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STUDIES ON PHARMACOGNOSTIC PHYSICO-CHEMICAL PROPERTIES OF *MALACHRA CAPITATA* L.

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ABSTRACT

Evaluation of the physico-chemical properties of *Malachra capitata* root, stem and leaf samples indicate that the dry weight and acid soluble ash was found maximum in stem sample, while water soluble ash value was higher in root and stem samples; the ash properties show variation among the dry powdered samples of *Malachra capitata*. Water insoluble ash value, acid insoluble ash, sulphated ash and residue on ignition were shows maximum value in the leaf sample; the fluorescent properties of the *Malachra capitata* dry powdered samples (root, stem and leaf) emit various colours under day light and UV-light conditions and the extractive value of different parts of *Malachra capitata* stem and leaf showed the highest extractive value in water while in root it was more in methanol extract.

INTRODUCTION

Pharmacognostic physico-chemical properties analysis over ash values used to determine quality and purity of crude drug. It indicates the presence of various impurities like carbonate, oxalate and silicate.[1, 2] The total ash is particularly important in the evaluation of purity of drugs, ***i.e.*** the presence or absence of foreign inorganic matter such as metallic salts and/or silica. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol soluble components. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. The water soluble ash is used to estimate the amount of inorganic elements present in drugs. The moisture content of the drug is not too high, thus it could discourage bacterial, fungal or yeast growth, as the general requirement for moisture content in crude drug is not more than 14%w/w (Anonymous, 1985).[3] The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent. The plants belongs to family Malvaceae, commonly referred to as the Mallow family, consists of 243 genera and 4225 species that are found worldwide.[4] Malvaceae is comprised of many well known genera including *Gossypium* (cotton) and *Hibiscus* (ornamental) and many of these genera are known to contain biologically active constituents.[5, 6] The present study was carried out to record the various physico-chemical properties of *Malachra capitata* plant (root, stem and leaf) samples.

MATERIAL AND METHODS

Plant material: *Malachra capitata* was collected from Komaneri village of Thoothukudi district, Tamil Nadu, India and identified by Dr. Chelladurai, Research Officer, at Central Council for Research in Ayurveda and Siddha, Palayamkottai (Figure 1).

Figure 1: *Malachra capitata* plant –Habit and the dry powdered plant samples used.

Preparation of Dry Powder of *Malachra capitata* samples

The different parts (root, stem and leaf) of *M. capitata* collected were dried separately at room temperature (30°C±2°C) for about two weeks to get a constant weight. The dried plant materials were ground to powder by mechanical device and stored for further biochemical and bioactive analysis (Figure 1).

Pharmacognostic Physico-chemical Properties

Determination of loss of weight on drying: A known quantity of the dry powder samples of *M. capitata* root, stem and leaf were dried separately and allowed to dry until a constant weight was obtained. From the initial and final weight, the loss of weight on drying was determined.

Determination of Total ash: A known quantity of dry powdered samples (2g) of different parts of *M. capitata* was taken in a pre-weighed crucible (china clay dish). The crucible with plant material was gently heated until the temperature rise to 600°C and maintain for 10 minute to ash. Then the crucible was cooled to room temperature and weighed. The total-ash value was calculated based on the amount of dry matter used and the data recorded on percentage.

Determination of water-soluble/insoluble ash: The total ash of the *M. capitata* plant samples prepared was transferred separately into 50ml boiling tubes containing 25ml of distilled water, boiled for 5min, and filtered through a pre-weighed ash less filter paper. The boiling tube was washed and passed through the filter paper. The washing was repeated 2 to 3 times. The filter paper was then removed, dried in a hot air oven at 105°C for few hours and weighed the insoluble matter with filter paper. The percentage of insoluble matter was determined based on the plant sample used.

Determination of acid-soluble/insoluble ash: The total ash of the *M. capitata* plant samples prepared was transferred separately into a beaker containing 25ml of hydrochloric acid, boiled for 5min, and filtered through a pre-weighed ash less filter paper. The boiling tube was washed with hydrochloric acid and passed through the filter paper. The washing was repeated 2 to 3 times. The filter paper was then removed, dried in a hot air oven at 105°C for few hours and weighed the insoluble matter with filter paper. The percentage of acid insoluble matter was determined based on the plant sample used.

