Research Article

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HPTLC FINGERPRINTING AND *IN VITRO* ANTIOXIDANT STUDIES OF *ARGYREIA SPECIOSA* SWEET LEAVES AND *MESUA FERREA* LINN. FLOWERS

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ABSTRACT

Natural antioxidants present in plants have attracted interest because of their safety and potential nutritional and therapeutic effects. The plant kingdom offers a wide range of natural antioxidants. The HPTLC fingerprinting was done for methanolic extracts of *Argyreia speciosa* leaves and *Mesua ferrea* flowers. The solvent system developed were Chloloroform: Methanol:Ethyl acetate:: 9:1.1:0.5 and Chloloroform: Methanol:: 9:1 for *Argyreia speciosa* and *Mesua ferrea* respectively. For *Argyreia speciosa* the prominent Rf and area % obtained were 0.79 and 42.51 at 200 nm; 0.87 and 25.63 at 250 nm; 0.88 and 16.33 at 300 nm; 0.85 and 11.53 at 350 nm; 0.12 and 11.93 at 400 nm; 0.12 and 12.62 at 450 nm and for *Mesua ferrea* the prominent Rf and area % obtained were 0.89 and 20.73 at 200 nm; 0.89 and 15.70 at 240 nm; 0.78 and 15.98, 0.90 and 12.58 at 280 nm; 0.15 and 10.68, 0.30 and 22.37 at 320 nm; 0.29 and 20.49 at 400 nm. The Total antioxidant capacity of *Argyreia speciosa* was found to be 108.33 ± 1.59 and of *Mesua ferrea* was 91.67 ± 2.16. The percentage Inhibition (IC_{50}) of *Argyreia speciosa* was 168.46 ± 1.03 µg /ml and of *Mesua ferrea* was 300.01 ± 1.59 µg / ml in DPPH free radical scavenging activity. In scavenging of superoxide radical by alkaline DMSO method IC_{50} of *Argyreia speciosa* was 148.63 µg / ml and of *Mesua ferrea* was 273.56 µg / ml. In scavenging of hydrogen peroxide method IC_{50} of *Argyreia speciosa* and *Mesua ferrea* were 30.47 µg / ml and 21.70 µg / ml respectively.

Keywords: HPTLC fingerprinting, *In vitro* antioxidant studies, *Argyreia speciosa*, *Mesua ferrea*.

INTRODUCTION

The plant kingdom offers a wide range of natural antioxidants. However, there is still not enough knowledge about the practical usefulness of most of them. The *in vitro* methods provide easy and useful indications of antioxidant activities. *Argyreia speciosa* Sweet is a woody climber which belongs to the family Convulvulaceae. The plant is commonly known as Elephant creeper in English and in Hindi, it is known as 'Vryddhadaru". It has been used as a "rasayana" drug (rejuvenating drugs) in the traditional Ayurvedic system of medicines[1]. *Mesua ferrea* Linn. belongs to the family Clusiaceae is known as 'Nagkeshara' in Hindi and in English as Ceylon Ironwood. It is a genus of trees or shrubs distributed chiefly in tropical Asia. It is a medium to large evergreen tree with short trunk, often buttressed at the base[1].

MATERIALS AND METHODS

The plant twigs of *Argyreia speciosa* Sweet and *Mesua ferrea* Linn. have been identified by Prof. N. K. Dubey, Reader, Department of Botany, Banaras Hindu University. The herbarium COG/H.No. – 024 of *Argyreia speciosa* leaves and COG/H.No. – 025 of *Mesua ferrea* flowers have been deposited in the Pharmacognosy division of Department of Pharmaceutics, IIT (BHU) Varanasi, India.

Successive extraction

All the solvents, chemicals, reagents and HPTLC pre-coated plates of silica Gel 60F254 were procured from Merck India. The powdered leaves of *Argyreia speciosa* and powdered *Mesua ferrea* flowers were successively extracted by using petroleum ether (60º-80º), chloroform and methanol. The methanolic extracts of *Argyreia speciosa* leaves and *Mesua ferrea* flowers were used for HPTLC fingerprinting and *in vitro* antioxidant studies.

