture content, pH value, ash value, acid insoluble ash, water soluble ash, HPTLC etc. The extractive value in various solvents and ash value are important in identification and standardization of single drug.

2. METHODOLOGY

2.1 Determination of Moisture Content

Five grams of the powdered drug was taken in a weighed flat porcelain dish and was dried in the oven at 100-105° C for five hours and weighed. Repeated the drying and weighing at one-hour interval until difference between two successive weightings corresponds not

more than 0.25 %. Constant weight is reached when two consecutive weighing, after drying for 30 minutes and cooling for 30 minutes in desiccators, showed not more than 0.01 g difference[7].

2.2 Determination of Total Ash

Five grams of powdered *Osbeckia muralis Naudin.*was taken in a weighed crucible. It was then heated over a burner until all the carbon was burnt off at a temperature not exceeding 450^0 C. Then it was cooled in a desiccator and weighed, exhaust the charred mass with hot water, collect the residue on ash less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dry, and ignite and calculated the percentage of total ash with reference to the air-dried sample of the crude drug[8].

2.3 Determination of Acid Insoluble Ash

The ash obtained in the previous experiment was boiled with 25 ml of dilute Hydrochloric acid. The solution was filtered using an ash less filter paper. The residue was washed with hot water and ignited in a weighed silica crucible with the filter paper. The weight of the crucible was measured to get acid insoluble ash[8].

2.4 Determination of Water Soluble Ash

5 grams of air-dried powder of the test drug was incinerated in a weighed crucible. The ash thus obtained was boiled with 25 ml of distilled water for 5 minutes. The solution was filtered using an ash less filter paper. The residue was washed with warm distilled water and ignited in a weighed silica crucible[8].

2.5 Determination of pH value

Operate the pH meter and the electrode system according to the manufacturer's instructions. Standardize the meter and electrodes with 0.05 M Potassium hydrogen phthalate (pH 4.0). when measuring an acid solution or with 0.05 M Sodium Borate when measuring in alkaline solution. At the end of a set of measurements, take a reading of the solution used to standardize the meter and electrodes. This reading should not differ by more than 0.02 as that of original value with which the apparatus was standardized.

Preparation of solutions: A 10 % w/v solution in water filtered through a filter paper[9].

2.6 Extraction using in different solvents

Different solvents like Aqueous, Ethanol, Methanol, Acetone, Chloroform, Petroleum ether, has been used for the extraction of the drug. Two different methods have been adopted. One with Standard procedure using dry drug and other using fresh drug has been followed for the extraction[10].

2.7 Ash Analysis

The air-dried powdered drug was taken in a crucible and heated in an electric Bunsen burner to make the ash. Then it was diluted with distilled water, boiled and filtered[10].

2.8 HPTLC

One gram of powdered sample was extracted with 10 ml ethanol and kept for cold percolation for 24h and filtered. Five, ten and fifteen µl of the above samples were applied on a pre-coated silica gel F254 on aluminum plates to a band width of 6 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (9:1). The developed plates were visualized and scanned under 254 and 366 nm. It was derivatised with vanillin sulphuric acid reagent, visulaised and scanned under 620 nm after heating the plate until the development of colours. R_f, colour of the spots and densitometric scan were recorded.

3. RESULTS AND DISCUSSION

The result of the preliminary study shows the moisture content(Table No.1), Total Ash(Table No.2), Acid insoluble ash(Table No.3), Water soluble ash(Table No.4), pH value(Table no.5), the extractive values in different solvents(Table No.6), preliminary Phytochemical study(Table No.7), results of ash analysis(Table No.8), TLC photo documentation of ethanolic extract of leaf of *Osbeckia muralis,* Under short UV(Figure No.1.1) Under long UV (Figure No.1.2)Under white light after derivatisation with Vanillin/sulphuric acid(Figure No.1.3), R_f values of ethanolic extract of leaves of *Osbeckia muralis*(Table No.9)Densitometric Scan of ethanolic extract of leaves of *Osbeckia muralis***(**Figure No. 2) At 254 nm(Figure No.2.1), At 366 nm (Figure No.2.2) At 620 nm post derivatisation with vanillin/sulphuric acid.(Figure No.2.3)

The study reports the presence of proteins, carbohydrates, tannins, flavanoids, alkaloids, Chloride, Sulphate, Potassium, Sodium.