Determination of residue on ignition: A known amount of *M. capitata* powdered samples was taken separately in a previously weighed silica crucible, carefully ignited, cooled and weighed. The percentage of residue on ignition was determined separately.

Determination of sulphated ash: A known amount of *M. capitata* powdered samples was taken separately in a previously weighed silica crucible and moistened with concentrated sulphuric acid. It was ignited gently, moistened once again with conc. H_2SO_4 and re-ignited. The crucible was cooled and weighed. The percentage of sulphated ash with reference to the air dried sample of *M. capitata* was calculated separately.

Determination of Extractive Value: One gram of the powder samples of *M. capitata* were macerated separately with 50ml of different solvent such as petroleum ether, chloroform, ethyl acetate, methanol, ethanol and water and kept for 6hrs with intermittent shaking. Then, the extracts were allowed to stand for 18hrs, filtered and 25ml of the extracts was transferred to 50ml test tube separately. The extracts containing test tubes were kept in hot air oven for 6hr at 105°C to evaporate the water. Then, the test tubes were taken out, cooled and weighed the content. The difference in weight was noted. The solvent extractive values were calculated based on the weight of air dried samples taken and the data are recorded in percentage.

Fluorescence Analysis: The fluorescence analysis of dry plant samples, the percentage loss of weight on drying, total ash, acid soluble/insoluble ash, water soluble/insoluble ash, sulphated ash and residue on ignition were obtained from different parts of the plant *M. capitata* by employing standard methods of analysis as described. The dry powdered plant samples and the extract of the dry powder plant sample in various solvents (Table 3 to 5) were examined under day light, fluorescent light and ultra violet light (365nm-254nm). The powder was also treated with various chemical reagents and the change in colour was recorded and the fluorescence characters were determined.

RESULTS AND DISCUSSION

The herb *M. capitata*, belongs to Malvaceae, is medicinally important as they contain biologically active compounds. The plant is mostly erect, coarse, annual or perennial herb, 1-2m tall, throughout densely yellowish -tomentose and usually also moderately to copiously hispid with simple or stellate hairs of 2mm long; leaf long-petioled; stipules lanceolate, 5-15mm long; blades orbicular to ovate, 2-10cm long, palmatelysinuate to 3-, 5-, or 7-lobed, lobes mostly obtuse, crenate to serrate, the base obtuse or truncate; flowers in axillary, pedunculate, bracteates heads, bracts 1-2cm long, stipitate and subtended by paired, filiform bracteoles, conduplicate, suborbicular to ovate, obtuse or acute, entire or once or twice dentate, obtuse to cordate at base, prominently veined and whitish basocentrally; involucral bracts wanting; calyx tubular-campanulate, 4-8mm long, 5-lobed to below middle, lobes

ovate-lanceolate, white with brownish or reddish nerves; petals yellow, obovate, 10-15mm long, slightly exceeding stamina column; mericarps 3-3.5mm long, muticous, reddish veined, puberulent; seed obovoid-cuneate, about 2.5mm long, black, whitish –pubescent about hilum (Figure 1).

The physical properties of various parts (root, stem and leaf) of *M. capitata* were determined and the data are presented in Table 1 and in Figure 2. The dry weight of different parts of *Malachra capitata* showed the maximum dry weight (10.33±0.47%) in stem followed by (10±0.00%) in root, and (7.67±0.47%) in leaf sample.