HPTLC fingerprinting

The solvent system for *Argyreia speciosa* and *Mesua ferrea* was developed by the help of Nyiredy's prism system and trial and error method[2]. CAMAG (Switzerland) HPTLC was used with automatic TLC Sampler III, AMD 2 automatic developing chamber, ascending mode of development and CAMAG TLC scanner 3 with Cats software. Multi wavelength scanning was conducted separately for *Argyreia speciosa* and *Mesua ferrea*. For *Argyreia speciosa* it was done at 200, 250, 300, 350, 400 and 450 nm. For *Mesua ferrea* it was done at 200, 240, 280, 320 and 400 nm. The measurement type was remission and measurement mode was absorption.

In vitro antioxidant studies

The study dealt with Total antioxidant capacity, DPPH radical scavenging activity, Alkaline dimethyl sulfoxide (DMSO) method for scavenging of the superoxide radical and Hydrogen peroxide scavenging activity.

Total antioxidant assay

This was done according to the procedure of Prieto P., *et al.,* (1999)[3].

DPPH free radical scavenging activity

This was determined by the method described by Braca A., *et al*. (2001)[4].

Table 1 and Graph 1: Observation Table and Standard Calibration Curve for Ascorbic acid

Concentration (µg/ml)	Absorbance (695 nm)
0	0
25	0.112
50	0.185
75	0.196
100	0.266
125	0.419

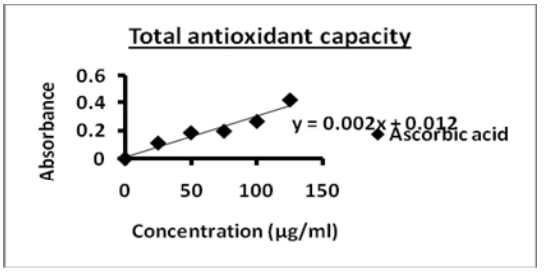

Table 2: Observation Table for the methanolic extracts *Argyreia speciosa* and *Mesua ferrea*

Absorbance (695 nm) (Mean ± S.E.M.*)	
Argyreia speciosa	*Mesua ferrea*
0.033 ± 0.003	0.030 ± 0.004

Table 3: Results for Total Antioxidant Capacity

Total antioxidant capacity (Mean ± S.E.M*)	
Argyreia speciosa	*Mesua ferrea*
108.33 ± 1.59	91.67 ± 2.16

Table 4: Observation Table for Percentage Inhibition (Absorbance)

Concentration (µg/ml)	Absorbance or percentage inhibition (Mean ± S.E.M*)		
	Rutin	*Argyreia speciosa*	*Mesua ferrea*
0	0	0	0
25	24.80 ± 0.166	9.73 ± 0.40	7.31 ± 0.40
50	46.00 ± 0.220	17.29 ± 0.26	11.18 ± 0.16
75	69.08 ± 0.245	23.94 ± 0.03	15.45 ± 0.37
100	89.073 ± 0.190	29.16 ± 0.16	16.18 ± 0.15
125	103.14 ± 0.156	37.70 ± 1.15	21.97 ± 0.95

Table 5: Results for Percentage Inhibition (IC_{50})

Percentage Inhibition (IC_{50}), Mean ± S.E.M*. (µg/ml)			
Sample	Rutin	*Argyreia speciosa*	*Mesua ferrea*
IC_{50} (µg/ml)	54.77 µg / ml	168.46 ± 1.03	300.01 ± 1.59

Table 6: Observation Table for Percentage Inhibition (Absorbance)

Concentration (µg/ml)	Absorbance or percentage inhibition (Mean ± S.E.M*)		
	Ascorbic acid	*Argyreia speciosa*	*Mesua ferrea*
0	0	0	0
10	27.53	7.96 ± 0.16	2.97 ± 0.46
20	41.76	10.34 ± 0.58	5.37 ± 0.54
30	51.83	16.67 ± 0.59	7.49 ± 0.50
40	55.67	18.33 ± 0.49	10.32 ± 0.30
50	58.79	23.55 ± 0.50	11.99 ± 0.58
60	-	25.47 ± 0.42	14.40 ± 0.35
80	-	28.28 ± 0.24	15.92 ± 0.17
100	-	31.64 ± 0.47	17.22 ± 0.73

Table 7: Results for Percentage Inhibition (IC_{50})

Percentage Inhibition (IC_{50})			
Sample	Ascorbic acid	*Argyreia speciosa*	*Mesua ferrea*
IC_{50} (µg/ml)	34.71 µg / ml	148.63 µg / ml	273.56 µg / ml

