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Morphological characteristics and antibacterial activity of mulberry fruit varieties

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Abstract
The present study was carried out to characterize the mulberry fruit yielding varieties (G4, MR2, G2 and S36) by its morphological characters and analyze antibacterial activity of fruit extracts of G4 and MR2. Among the morphological characters, fruit colour ranged from purple, deep purple, red and dark red. Similarly, fruit weight ranged from 0.5 to 1.52g in which G4 recorded highest fruit weight of 1.52 g followed by MR2 (1.10g), G2 (0.713 g) and S36 (0.5g). Based on the availability of the fruit quantum G4 and MR2 was selected for antibacterial activity against the human bacterial pathogens such as *Staphylococcus aureus ATCC11632*, *Streptococcus pneumonia ATCC*6301 and *Staphylococcus epidermidis ATCC12228.* Among the different concentrations (20, 40, 60, 80, 100 µl) of mulberry fruit extracts, 100 µl exhibited highest zone of inhibition of 17.0±1.25 to 20.0±0.81 mm which showed antibacterial response against bacterial pathogens. G4 fruit extract showed highest antibacterial activity against *S. aureus* with inhibition of 19.0±0.49 mm. Similarly, MR2 fruit extract showed highest antibacterial activity against *S. epidermidis* with inhibition of 20.0±0.73 mm. The present findings indicated that mulberry fruit extracts have medicinal choice for the development of alternative antimicrobial drug without any side effects as seen in chemical drugs.

Keywords: Mulberry fruit varieties, qualitative, quantitative, morphological characters, *in vitro* antibacterial assay

Introduction
Sericulture is one of the leading agriculture based rural industry that being practiced worldwide due to its revenue generating capacity. Mulberry (*Morus* sp.) is a multipurpose plant with high potential economic value and widely distributed in Asia, Europe, North America, South America and Africa (Imran *et al.*, 2010) [8]. In India, mulberry plant is economically important and predominantly used as a food source of silkworm (*Bombyx mori*), commercially reared for silk production and widely distributed in Jammu and Kashmir, UP, Karnataka, Tamil Nadu, West Bengal, Kerala and to a lesser extent of Himachal pradesh. In countries like Turkey and Greece, mulberry trees are grown especially for its fruit production instead of its foliage (Gungor *et al.*, 2008) and it is utilized in food industries for the production of mulberry juices, paste, marmalade, jam, jelly and several traditional products such as pekmez, pestil and kome are also made using mulberry fruits. Chinese utilized mulberry fruit as a natural traditional medicine to strengthen the joints, lower the blood pressure, treat fever, protect liver damage and assist in discharge of urine. In Pakistan, *Morus laevigata* is highly appreciated for its delicious fruit which is eaten fresh as well as in dried forms and consumed in marmalades, juices, liquors, natural dyes and cosmetic industries. Mulberry is known as “Kalpa Vruksha” in India which means all parts of the plant are used for various purposes and also its fruit is commonly named as toot and shahtoot (King’s or ‘‘superior” mulberry). Mulberry fruit contains larger amounts of primary metabolites in the form of carbohydrate, protein, lipid, vitamins, minerals and fiber that provide a healthy dietary option to the consumers. Mulberry fruit has a rich source of bioactive components like polyphenols (phenolic acids, flavonoids like anthocyanin, flavonol and flavanol) alkaloids and melatonin (Natic *et al.*, 2015) [11]. Conventionally, it is believed that fruits of mulberry are advantageous to the human body (Ercisli and Orhan, 2007) [6]. Unani medicine known as Tutiaswad contains *M. nigra* fruits which are believed to have anti-cancerous activities (Nursalam, 2016). Despite having immense nutraceutical and pharmaceutical properties these mulberry fruits are underutilized in India due to lack of awareness, research and development.

So it is essential to study the nature and biological activities of the mulberry fruit. With this background, the present study was focused on morphological characters of mulberry varieties and antibacterial activity of fruit extracts.