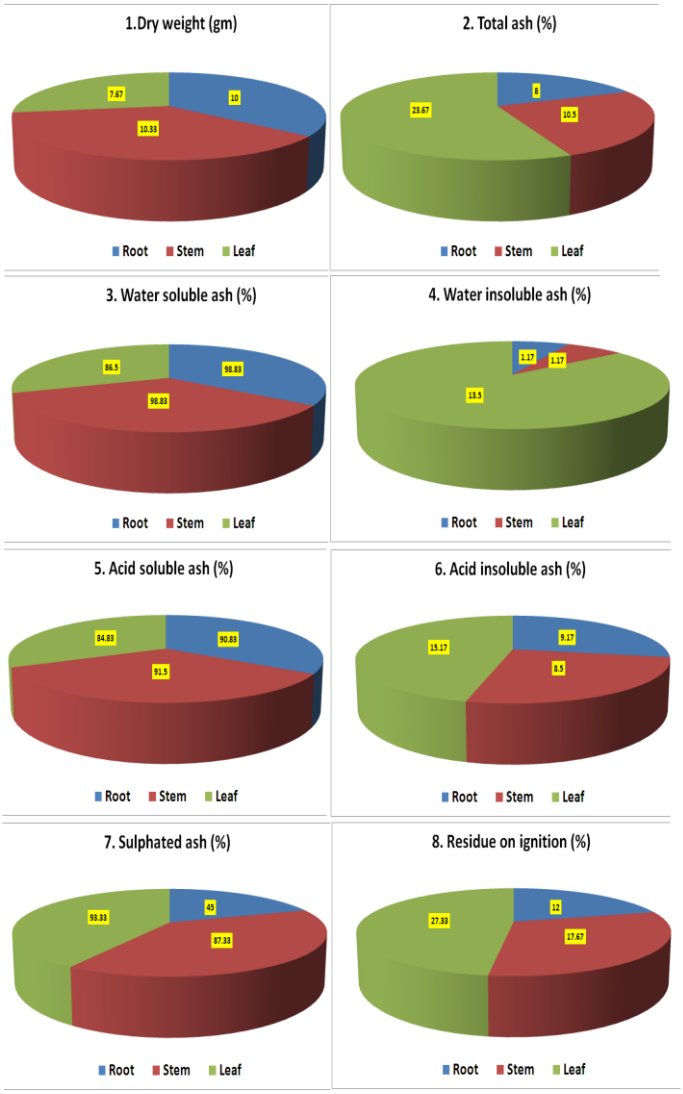

Figure 2: Pharmacognostic physical properties (1 to 8) of *Malachra capitata* dry samples.

The ash value furnishes a basis for judging the identity and cleanliness of a drug in powder form. Ash values were determined with a purpose to find out the total amount of inorganic solutes present in the medicinal plant materials. It is known that ash of any plant does not contain any organic material and therefore inorganic salts are used medicinally. Only a few herbal therapies make use of ash.

Ash properties of *M. capitata* dry powder shows variations between samples of different parts (Table 1). The leaf sample shows maximum amount of total ash (23.67±0.97%), sulphated ash (93.33±2.05%) and residue on ignition (27.33±3.09%) when compared with the other two samples. High amount of water soluble ash value (98.83±0.94%) was recorded in root and stem dry powder sample of *M. capitata*. Water insoluble and acid insoluble ash value was maximum (15.17±0.6% and13.5±0.00%, respectively) in leaf dry powdered sample. Acid soluble ash value was high (91.5±1.47%) in stem of *M. capitata.*

Table 1: Pharmacognostic physical property of the dry samples of *Malachra capitata*

Pharmacognostic characters Analyzed	Different parts of *Malachra capitata*		
	Root	**Stem**	**Leaf**
1. Dry weight (%)	10.00 ± 0.00^{lmn}	10.33 ± 0.47^{lmn}	7.67 ± 0.47^{n}
2. Total ash (%)	8.00 ± 0.91^{mn}	10.50 ± 1.26^{lmn}	23.67 ± 0.89^{h}
3. Water soluble ash (%)	98.83 ± 0.94^{a}	98.83 ± 0.94^{a}	86.50 ± 0.00^{e}
4. Water insoluble ash (%)	1.17 ± 0.94^{op}	1.17 ± 0.94^{op}	13.50 ± 0.00^{jkl}
5. Acid soluble ash (%)	90.83 ± 1.70^{cd}	91.50 ± 1.47^{c}	84.83 ± 0.62^{e}
6. Acid insoluble ash (%)	9.17 ± 1.69^{mn}	8.50 ± 1.47^{mn}	15.17 ± 0.69^{jk}
7. Sulphated ash (%)	45.00 ± 2.16^{f}	87.33 ± 7.72^{de}	93.33 ± 2.05^{bc}
8. Residue on ignition (%)	12.00 ± 1.41^{klm}	17.67 ± 0.47^{ij}	27.33 ± 3.09^{gh}

Mean ± Standard Deviation (n=3); Similar alphabets between rows and columns indicate non-significance at 5% level CD (P=0.05) value (PS =4.31).