Table-1: Moisture content

Wt. of Drug	*Loss of Wt. After Drying*	*Moisture Content*
5 g	0.22 g	**4.4 %**

Table-2: Total Ash

Weight of Drug	*Weight of Ash*	*Total Ash*
5 g	0.48g	**9.6 %**

Table-3: Acid Insoluble Ash

Weight of Drug	*Weight of Ash*	*Weight of acid insoluble ash*	*Acid insoluble ash (% w/w)*
5 g	0.48 g	0.2 g	**0.4 %**

Table-4: Water soluble Ash

Weight of Drug	*Weight of Ash*	*Weight of Water soluble ash*	*Water soluble ash (% w/w)*
5 g	0.48 g	0.35 g	**7 %**

Table-5: pH value

pH Value	**5.8**

Table-6: Results of Extractive values in different solvents

Sl.No:	*Solvent*	*Percentage of Extract of Osbeckia muralis Naudin.*(fresh)	*Percentage of Extract of Osbeckia muralis Naudin.*(dry)
1	***Water***	3.2	14
2	***Ethanol***	2.2	5.2
3	***Methanol***	2.2	5
4	***Chloroform***	12.11	2
5	***Petroleum Ether***	2.8	3.3
6	***Acetone***	3.2	2.4

Table-7: Results of Phytochemical Evaluation[13]

Sl No	Name of the Tests	Observation	Results
1.	Proteins		
	Biuret test	Violet colour precipitate	Present
	Millon's test	White ppt formed, turned red on heating	Present
2.	Carbohydrate test		
	Benedict's test (Reducing sugar)	Green colour precipitate	Present
	Benedict's test (Non-reducing sugar)	Formation of a coloured precipitate	Present
3.	Tannins	A green colour seen	Present
4.	Saponin		
	Foam test	Foam seen, but did not persist	**Absent**
5.	Flavanoids		
	Shinoda test	Scarlet colour seen	Present
6.	Steroids		
	Salkowski's test	No colour change	**Absent**
7.	Alkaloids		
	Mayer's test	Yellow precipitate	Present
8.	Tritrepenoides		
	Liebermann Burchard's test	No Change	Absent
9.	Starch	No colour change	**Absent**
10.	Resin	No change	**Absent**
11.	Phenols		
	Phenol test	No intense colour	**Absent**

Table-8: Results of Ash Analysis

Sl.No	*Components*	*Observations*	*Coleus aromaticus*
1	***Carbonates***	No colour change	Absent
2	***Fluorides***	No precipitate	Absent
3	***Chlorides***	Curdy white precipitate	**Present**
4	***Sulphates***	White precipitate	**Present**
5	***Chromates***	No yellow precipitate	Absent
6	***Phosphates***	No precipitate	Absent
7	***Potassium***	Yellow precipitate	**Present**
8	***Sodium***	White colour precipitation	**Present**
9	***Aluminium***	No gelatinous precipitate	Absent
10	***Calcium***	No white colour precipitation	Absent