Materials and Methods

Qualitative and quantitative morphological characters

The morphological characters of different mulberry varieties such as G4, MR2, G2 and S36 grown in the mulberry garden of Department of sericulture, Forest College and Research Institute, Mettupalayam were observed and the garden is located at an altitude of 320 m above MSL, 11.20 °N latitude and 76.56 °E longitude. Qualitative morphological characters of mulberry varieties were visually observed such as growth habit, branch colour**,** shoot type, bud colour, bud attachment, leaf colour, leaf shape, fruit colour and fruit shape. Quantitative morphological characters such as leaf length, leaf width, fruit and pedicel length, width of the fruit and fruit weight were also observed.

In vitro evaluation of Antibacterial activity of mulberry fruit extracts

Extraction of fruit extracts from mulberry fruits

Mulberry fruits from G4 and MR2 were collected from the farmer's fields in Annur (Kariyakoundanpudhur, Kariampalayam), Coimbatore, Tamil Nadu**.** Mulberry fruits were collected and stored in the food grade containers to avoid fungal contamination and kept in refrigerator for further use. The mulberry fruit juice was extracted with stainless steel manual hand juicer. The extracted juice was filtered twice and used for analyzing antibacterial activity against human pathogens.

In vitro antibacterial assay

Six different concentrations viz., 20, 40, 60, 80 and 100 µl of the fruit extracts of G4 and MR2 varieties were prepared and used for assay. Bacterial cultures were procured from ATCC, and inoculated in 10 ml test tube and incubated in an orbital incubator shaker for overnight at 37°C (120 rpm). Nutrient broth was used for culturing different microbes. The viability of the cells is checked by further plating and found to be viable. The sterile nutrient agar media was used to culture the bacterial pathogens overnight at 37 °C. Pathogens *Staphylococcus aureus*, *Streptococcus pneumonia*, *Staphylococcus epidermidis* were spread plated using a L-rod on to the NB agar plates. Sterile well cutter was used to make holes on the agar plate. 100 µl of the dissolved samples at different concentrations (20, 40, 60, 80,100 µl) were poured onto the wells. After the incubation period, the zone of inhibition was measured and reported in millimeters mm (Seeley *et al.*, 2001). Diameter of the clear zones (greater than 5mm) around each well was measured (Hammer *et al.*, 1999). Chloramphenicol was served as a control. All assays were performed in triplicate and for measuring activity index, following formula was used.

$$\text{Activity index} = \frac{\text{Zone of inhibition of fruit extract}}{\text{Zone of inhibition of antibiotic}}$$

Statistical analysis

The obtained results are the means of independent values and statistical significance was analyzed with $p \leq 0.05$ level of significance (Gupta, 2002) by Analysis of variance (ANOVA) for different observation made on the treatments was performed and means were compared using Completely Randomized Design (CRD) or Randomized Block Design (RBD) as described by Panse and Sukhatme (1957).

Results and Discussion

Qualitative morphological characters

The qualitative morphological characters analysis is considered as a primary approach towards the assessment of genetic diversity in a plant species (Boubaya *et al.*, 2009) [3]. In this current study, the growth habit of four mulberry varieties (G4, MR2, G2 and S36) exhibited predominantly semi erect except S36 which showed erect growth habit. Branch colour is similar for all the varieties which was greyish green. Similarly, slightly curved shoot type and brown bud colour was also similar for all the four varieties. Bud was attached closely with the branch for all the four varieties. Leaf colour is varied from light green to dark green. Cordate shape of leaf was observed in G4 and G2 varieties, acute shape in MR2 variety and wide acute shape in S36 variety (Table 1).