Two-way ANOVA Table:

Source	df	SS	MS	F	PROB
TOT	71	102297.583061	1440.811029	209.6496	
Rep	2	1.632669	0.816335	0.1188	
Trt	23	101979.816728	4433.905075	645.1690	0.000 **
Err	46	316.133664	6.872471	1.0000	
P	7	96049.754483	13721.393498	1996.5735	0.000 **
S	2	1243.827919	621.913960	90.4935	0.000 **
Ps	14	4686.234325	334.731023	48.7061	0.000 **
Err	46	316.133664	6.872471	1.0000	
CV = 6.11%					
	SED	**CD(0.05)**	**CD(0.01)**		
P	1.23581	2.48760	3.32123		
S	0.75677	1.52334	2.03383		
Ps	2.14048	4.30865	5.75254		

** =Significance at 1% level.

Studies of Ramasubramaniaraja,[7] indicate the presence of 5% total ash, 3.6% acid insoluble ash, 4% sulphated ash, 2.5% water soluble ash and 6% weight loss on drying in the ethanol extract of *A. indicum* leaves; Chumbhale and Upasani[8] reported the ash value of total ash (9.03±0.05% w/w), acid insoluble (1.50±0.01% w/w), water soluble (2.51±0.02% w/w) and sulphated ash (7.50±0.01%w/w) values in *Thespesia lampas* on dry weight basis and in *Hibiscus sabdariffa* leaves, Manish Kumar *et al*.[9] analyzed the total ash (7.55%), acid

insoluble ash (1.06%) water insoluble ash (2.12%) and sulphated ash (8.25%). In the present study, the ash values of different parts of *M. capitata* dry weight and acid soluble ash was found maximum in stem sample, while water soluble ash value was maximum in root and stem sample. The remaining water insoluble ash value, acid insoluble ash, sulphated ash and residue on ignition were shows maximum value in the leaf sample (Table 1; Figure 3).

Extractive values

Extractive values of different solvent extract help to determine the amount of active constituents in a given amount of the plant material when extracted with solvents. These values provide an indication of the extent polar, medium polar and non-polar components present in the plant material. It is employed for those plant materials in which no suitable or biological assay method exists.

Table 2: Determination of extractive values (%) of root, stem and leaf of *Malachra capitata* in various solvent extracts.

Solvent extracts Analyzed	Extractive values (%)		
	Root	Stem	Leaf
1. Petroleum ether	9.33 ± 4.99^{ghi}	8.00 ± 3.27^{hi}	5.33 ± 1.51^{hi}
2. Chloroform	4.00 ± 0.00^{i}	7.47 ± 1.51^{hi}	5.33 ± 1.51^{hi}
3. Ethyl acetate	14.67 ± 1.89^{efg}	10.67 ± 1.89^{fgh}	9.33 ± 1.89^{ghi}
4. Methanol	32.00 ± 4.53^{b}	17.00 ± 1.41^{de}	14.93 ± 1.51^{ef}
5. Ethanol	10.67 ± 3.78^{fgh}	7.33 ± 0.94^{hi}	6.67 ± 0.94^{hi}
6. Water	25.33 ± 1.89^{c}	32.00 ± 0.00^{b}	45.33 ± 4.99^{a}

Mean ± Standard Deviation (n=3); Similar alphabets between rows and columns indicate non-significance at 5% level CD (P=0.05) value (TS =5.43).

Two-way ANOVA Table:

Source	df	SS	MS	F	PROB
TOT	53	7121.153333	134.361384	12.5360	
Rep	2	2.333333	1.166667	0.1089	
Trt	17	6754.406667	397.318039	37.0700	0.000 **
Err	34	364.413333	10.718039	1.0000	
T	5	5494.628889	1098.925778	102.5305	0.000 **
S	2	47.551111	23.775556	2.2183	0.124 NS
TS	10	1212.226667	121.222667	11.3102	0.000 **
Err	34	364.413333	10.718039	1.0000	
CV = 19.33%					
	SED	**CD(0.05)**	**CD(0.01)**		
T	1.54330	3.13785	4.21093		
S	1.09128	2.21880	2.97758		
TS	2.67308	5.43492	7.29354		

NS =Non significance; ** =Significance at 1% level.