Table 8: Observation Table for Percentage Inhibition

Concentration (µg/ml)	Absorbance or percentage inhibition (Mean ± S.E.M*)		
	Ascorbic acid	*Argyreia speciosa*	*Mesua ferrea*
0	0	0	0
5	12.86	14.43 ± 2.72	5.04 ± 0.89
10	29.58	19.93 ± 2.38	15.24 ± 3.20
15	43.34	27.22 ± 3.05	18.70 ± 1.06
20	52.78	35.04 ± 1.35	21.17 ± 1.56
25	61.46	39.44 ± 1.10	25.55 ± 1.63

Table 9: Results for Percentage Inhibition (IC_{50})

Percentage Inhibition (IC_{50})			
Sample	Ascorbic acid	*Argyreia speciosa*	*Mesua ferrea*
IC_{50} (µg/ml)	16.02 µg / ml	30.47 µg / ml	21.70 µg / ml

***:** Average of three determinations

Scavenging of superoxide radical by alkaline DMSO method

This was done by the method proposed by Elizabeth K., *et.al,* (1990)[5].

Scavenging of hydrogen peroxide

The scavenging of hydrogen peroxide radical was carried based on the method proposed by Jayaprakasha G.K., *et al.,* (2004)[6].

RESULTS

HPTLC fingerprinting

The solvent system Chlololoroform: Methanol:Ethyl acetate:: 9:1.1:0.5 had shown good separation of spots in *Argyreia speciosa*. The solvent system Chlololoroform: Methanol:: 9:1 had shown good separation of spots in *Mesua ferrea*. For *Argyreia speciosa* the prominent Rf and area % obtained were 0.79 and 42.51 at 200 nm; 0.87 and 25.63 at 250 nm; 0.88 and 16.33 at 300 nm; 0.85 and 11.53 at 350 nm; 0.12 and 11.93 at 400 nm; 0.12 and 12.62 at 450 nm. For *Mesua ferrea* the prominent Rf and area % obtained were 0.89 and 20.73 at 200 nm; 0.89 and 15.70 at 240 nm; 0.78 and 15.98, 0.90 and 12.58 at 280 nm; 0.15 and 10.68, 0.30 and 22.37 at 320 nm; 0.29 and 20.49 at 400 nm.

***In vitro* antioxidant studies**

All the experiments were carried out in triplicates and were expressed as Mean ± S.E.M. The amount of extract needed to inhibit free radical concentration by 50 % (IC_{50}), was graphically determined by a linear regression method using MS Windows based Graphpad Instat (Version 3) software.

Total antioxidant capacity

From the standard graph plotted the results for the samples were interpolated and given in Table 1, 2, 3 and Graph 1.

DPPH free radical scavenging activity

From the standard graph plotted the results for the samples were interpolated and given in Table 4 and 5.

Scavenging of superoxide radical by Alkaline DMSO method

From the standard graph plotted the results for the samples were and given in Table 6 and 7.

Scavenging of Hydrogen peroxide

From the standard graph plotted the results for the samples were interpolated and given in Table 8 and 9.