Table 1: Qualitative morphological characters of mulberry fruit varieties

S. No	Characters	G4	MR2	G2	S36
1.	Growth habit	Semi erect	Semi erect	Semi erect	Erect
2.	Branch colour	Greyish green	Greyish green	Greyish green	Greyish green
3.	Shoot type	Slightly curved	Slightly curved	Slightly curved	Slightly curved
4.	Bud colour	Brown	Brown	Brown	Brown
5.	Bud attachment	Adhering to branch	Adhering to branch	Adhering to branch	Adhering to branch
6.	Leaf colour	Dark green	Green	Dark green	Light green
7.	Leaf shape	Cordate	Acute	Cordate	Wide acute
8.	Fruit shape	Oblong	Pendulous	Pendulous	Round
9.	Fruit colour	Purple	Deep purple	Red	Dark Red

Fruit shape was similar for MR2 and G2 varieties whereas oblong shape for G4 and round shape for S36 variety. Fruit colour was observed and recorded as purple (G4), deep purple (MR2), red (G2) and dark Red (S36). Previously Krishna *et al.* (2018) [10] observed the morphological characters of different genotypes of mulberry in which the qualitative traits such as growth habit was found to be erect or upright, shoot was either slightly curved or straight, bud attachment was observed as slanting outward or adhering to branch, bud shape, leaf colour, leaf shape, leaf apex, leaf base, fruit shape was found to be either pendulous or oblong and fruit colour varied from red to white resulted in distinct variations among the genotypes. Similarly, Chanotra *et al.* (2019) [4] studied the leaf shape and several morphological characters of mulberry genotypes for morpho-physiological characterization for breeding programme. Several other studies have also been carried out on morphological characters of Mulberry by many authors Vijayan *et al.* (2004) [14], Peris *et al.* (2014) [12],

Erarslan *et al*. (2021) [5].

Quantitative morphological characters

The variations in quantitative morphological characters among the of four mulberry varieties were also assessed. Highest leaf length of 28.0 cm was recorded in G4 followed by MR2 (26.4 cm) and G2 (25.5 cm) which was on par with each other whereas S36 (23.4 cm) was significantly different from the others. Similarly, leaf width was observed as in G2 (18.3 mm), G4 (17.5 mm) and S36 (17.0 mm) which was on par with each other whereas MR2 (14.7 mm) (Table 2) was significantly different from the others. Likewise leaf length and width was recorded and reported as 11.3 to 20 cm and 10.08 to 15.86 cm in ten mulberry genotypes by Jalikop *et al.* (2011) [9]. Krishna *et al*. (2018) [10] studied the quantitative morphological characters includes leaf length and width, fruit length and width, pedicel length and leaf lamina length of mulberry genotypes. Pedicle length varied from 0.35 cm to 1.13 cm in which G2 (1.13 cm) recorded highest length followed by MR2 (0.96 cm), G4 (0.7 cm) and S36 (0.35 cm). Similarly, pedicel length ranging from 0.5 cm to 1.5 cm was reported in ten mulberry genotypes (Krishna *et al.*, 2018) [10]. Fruit length and width was measured and recorded in the range of 2 cm to 3.14 cm and 9 to 18 mm respectively.

Table 2: Quantitative morphological characters of mulberry fruit varieties

S. No	Mulberry varieties	Characters					
		Leaf length (with petiole) (cm)	Leaf width (mm)	Fruit length (with pedicle) (cm)	Fruit width (mm)	Pedicel length (cm)	Fruit weight (g)
1.	G4	28.0±1.66 ^{a}	17.5±0.74^{a}	2.58±0.20 ^{b}	18.0±1.18 ^{a}	0.7±0.06 ^{c}	1.52±0.04 ^{a}
2.	MR2	26.4±2.37 ^{a}	14.7±0.01^{b}	3.14±0.26 ^{a}	16.0±1.27 ^{b}	0.96±0.06 ^{b}	1.10±0.05 ^{b}
3.	G2	25.5±0.18 ^{a}	18.3±0.57^{a}	3.03±0.25 ^{a}	15.0±0.28 ^{b}	1.13±0.07 ^{a}	0.713±0.04 ^{b}
4.	S36	23.2±1.58 ^{b}	17.0±1.47^{a}	2.0±0.14^{c}	9.0±0.77 ^{c}	0.35±0.01^{d}	0.5±0.01 ^{c}
	SEd	1.3517	0.6429	0.1902	0.7177	0.0540	0.7177
	CD (0.05)	3.1171	1.5732	0.4655	1.7563	0.1322	1.7563
		S**	S**	S**	S**	S**	S**

