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COMPARATIVE ANTIMICROBIAL ACTIVITIES OF *MORUS ALBA* CRUDE EXTRACT AND FRACTION AGAINST *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

The comparative antimicrobial activities of the *Morus alba* crude extract and fraction of various solvent were tested against *Staphylococcus aureus*. The water, ethanol, methanol, acetone, hexane and butanol solvent were used for the extraction and fractionalization. Antimicrobial susceptibility test showed that both the crude extract and fraction inhibited the growth of *Staphylococcus aureus* (0.5 – 3 mm, and 0.5 – 4 mm, respectively). However, the ethanol with methanol fraction highly inhibited the growth of *Staphylococcus aureus* at 18:2 combinations (8 mm zone). In GC-MS 39 bioactive phytochemical compounds were identified in the ethanolic fraction. The results of this study tend to give credence to the popular use of both crude extracts and fraction.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become an increasingly aggressive and prevalent pathogen in medicine (1). These express mecA gene to produce altered penicillin binding protein. Now-a-day, it has been increasing as a serious nosocomial and community pathogen having the property of multi drug resistant (2). Enhanced secondary infections of *Staphylococcus aureus* infections is due to bacterial dissemination from the blood into surrounding tissues and is associated with significantly increased morbidity and mortality (3). Hence, search for effective and safer antimicrobial agents has become an area of current research.

Morus alba (Linn) belongs to the family of Moraceae, its fruit and leaves are used to treat prematurely grey hair to tonify the blood and treat constipation and diabetes as well as the root bark has been used by human beings for at least 4000 years (4, 5). It have active ingredients mainly comprise of polysaccharides, alkaloids, peptides, flavonoids, polyphenols and soon (6). Hence the present study was aimed at investigating the anti-microbial activity of *Morus alba* various crude extract and active fraction against clinically isolate hazardous bacteria *Staphylococcus aureus.*

MATERIALS AND METHODS

Plant material and Extraction

Morus alba leaves were shade dried, crushed and powdered. This powder of plant material was subjected to soxhlet for extraction (48h) with absolute water, ethanol, methanol, acetone, hexane and butanol solvent and extract was concentrated to dryness in rotary vacuum evaporate under reduced pressure and stored refrigerator.

Fractionation

For fractionation, the plant crude extract was submitted to chromatography on a silica gel column (9.7 cm x 15 cm) with different concentration of water, ethanol, methanol, acetone, hexane and butanol solvent. After fractionation, pure compound were stored in an air-tight container for further use.

Micro organisms

The bacterial strain used in this test was *Staphylococcus aureus*, which was clinically obtained from clinical patients at dental clinics in and around Thanjavur and Chennai, Tamil Nadu, India.

Determination of antimicrobial activity

Culture supernatants with fractions and crude extract of the plants were used in the disc-diffusion method separately. *Staphylococcus aureus* swabbed on the surface of the sabouraud agar plates and discs (Whatman No.1 filter paper with 9 mm diameter) impregnated with the 50 µl of each plant sample was place on the surface individually. To compare the anti-bacterial activities, Nystatin (20 µg/disc) used as standard antibiotic and negative control, a blank disc impregnated with solvent followed by drying was used. The plates (triplicates) were incubated 28°C for 72 h. The antimicrobial potency of the test samples was measured by determining the diameter of the zones of inhibition in millimeter.

GC-MS analysis

30 g powdered sample of *Morus alba* were soaked and dissolved in 75 ml of methanol for 24 h. Then the filtrates were collected by evaporated under liquid nitrogen. The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm df capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised upto 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Table 1: Antimicrobial activity of individual extract and fraction of *Morus alba* tested against *Staphylococcus aureus* by disk diffusion method.

Plant sample / Solvent	Zone of inhibition (mm)					
	Water	**Ethanol**	**Methanol**	**Acetone**	**Hexane**	**Butanol**
***Morus alba* extract**	0.5	3	1	0.5	2	2
***Morus alba* fraction**	4	1	2	2	3	0.5

Table 2: Antimicrobial activity of ethanol and methanol combined fractions of *Morus alba* tested against *Staphylococcus aureus* by disk diffusion method.