The extractive values of different parts of *M. capitata* (root, stem and leaf) were calculated in various solvents and the data are presented in Table 2 and Figure 3. The extractive value

varies in different solvents as well as in different plant parts tested. The extractive value ranges from 4.0±0 % to 32.0±4.53% in root, 7.33±0.94% to 32.0±0% in stem and 5.33±1.51% to 45.33±4.99% in leaf sample of *M. capitata.* In root dry powder, the extractive value was more (32.0±4.53%) in methanol extracts, while in stem and leaf dry powdered sample shows maximum extractive value in water extract (32.0±0% and 45.33±4.99%), respectively. The extractive value of *M. capitata* samples recorded in different solvent extracts is arranged in the following descending order: methanol > water > ethyl acetate > ethanol > petroleum ether > chloroform in root sample; water > methanol > ethyl acetate > petroleum ether > chloroform > ethanol in stem sample; and water > methanol > ethyl acetate > ethanol > petroleum ether = chloroform in leaf sample (Table 2).

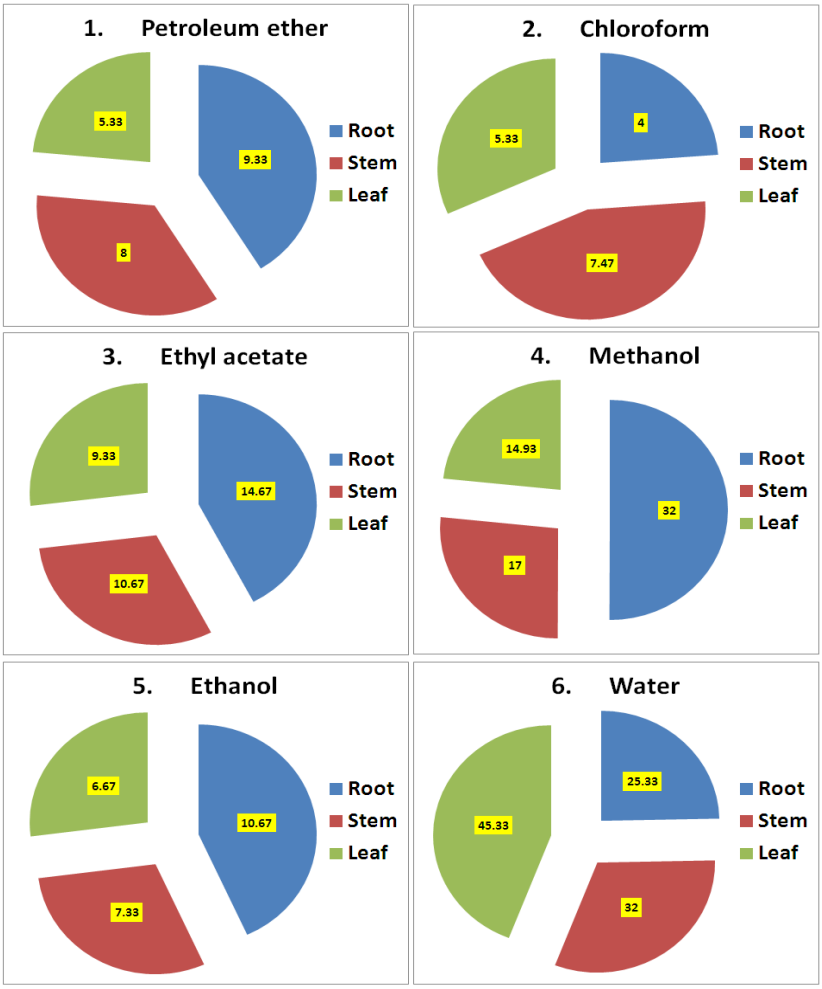

Figure 3: Extactive values (%) of *Malachra capidata* samples in different solvant extracts (1 to 6).