Values are expressed in mean ± SD with three replications (n=3). Means followed by different small superscript in a Coloumn are statistically different at $p \leq 0.05$

S** - Significant difference

MR2 (3.14 cm) and G2 (3.03 cm) varieties recorded highest fruit length followed by G4 (2.58 cm) and S36 (2.0 cm). Fruit width of 18 mm was measured in G4 was the highest followed by MR2 (16 mm), G2 (15 mm) which was on par with each other and the least best was recorded in S36 (9 mm). Fruit weight was in the range of 0.5 g to 1.52 g in the four mulberry varieties in which G4 recorded highest fruit weight of 1.52 g followed by MR2 (1.10g), G2 (0.713) which was on par with each other and S36 (0.5g) (Table 2) was the least recorded. In different studies, the fruit weight was reported as similar as our results such as 0.56 g (Jalikop *et al.*, 2011) [9], 0.66 g (Yilmaz *et al.*, 2012) [17], 1.091 g (Boubaya *et al.*, 2009) [3].

Fig 1: Fruits of different mulberry varieties. (a – G4 variety fruit; b – MR2 variety fruit; c - G2 variety fruit; d – S36 variety fruit)

***In vitro* Antibacterial activity of fruit extracts**

Aqueous fruit extract of MR2 and G4 mulberry varieties were used to analyze antibacterial activity with different concentrations ranged from (20, 40, 60, 80 and 100µl) and the zone of inhibition was measured against bacterial pathogens. Table 3 showed that there was significant variation between G4and MR2 mulberry fruit extracts against *S. aureus* in all different concentrations (20, 40, 60, 80, 100 µl). In G4, highest zone of inhibition was recorded in the concentration of 100 µl (19.0±0.49 mm) followed by 80 µl (15.0±0.89 mm), 60 µl (14.0±0.27 mm), 40 µl (13.0±1.0 mm) and the least inhibition zone was obtained in the concentration of 20 µl (12.0±0.98 mm) (Fig 2a, Table 3). MR2 showed highest zone of inhibition in the concentration of 100 µl (17.0±1.25 mm) followed by 80 µl (13.0±0.36 mm), 60 µl (12.0±0.54 mm), 40 µl (11.0±0.53 mm) and the least inhibition zone was obtained in the concentration of 20 µl (10.0±0.15 mm) (Fig 2b, Table 3). G4 exhibited highest zone of inhibition against *S. aureus* than MR2 (Fig 2a, Fig2b). Yigit and Yigit (2009) [16] reported the antibacterial activities of methanol and aqueous extracts of *Morus nigra* fruits against bacterial pathogens such *as S.aureus, Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis* by disc diffusion method.

Table 3: Antibacterial activity of G4 and MR2 mulberry fruit extract against *Staphylococus aureus* ATCC11632

S. No		Zone of inhibition (mm) Concentration of the sample(µl)				
		20 µl	40 µl	60 µl	80 µl	100 µl
1.	G4 mulberry variety fruit extract	12 .0±0.98[a]	13.0±1.0 [a]	14.0±0.27 [a]	15.0±0.89 [a]	19.0±0.49 [a]
2.	MR2 mulberry variety fruit extract	10.0 ± 0.15[b]	11.0± 0.53 [b]	12.0± 0.54 [b]	13.0± 0.36 [b]	17.0± 1.25 [b]
	SEd	0.5776	0.7053	0.3494	0.5569	0.7783
	CD (0.05)	1.6037	1.9581	0.9700	1.5462	2.1610
		S**	S**	S**	S**	S**
3.	Standard (Chloramphenicol)	17.0± 0.27				

Values are expressed in mean ± SD with three replications (n=3). Means followed by different mall superscript in a Coloumn are statistically different at $p \leq 0.05$
S** - Significant