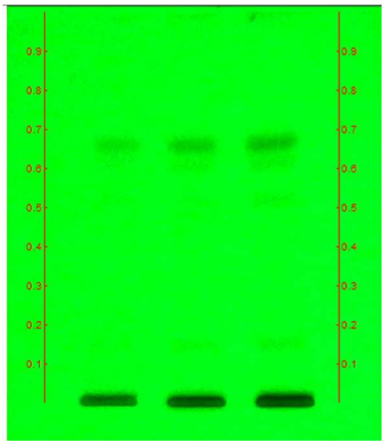


Fig 1.1: Under short UV

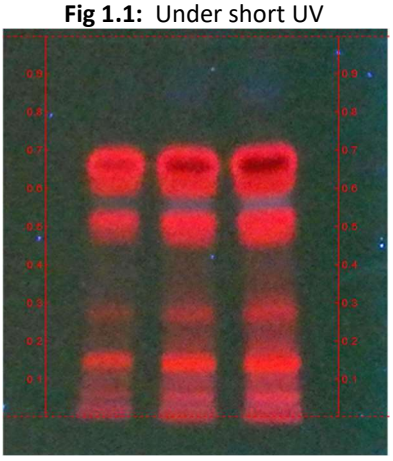


Fig 1.2 : Under long UV

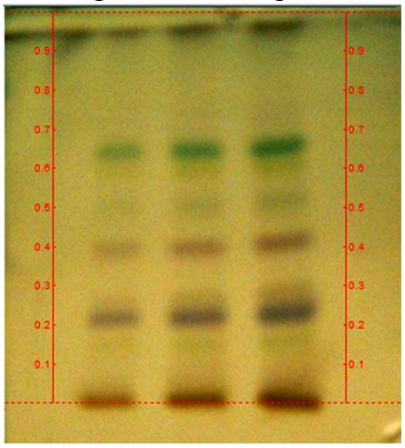


Fig 1.3 : Under white light after derivatisation with Vanillin/sulphuric acid

Track 1- *Osbeckia muralis*– 3 µl

Track 2– *Osbeckia muralis*– 6 µl

Track 3– *Osbeckia muralis*– 9 µl

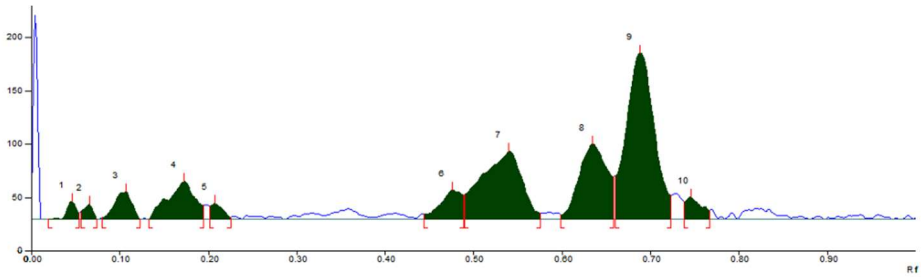
Solvent system: Toluene: Ethyl Acetate (9:1)

Figure 1: TLC photo documentation of ethanolic extract of leaf of *Osbeckia muralis*

Table 9: R_f values of ethanolic extract of leaves of *Osbeckia muralis*

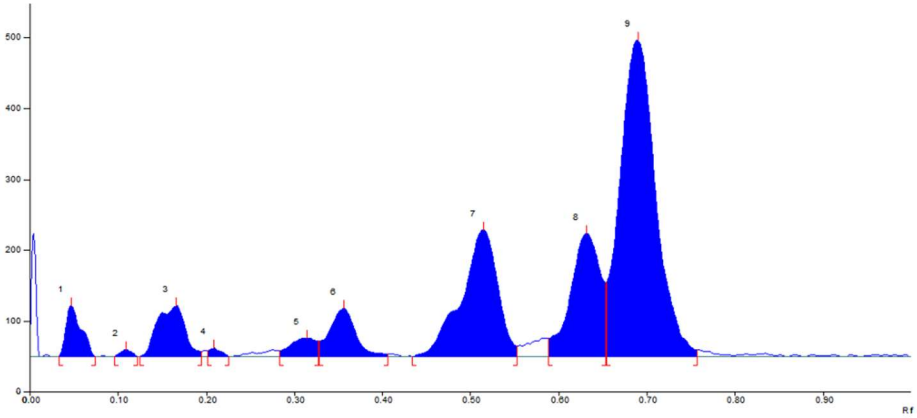
Under short UV	Under long UV	Post derivatization
-	0.05 (F Red)	-
-	0.13 (F Red)	-
0.15 (DL Green)	-	0.15 (Lemon Yellow)
-	-	0.23 (Violet)
-	0.27 (F Red)	-
-	-	0.41 (Purple)
-	0.47 (F Red)	-
0.52(L Green)	0.52(F Red)	0.52 (L Green)
-	0.57 (F Red)	-
0.61(L Green)	-	0.61 (Lemon Yellow
0.66 (L Green)	0.67 (F Red)	0.66 (D Green)