Plant sample/ Fraction concentration	Zone of inhibition (mm)								
	18:2 (E/M)	**16:4 (E/M)**	**14:6 (E/M)**	**12:8 (E/M)**	**10:10 (E/M)**	**8:12 (E/M)**	**6:14 (E/M)**	**4:16 (E/M)**	**2:18 (E/M)**
Morus alba	8	5	3	2	1	0.5	6	5	2

Table 3: The main compounds identified by GC-MS in the extracts of *Morus alba*

S.No.	Peak name	Retention time	Peak Area	%Peak Area
1.	Name: 2-Propanone, 1-(acetyloxy)- Formula: $C_5H_8O_3$ MW: 116	3.83	2504329	2.5417
2.	Name: 2-Propanone, 1,1-dipropoxy- Formula: $C_9H_{18}O_3$ MW: 174	4.01	8004537	8.1241
3.	Name: Propanoic acid, 2-hydroxy-, pentyl ester Formula: $C_8H_{16}O_3$ MW: 160	4.64	1009481	1.0246
4.	Name: Ethane, 1,2-bis[(4-amino-3-furazanyl)oxy]- Formula: $C_6H_8N_6O_4$ MW: 228	4.73	133949	0.1359
5.	Name: 2-Furanmethanol Formula: $C_5H_6O_2$ MW: 98	5.17	1687941	1.7132
6.	Name: 1,2-Ethanediol, diacetate Formula: $C_6H_{10}O_4$ MW: 146	5.26	1388690	1.4094
7.	Name: Methyl N-cyclohexyl-3-phenylpropanimidate Formula: $C_{16}H_{23}NO$ MW: 245	5.38	740362	0.7514
8.	Name: Ethanamine, N-ethyl-N-[(1-methylethoxy)methyl]- Formula: $C_8H_{19}NO$ MW: 145	6.25	1601345	1.6253
9.	Name: 2-Cyclopenten-1-one, 2-hydroxy- Formula: $C_5H_6O_2$ MW: 98	6.50	2678416	2.7184
10.	Name: Furan, 2,3-dihydro-2,5-dimethyl- Formula: $C_6H_{10}O$ MW: 98	6.71	178469	0.1811
11.	Name: 2-Hydroxy-gamma-butyrolactone Formula: $C_4H_6O_3$ MW: 102	7.97	6847502	6.9498

12.	Name: 2-Furanethanol, à-methyl-, acetate Formula: $C_9H_{12}O_3$ MW: 168	8.72	294428	0.2988
13.	Name: Piperazine, 1,4-dimethyl- Formula: $C_6H_{14}N_2$ MW: 114	8.92	2492261	2.5295
14.	Name: Cyclopentane, 1-acetyl-1,2-epoxy- Formula: $C_7H_{10}O_2$ MW: 126	9.66	8063103	8.1835
15.	2-Propanamine, N-methyl-N-nitroso- Formula: $C_4H_{10}N_2O$ MW: 102	10.80	1015530	1.0307
16.	Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: $C_6H_8O_4$ MW: 144	10.89	2652473	2.6921
17.	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl- Formula: $C_6H_6O_4$ MW: 142	11.69	1011354	1.0265
18.	1,2-Benzenediol Formula: $C_6H_6O_2$ MW: 110	12.29	1680378	1.7055
19.	Name: Acetic acid, 3,4-dihydroxy-3-methyl-butyl ester Formula: $C_7H_{14}O_4$ MW: 162	13.53	1503270	1.5257
20.	2-Methoxy-4-vinylphenol Formula: $C_9H_{10}O_2$ MW: 150	13.92	653288	0.6630
21.	Methyl 4-nitrohexanoate Formula: $C_7H_{13}NO_4$ MW: 175	13.97	891276	0.9046
22.	2-Propenal, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)- Formula: $C_{12}H_{18}O$ MW: 178	14.72	377660	0.3833
23.	(+)-3-Carene, 10-(acetylmethyl)- Formula: $C_{13}H_{20}O$ MW: 192	15.11	155959	0.1583
24.	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone	15.22	1211304	1.2294