Table 4: Antibacterial activity of G4 and MR2 mulberry fruit extract against *Streptococcus pneumonia* ATCC6301

S.NO		Zone of inhibition (mm) Concentration of the sample(µl)				
		20 µl	40 µl	60 µl	80 µl	100 µl
1.	G4 mulberry variety fruit extract	10.0±0.22[a]	12.0±0.73	13.0±0.47	15.0±1.31	20.0±0.81 [a]
2.	MR2 mulberry variety fruit extract	12.0±0.28[b]	13.0±0.26	14.0±0.56	16.0±0.80	18.0±1.43 [b]
	SEd	0.2105	0.4532	0.4278	0.8909	0.4854
	CD (0.05)	0.5843	1.2582	1.1877	2.4736	1.3476
		S**	NS	NS	NS	S**
3.	Standard (Chloramphenicol)	18.0± 0.79				

Values are expressed in mean ± SD with three replications (n=3). Means followed by different small superscript in a Coloumn are statistically different at $p \leq 0.05$
S** - Significant, NS – Non-Significant

G4 and MR2 mulberry fruit extract exhibited significant antibacterial activity against *S. pneumonia.* The highest zone of inhibition was obtained in G4 fruit extract in the concentration of 100 µl (20.0± 0.81mm) (Fig 3a) followed by MR2 in the concentration of 100 µl (18.0± 0.43 mm) (Fig 3b, Table 4). The lowest inhibition zone was recorded in the G4 fruit extract in the concentration of 20 µl (10.0± 0.22 mm) (Fig 3a) followed by MR2 fruit extract in the concentration of 20 µl (12.0± 0.28 mm) (Fig 3b). Whereas all the other concentrations (40, 60 & 80 µl) of the G4 and MR2 mulberry fruit extract does not have any significant difference between each other. Overall, G4 exhibited highest zone of inhibition against *S. pneumonia* than MR2 (Table 4). Yang *et al.* (2012) reported the antibacterial activity of morin isolated from mulberry fruits which showed moderate inhibition (++) against *Streptococcus mutans*.

Table 5: Antibacterial activity of G4 and MR2 mulberry fruit extract against *Staphylococcus epidermidis* ATCC12228

S. No		Zone of inhibition (mm) Concentration of the sample(µl)				
		20 µl	40 µl	60 µl	80 µl	100 µl
1.	G4 mulberry variety fruit extract	NA [b]	13.0± 0.55	14.0±0.71	15.0±1.10	18.0±0.22[b]
2.	MR2 mulberry variety fruit extract	12.0±0.25 [a]	13.0± 0.21	14.0±0.11	16.0±1.22	20.0±0.73[a]
	SEd	0.1457	0.3440	0.4162	0.9496	0.4423
	CD (0.05)	0.4046	0.9552	1.1557	2.6367	1.2280
		S**	NS	NS	NS	S**
3.	Standard (Chloramphenicol)	17.0± 1.19				

Values are expressed in mean ± SD with three replications (n=3). Means followed by different small superscript in a Coloumn are statistically different at $p \leq 0.05$
NA – No activity, S** - Significant, NS – Non-Significant