DISCUSSION

These data implies that the phytoconstituents of *Argyreia speciosa* extract absorbs at lower wavelengths. The total antioxidant capacity of *Argyreia speciosa* and *Mesua ferrea* has been done by the phosphomolybdenum method and was expressed as the number of equivalent of ascorbic acid (mg/g of the plant extract). This assay is simple and inadequate of other antioxidant measurement. The basic principle behind this is the simple reduction of Mo (IV) to Mo (V) which leads to the formation of the Mo (V) complex that produces a green color complex of phosphates/Mo (V) which has the maximal absorbance at 695 nm. The total antioxidant capacity (Graph: 1) in *Argyreia speciosa* and *Mesua ferrea* was determined using the linear regression equation of the calibration curve ($y=0.002x+0.012$, $r^2 = 0.940$) and were expressed as ascorbic acid equivalent. DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants. It is a convenient screening tool for quickly determining antioxidant content of food products and food ingredients[7]. The DPPH molecule is a stable molecule that accepts electron from the analyte. The ability to scavenge stable DPPH molecule is a widely used method to evaluate radical-scavenging capacity (RSC) in a relatively short time compared to other methods[7]. The basic principle for the reduction in DPPH free radicals is that, the antioxidant reacts with stable free radical, DPPH and converts it to 1, 1-diphenyl-2-picryl hydrazine. This happens when only it reacts with hydrogen donors. The DPPH radical is purple in color and upon reaction with a hydrogen donor changes to yellow in colour. This assay provides the information on the reactivity of compound on the stable free radical. Because at 517 nm wavelength; the odd electrons of DPPH shows a strong absorption band. As this electron becomes paired off in the presence of free radical scavenger, the absorption vanishes and decreases, and the resulting decolorisation is stochiometric with respect to the number of electrons taken up[8]. The evaluation of scavenging effects of *Argyreia speciosa* and *Mesua ferrea* on the DPPH radical was investigated using rutin as the standard. The results were showed in the Table 5, rutin was found to have an IC_{50} of 54.76 µg / ml whereas *Argyreia speciosa* has moderate antioxidant activity of (168.46 µg / ml) and less occurs with *Mesua ferrea* (300.01 µg / ml). Alkaline dimethyl sulfoxide (DMSO) method was used as a superoxide generation system. Superoxide is a highly reactive molecule that can react with many substrates, produced in various metabolic processes, including phagocytosis. It can cause oxidation or reduction of solutes depending on their reduction potential. It is well known that superoxide anions damage bio macromolecules directly or indirectly by forming H_2O_2, OH, peroxyl nitrite or singlet oxygen during pathophysiologic events such as ischemic reperfusion injury[8]. DMSO method is a well known method for it scavenging activity of the $OH^{\bullet}$ radical. Alkaline DMSO, used as a superoxide generating system reacts with NBT to give colored diformazan. In the presence of scavenger the reduction of NBT can be measured at 560 nm. As compared to the standard ascorbic acid *Argyreia speciosa* and *Mesua ferrea* have been seen to have a moderate scavenger activity of the superoxide generated in the solution. Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by the oxidation of the essential thiol (-SH) group. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe_2^+ and possibly Cu_2^+ ions to form hydroxyl radical and this may be the origin of many of its toxic effects[9]. It is therefore

biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. *Argyreia speciosa* shows a quite acceptable activity whereas *Mesua ferrea* shows a very less activity as compared to that of the standard ascorbic acid.

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REFERENCES

1. Kirtikar KR, Basu BD. Indian Medicinal Plants, Vol III 2nd ed. Allhabad: India; 1956.
2. Wagner H, Bladt S., Ed. Plant drug analysis - A Thin Layer Chromatography Atlas. Springer-Verlog Berlin Heidelberg, Germany; 1996. http://dx.doi.org/10.1007/978-3-642-00574-9
3. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of Vitamin E. Anal Biochem 1999; 269: 337–341. http://dx.doi.org/10.1006/abio.1999.4019 PMid:10222007
4. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from Bauhinia terapotensis. Journal of Natural Products 2001; 64: 892-895. http://dx.doi.org/10.1021/np0100845 PMid:11473417
5. Elizabeth K, Rao MNA. Oxygen radical scavenging activity of curcumin. International Journal of Pharmaceutics 1990; 58: 237-240. http://dx.doi.org/10.1016/0378-5173(90)90201-E
6. Jayaprakasha GK, Jaganmohan Rao, L Sakariah KK. Antioxidant activities of flavidin in different *in vitro* model systems. Bioorganic and Medicinal Chemistry 2004; 12: 5141-5146. http://dx.doi.org/10.1016/j.bmc.2004.07.028 PMid:15351397
7. Miller HE, Rigelhof F, Marquart L, Prakash A, Kanter M. Antioxidant Content of Whole Grain Breakfast Cereals, Fruits and Vegetables. Journal of the American College of Nutrition 2000; 19(3): 3125–3195.
8. Shirwaikar A, Shirwaikar A, Rajendran K, Punitha ISR. *In Vitro* Antioxidant Studies on the Benzyl Tetra Isoquinoline Alkaloid Berberine, Biol. Pharm Bull 2006; 29(9): 1906-1910. http://dx.doi.org/10.1248/bpb.29.1906 PMid:16946507
9. Miller MJ, Sadowska krowicka, Chotinaruemol H, Kakkis S, JL Clark DA. Amelioration of chronic ileitis by nitric oxide synthase inhibition. The J. Pharmac. and Experi. Thera 1993; 264: 11-16.

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