	Formula: $C_{13}H_{18}O_2$ MW: 206			
25.	2,4-Imidazolidinedione, 3-(2-hydroxyethyl)-5,5-dimethyl- Formula: $C_7H_{12}N_2O_3$ MW: 172	15.33	780753	0.7924
26.	Sucrose Formula: $C_{12}H_{22}O_{11}$ MW: 342	16.41	6831613	6.9336
27.	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one Formula: $C_{13}H_{18}O$ MW: 190	16.61	6419206	6.5151
28.	1,6-Anhydro-á-D-glucopyranose (levoglucosan) Formula: $C_6H_{10}O_5$ MW: 162	17.32	5342771	5.4226
29.	Undecanoic acid Formula: $C_{11}H_{22}O_2$ MW: 186	17.87	476535	0.4837
30.	3',5'-Dimethoxyacetophenone Formula: $C_{10}H_{12}O_3$ MW: 180	18.00	1242820	1.2614
31.	Megastigmatrienone Formula: $C_{13}H_{18}O$ MW: 190	19.00	1274930	1.2940
32.	Name: Tridecanoic acid Formula: $C_{13}H_{26}O_2$ MW: 214	20.81	1294661	1.3140
33.	1,4-Naphthalenedione, 2,3,6-trimethyl- Formula: $C_{13}H_{12}O_2$ MW: 200	21.39	1166977	1.1844
34.	3-methyl-5-(2,6-dimethylheptyl)-1,5-Pent-2-enolide Formula: $C_{15}H_{26}O_2$ MW: 238	21.50	786479	0.7982
35.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	21.69	6974061	7.0782
36.	Pentadecanoic acid, 14-methyl-, methyl ester Formula: $C_{17}H_{34}O_2$ MW: 270	23.12	632598	0.6420
37.	Phytol	27.17	10332326	10.4866

	Formula: $C_{20}H_{40}O$ MW: 296			
38.	Methyl 2-O-benzyl-d-arabinofuranoside Formula: $C_{13}H_{18}O_5$ MW: 254	30.76	1964470	1.9938
39.	Squalene Formula: $C_{30}H_{50}$ MW: 410	39.24	4230941	4.2941

RESULT AND DISCUSSION

Morus alba water, ethanol, methanol, acetone, hexane and butanol crude extract and fraction individually showed normal anti-bacterial activity. The average zone of 0.5, 3, 1, 0.5, 2, 2 mm of extracts inhibitions and 2, 5, 3, 2, 1, 0.5 mm inhibitions of fractions were analyzed (Table 1). The observed active fraction of ethanol and methanol were further combining various concentrations and over again treated with novel *Staphylococcus aureus* strain (Table 2). The 18:2 combinations of ethanol with methanol fraction showed the highest activity against the growth of *Staphylococcus aureus* having the zone of inhibition of 8 mm. Besides this, the fraction showed good activity against the growth of *Staphylococcus aureus* than the all individual fraction as well as crude extracts.

In addition, GC-MS analysis, totally 39 compounds identified from the methanol fractions of the *Morus alba* are presented in Table 3. The plant samples revealed the synthesis of 2-Propanone, 1-(acetyloxy)-; 2-Propanone, 1,1-dipropoxy-; Propanoic acid, 2-hydroxy-, pentyl ester; Ethane, 1,2-bis[(4-amino-3-furazanyl)oxy]-; 2-Furanmethanol; 1,2-Ethanediol, diacetate; Methyl N-cyclohexyl-3-phenylpropanimidate; Ethanamine, N-ethyl-N-[(1-ethylethoxy)methyl]-; 2-Cyclopenten-1-one, 2-hydroxy-; Furan, 2,3-dihydro-2,5-dimethyl-; 2-Hydroxy-gamma-butyrolactone; 2-Furanethanol, à-methyl-, acetate; Piperazine, 1,4-dimethyl-; Cyclopentane, 1-acetyl-1,2-epoxy-;2-Propanamine, N-methyl-N-nitroso-; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-;4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-;1,2-Benzenediol; Acetic acid, 3,4-dihydroxy-3-methyl-butyl ester;2-Methoxy-4-vinylphenol;Methyl 4-nitrohexanoate;2-Propenal, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-;(+)-3-Carene, 10-(acetylmethyl)-;1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone;62,4-Imidazolidinedione,3-(2hydroxyethyl)-5,5-dimethyl-;Sucrose;4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one; 1,6-Anhydro-á-D-glucopyranose

(levoglucosan). All these compounds are of pharmacological importance as they possess the properties such as analgesic, anti-diabetic, antibacterial, and antifungal. In conclusion, our investigation clearly indicate that the anti-bacterial activity vary with the plants extract and plant fraction used. Further research is necessary to establish the pharmacological mechanism of compound and toxicological profile is ongoing.

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