The extractive value in various solvents were reported as water (15.2%w/w), ethanol (12.8%w/w), ethyl acetate (11.2%w/w), methanol (10.4%w/w) and chloroform (6.4%w/w).[7] Extractive values of *Thespesia lampas* in various solvents like ethanol (0.24%), petroleum ether (0.08%), methanolic (3.75%), ethyl acetate soluble (8.0%), ethyl acetate insoluble

(4.2%) and aqueous extract (8.44%) w/w were reported by Chumbhale and Upasani.[8] Srividya *et al.*[10] reported the percentage yield of *Abutilon indicum* in various solvents like petroleum ether (2.18%), chloroform (2.04%), ethyl acetate (1.38%), *n*-butanol (3.84%), ethanol (5.81%) and water extracts (6.12%) w/v. According to Servin Wesley *et al.*[11] the maximum percentage yield in *Abutilon hirtum* was noted in ethanol extract (5.34%) followed by water extract (4.16%). In this study, the extractive values of different solvents were determined in dry powder samples of *M. capitata* and it was noted that aqueous extract of *M. capitata* leaf and stem showed maximum extractive value while it was maximum in methanol root extract (Table 2; Figure 3).

Fluorescence property

Fluorescence characteristic is a rapid method for resolution of doubtful specimen. When physical and chemical methods are inadequate, the plant material may be identified from their adulterants on the basis of fluorescence characteristics. Behaviors of the powdered drug with different chemical reagents and the preliminary phytochemical analysis are helpful for detection of various phytoconstituents.

Table 3: Fluorescence characters of *Malachra capitata* root dry powder* and extracts**

Root dry powder (RDP) + Solvents used	Fluorescence characters of root of *Malachra capitata*			
	Day light	**Fluorescent light**	**UV-254**	**UV-365**
1.Root dry powder(RDP)	Pale yellow	Pale yellow	Pale yellow	Pale yellow
2. RDP+H_2S0_4	Colourless	Light green	Green	Dark-green
3. RDP+CH_3COOH	Colourless	Colourless	Light-green	Colourless
4. RDP+HNO_3	Brown	Brown	Light-green	Black-green
5. RDP+NaOH	Pale yellow	Pale yellow	Light-green	Light-green
6.RDP+Petroleum ether	Pale yellow	Pale yellow	Colourless	Colourless
7. RDP+Chloroform	Pale yellow	Pale yellow	Light-green	Colourless
8. RDP+Ethyl acetate	Yellow	Yellow	Yellow	Pale yellow
9. RDP+Methanol	Pale yellow	Pale yellow	Pale yellow	Colourless
10. RDP+Ethanol	Pale yellow	Pale yellow	Colourless	Colourless
11. RDP+Water	Pale yellow	Pale yellow	Pale ellow	Yellow
12. RDP+Acetone	Colourless	Colourless	Pale yellow	Colourless
Solvent extracts used				
1.Petroleum ether	Colourless	Colourless	Colourless	Colourless
2.Chloroform	Pale yellow	Colourless	Colourless	Colourless
3.Ethyl acetate	Pale yellow	Pale yellow	Colourless	Colourless
4.Methanol	Pale yellow	Pale yellow	Colourless	Pale yellow
5.Ethanol	Pale yellow	Pale yellow	Colourless	Colourless
6.Water	Colourless	Colourless	Colourless	Colourless

*Fluorescent characters of the dry powder sample of the root were observed immediately in different solvents.
**Fluorescent characters of the dry powder sample of the root were observed in various solvent extracts after 24h of incubation.

Table 4: Fluorescence characters of *Malachra capitata* stem dry powder* and extracts.**