Table 5 represented that G4 and MR2 mulberry fruit extract exhibited significant antibacterial activity against *S. epidermidis.* The highest zone of inhibition was obtained in MR2 fruit extract in the concentration of 100 µl (20.0± 0.73 mm) (Fig 4b) followed by G4 in the concentration of 100 µl (18.0± 0.22 mm) (Fig 4a). The lowest inhibition zone was recorded in the MR2 fruit extract in the concentration of 20 µl (12.0± 0.25 mm) (Fig 4b). There was no antibacterial activity in the 20 µl concentration of G4 mulberry fruit extract against *S. epidermidis* (Fig 4a). Whereas all the other concentrations (40, 60 and 80 µl) of the G4 and MR2 mulberry fruit extract does not have any significant difference between each other (Fig 4a; Fig 4b). Overall, MR2 recorded highest zone of inhibition against *S. epidermidis.* Minhas *et al.* (2016) studied the antibacterial activity of *M. nigra* fruit extract by polar and non-polar solvents resulted in the maximum inhibition recorded in polar solvents over non-polar solvents against bacterial pathogens includes *E. coli, S. aureus, S. epidermidis* and *Serratia marcescens*. Mulberry fruits were found to have significant antibacterial activity due to the presence of flavonoids, a group of phenolic compounds, are widely included in fruits and vegetables. Previous studies have demonstrated its numerous positive effects such as antimicrobial, antioxidant and anticancer (Block, 1992) [2] effects in human health. In general, these activities are associated with free radical scavenging properties of flavonoids. In addition, flavonoid compounds may affect growth and metabolism of bacteria. They could have an

activation or inhibition effect on microbial growth according to their constitution and concentration (Alberto *et al.*, 2002) [1].

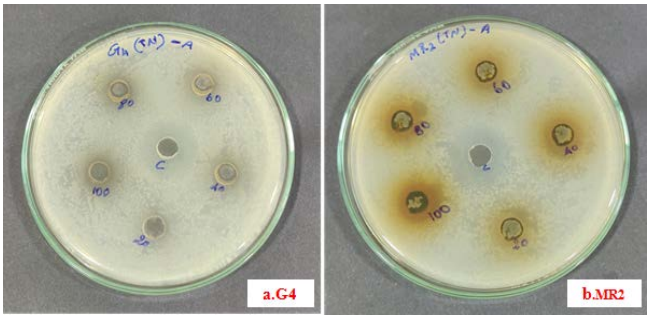

Fig 2: Inhibition of mulberry fruit extracts inhibition against *S. aureus* ATCC11632

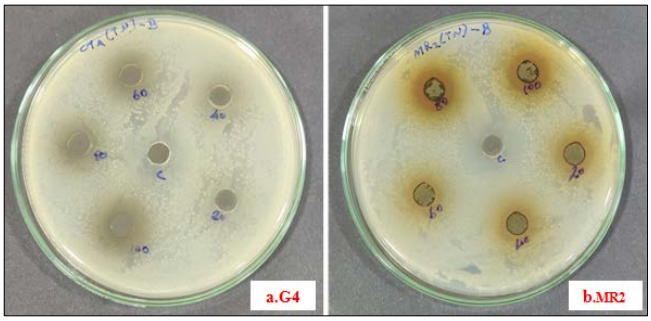

Fig 3: Inhibition of mulberry fruit extract inhibition against *S. pneumoniae* ATCC6301

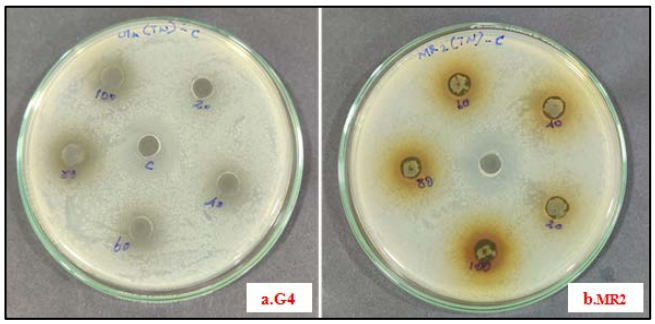

Fig 4: Inhibition of mulberry fruit extract inhibition against *S. epidermidis* ATCC12228

Conclusion

The present study characterized the morphological characters four mulberry fruit yielding varieties (G4, MR2, G2 and S36). The antibacterial activity of mulberry fruit extracts of G4 and MR2 showed highest antibacterial activity against human bacterial pathogens *Staphylococcus aureus*, *Streptococcus pneumonia* and *Staphylococcus epidermidis* due to the presence of high phenolic content, amino acids, vitamins and flavonoids. Hence the present findings indicated that there is a scope for promoting mulberry fruits for development of potent antibacterial drugs of plant origin.

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