*L-Light, D-dark, F-Fluorescence



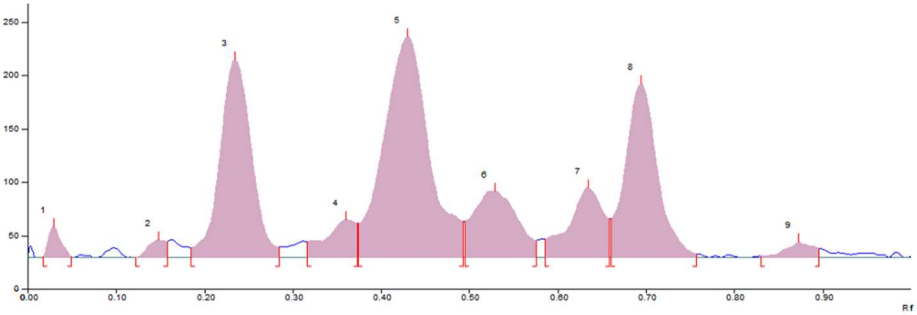
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.0 AU	0.05 Rf	16.8 AU	3.78 %	0.05 Rf	5.8 AU	141.7 AU	1.32 %
2	0.06 Rf	6.6 AU	0.07 Rf	13.8 AU	3.10 %	0.07 Rf	0.3 AU	119.1 AU	1.11 %
3	0.08 Rf	1.6 AU	0.11 Rf	25.9 AU	5.85 %	0.12 Rf	0.1 AU	410.2 AU	3.83 %
4	0.13 Rf	0.2 AU	0.17 Rf	35.3 AU	7.96 %	0.19 Rf	13.3 AU	875.9 AU	8.19 %
5	0.20 Rf	12.2 AU	0.21 Rf	14.8 AU	3.33 %	0.23 Rf	1.9 AU	172.2 AU	1.61 %
6	0.44 Rf	5.0 AU	0.48 Rf	27.4 AU	6.17 %	0.49 Rf	22.6 AU	539.9 AU	5.05 %
7	0.49 Rf	23.1 AU	0.54 Rf	63.4 AU	14.29 %	0.58 Rf	5.8 AU	2336.5 AU	21.84 %
8	0.60 Rf	4.3 AU	0.63 Rf	70.1 AU	15.81 %	0.66 Rf	39.8 AU	1811.9 AU	16.94 %
9	0.66 Rf	40.2 AU	0.69 Rf	155.7 AU	35.10 %	0.72 Rf	22.3 AU	3980.1 AU	37.20 %
10	0.74 Rf	16.5 AU	0.75 Rf	20.5 AU	4.62 %	0.77 Rf	8.1 AU	310.7 AU	2.90 %

Figure 2.1: Densitometric Scan of ethanolic extract of leaves of *Osbeckia muralis* (9 µl) (At 254 nm)



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.2 AU	0.05 Rf	72.4 AU	6.83 %	0.07 Rf	0.1 AU	1013.4 AU	3.31 %
2	0.10 Rf	0.1 AU	0.11 Rf	10.1 AU	0.95 %	0.12 Rf	0.5 AU	102.6 AU	0.33 %
3	0.12 Rf	0.2 AU	0.17 Rf	72.0 AU	6.79 %	0.19 Rf	7.9 AU	1838.9 AU	6.00 %
4	0.20 Rf	8.2 AU	0.21 Rf	11.8 AU	1.12 %	0.23 Rf	0.2 AU	132.0 AU	0.43 %
5	0.28 Rf	8.8 AU	0.31 Rf	26.4 AU	2.49 %	0.33 Rf	22.2 AU	640.4 AU	2.09 %
6	0.33 Rf	22.3 AU	0.36 Rf	67.9 AU	6.41 %	0.41 Rf	3.2 AU	1629.8 AU	5.32 %
7	0.43 Rf	0.0 AU	0.51 Rf	178.9 AU	16.88 %	0.55 Rf	14.8 AU	5959.1 AU	19.45 %
8	0.59 Rf	26.2 AU	0.63 Rf	173.9 AU	16.41 %	0.65 Rf	04.2 AU	4725.1 AU	15.42 %
9	0.65 Rf	104.7 AU	0.69 Rf	446.4 AU	42.12 %	0.76 Rf	9.4 AU	14602.2 AU	47.65 %