Stem dry powder (SDP) +Solvents used	Fluorescence characters of stem of *Malachra capitata*			
	Day light	Fluorescent light	UV-254	UV-365
1.Stem dry powder (SDP)	Light green	Light green	Light green	Light green
2. SDP+H_2S0_4	Pale green	Light green	Green	Dark green
3. SDP+CH_3COOH	Pale yellow	Pale green	Light green	Colour less
4. SDP+HNO_3	Brown	Brown	Light green	Black
5. SDP+NaOH	Pale yellow	Light green	Light green	Light green
6. SDP+Petroleum ether	Pale yellow	Pale yellow	Pale yellow	Light green
7. SDP+Chloroform	Pale yellow	Pale yellow	Light green	Light green
8. SDP+Ethyl acetate	Pale yellow	Light green	Light green	Yellow
9. SDP+Methanol	Light green	Pale yellow	Light green	Yellow
10. SDP+Ethanol	Light green	Light green	Light green	Yellow
11. SDP+Water	Pale yellow	Pale yellow	Pale yellow	Light green
12. SDP+Acetone	Pale yellow	Light green	Light green	Yellow
Solvent extracts				
1.Petroleum ether	Colourless	Colourless	Colourless	Colourless
2.Chloroform	Pale yellow	Pale yellow	Pale yellow	Colourless
3.Ethyl acetate	Pale yellow	Pale yellow	Pale yellow	Colourless
4.Methanol	Light green	Light green	Light green	Pale yellow
5.Ethanol	Light green	Light green	Light green	Pale yellow
6.Water	Colourless	Pale yellow	Pale yellow	Colourless

*Fluorescent characters of the dry powder sample of the stem were observed immediately in different solvents.
**Fluorescent characters of the dry powder sample of the stem were observed in various solvent extracts after 24h of incubation.

Table 5: Fluorescence characters of *Malachra capitata* leaf dry powder* and extracts.**

Leaf dry powder (LDP) +Solvents used	Fluorescence characters of leaf of *Malachra capitata*			
	Day light	Fluorescent light	UV-254	UV-365
1.Leaf dry powder (LDP)	Green	Green	Green	Green
2.LDP + H_2SO_4(1N)	Green	Green	Green	Dark green
3. LDP + CH_3COOH	Green	Green	Green	Green
4. LDP + HNO_3	Brown	Brown	Dark green	Black
5. LDP + NaOH	Green	Green	Dark green	Dark green
6. LDP + Petroleum ether	Green	Dark green	Dark green	Black
7. LDP + Chloroform	Green	Yellow green	Dark green	Dark green
8.LDP + Ethyl acetate	Green	Green	Dark green	Black
9. LDP + Methanol	Green	Dark green	Dark green	Black
10. LDP + Ethanol	Green	Dark green	Dark green	Black
11. LDP + Water	Green	Green	Green	Dark green
12. LDP + Acetone	Green	Dark green	Dark green	Dark green
Solvent extracts				
1. Petroleum ether	Light green	Yellow green	Light green	Colourless
2. Chloroform	Pale yellow	Yellow green	Light green	Orange red
3. Ethyl acetate	Light green	Light green	Light green	Orange red
4. Methanol	Dark green	Dark green	Light green	Orange red
5. Ethanol	Dark green	Dark green	Light green	Orange red
6. Water	Pale yellow	Pale yellow	Light green	Colourless

*Fluorescent characters of the dry powder sample of the leaf were observed immediately in different solvents.
**Fluorescent characters of the dry powder sample of the leaf were observed in various solvent extracts after 24h of incubation.

The fluorescence behavior of the dry powder samples of different parts (root, stem and leaf) of *M. capitata* was analyzed in different solvents and their extracts towards ordinary light, fluorescence light, UV- light (at 254nm and 365nm) and the results are presented in tables 3 to 5. The fluorescence analysis utilizes the fluorescence produced by the compounds in the ultraviolet light for analytical evaluation. The fluorescence behavior of the powdered plant material of different parts of *M. capitata* were observed in this study in different solution and their extracts towards ordinary light, fluorescent light and ultraviolet light can be used as a diagnostic tool for testing adulteration if any. Evaluation of the physical properties of *M. capitata* indicate that the dry weight and acid soluble ash was found maximum in stem sample, while water soluble ash value was maximum in root and stem samples; the ash properties show variation among the dry powdered samples of *M. capitata*. Water insoluble ash value, acid insoluble ash, sulphated ash and residue on ignition were shows maximum value in the leaf sample; the fluorescent properties of the *M. capitata* dry powdered samples (root, stem and leaf) emit various colours under day light and UV-light conditions and the extractive value of different parts of *M. capitata* stem and leaf showed the highest extractive value in water while in root it was more in methanol extract.

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