Fig 2.2: At 366 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.5 AU	0.03 Rf	29.1 AU	3.74 %	0.05 Rf	0.4 AU	329.7 AU	1.31 %
2	0.12 Rf	0.0 AU	0.15 Rf	16.3 AU	2.10 %	0.16 Rf	15.2 AU	266.2 AU	1.05 %
3	0.18 Rf	9.1 AU	0.23 Rf	185.2 AU	23.81 %	0.28 Rf	9.6 AU	5331.7 AU	21.12 %
4	0.32 Rf	14.7 AU	0.36 Rf	35.7 AU	4.59 %	0.37 Rf	32.1 AU	996.2 AU	3.95 %
5	0.37 Rf	32.1 AU	0.43 Rf	206.5 AU	26.55 %	0.49 Rf	33.7 AU	8589.6 AU	34.03 %
6	0.49 Rf	33.9 AU	0.53 Rf	62.3 AU	8.01 %	0.58 Rf	16.0 AU	2527.8 AU	10.02 %
7	0.59 Rf	17.4 AU	0.63 Rf	65.3 AU	8.40 %	0.66 Rf	36.1 AU	2047.9 AU	8.11 %
8	0.66 Rf	36.1 AU	0.69 Rf	162.6 AU	20.91 %	0.76 Rf	3.3 AU	4785.0 AU	18.96 %
9	0.83 Rf	1.1 AU	0.87 Rf	14.7 AU	1.89 %	0.90 Rf	8.5 AU	365.1 AU	1.45 %

Fig 2.3: At 620 nm post derivatisation with vanillin/sulphuric acid

4. CONCLUSION

The plant *Osbeckia muralis* has been studied to give the detail report of preliminary physico chemical and phytochemical analysis. Botanically identified genuine drug has been used for the study which showed the presence of proteins, carbohydrates, tannins, flavanoids, alkaloids, Chloride, Sulphate, Potassium, Sodium. This will help for the exact identification of the plant for the future references.

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REFERENCES

1. Ellof J.N. Which extractant should be used for the screening and isolation of antimicrobial components from plants. J. Ethnopharmacol. 1998; 60: p1–6.
2. Nascimento G.G.F., Lacatelli J., Freitas P.C. and Silva G.L. Antibacterial activity of plants extracts and phytochemical on antibioticresistant bacteria. Braz. J. Microbiol. 2000; 31: 886-891.
3. Edeoga HO, Okwu DE, Mbaebie BO, Phytochemical constituents of some Nigerian medicinal plants, Afr. J. Biotechnol. 2005;4(7): 685-688.
4. Mann J (1978). Secondary Metabolism. Oxford University Press, London, p. 154.
5. Gopalakrishna Bhat. K. Flora of Udupi. Manipal; Manipal Press Limited; 2003.p.219
6. *Osbeckia muralis*[cited 2013 November 13]. Available from: http://www.indiabiodiversity.org/species/show/230523
7. Anonymous, Ayurvedic Pharmacopeia.of India Publisher of , Dept. of F.H& W, Govt. of India, New delhi, 1970 Part 2, Vol 1, p.no.14
8. Anonymous, Ayurvedic Pharmacopeia.of India Publisher of , Dept. of F.H& W, Govt. of India, New delhi, 1970 Part 2, Vol 1, p.no. 13
9. Anonymous, Ayurvedic Pharmacopeia.of India Publisher of , Dept. of F.H& W, Govt. of India, New delhi, 1970 Part 2, Vol 1, p.no. 6
10. Anonymous, Ayurvedic Pharmacopeia.of India Publisher of , Dept. of F.H& W, Govt. of India, New delhi, 1970 Part 2, Vol 1, p.no.113,115 – 117,121