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Antimicrobial activities and GC-MS chemical profiling of methanol extracts of different parts of *Morus alba* (Tut) in Bangladesh

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Key words: *Morus alba*, GC-MS study, antimicrobial activity, anti-fungal activity, Methanollic extracts.

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Abstract

Morus alba has potential therapeutic value in different medicinal parts. We investigated the chemical differences presence in the methanolic extract (ME) of leaves, stem and root *M. alba* using GC-MS technique. GC-MS based phyto-chemical profiling revealed the presence of potent bioactive components in methanolic extracts of different parts (leaves, stem and root) of *M. alba*. Further, GC-MS study confirmed the occurrence of twenty to thirty bioactive constituents ranging from 0.06 to 32% based on their peak area. Among different methanolic extract of *M. alba* maximum number of phytocompounds were observed in leaves extract with glycerin (32.77%) as predominant compound and lowest in root bark (SBP) extract which is dominated hexadecanoic acid methyl ester (30.55%) respectively. Furthermore, all the study extracts were also screened for their antimicrobial activity and only stem bark extract (SBP) were found to active against both gram positive and gram negative bacteria but all are inactive against fungal strain.

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Introduction

Morus alba (*M. alba*) is a short lived, fast growing, broad leaved plant belongs to the family Moraceae known as white mulberry native to Indo-China and is widely distributed in the lower sub-Himalayan region. *M. alba* (locally known as tut in Bangladesh) is mainly cultivated in the northern zone of Bangladesh, particularly in Rajshahi, where Bangladesh Sericulture Research and Training Institute has been established. The genus Morus comprises of about 68 recognized species (Datta 2000), of which *M. alba* is widely grown for leaves to feed the caterpilars of silkworms (Vijayan *et al.* 2011). The main source of food for silkworm (Bombyx mori) is the leaf constitutes of *M. alba*. Silkworms take leaves of *M. alba* as a source of proteins to synthesize the two silk proteins, fribroin and sericin (Mahboubi 2019). *M. alba* flowers are small, sessile or shortly pedicellate, regular and cross-pollinated mainly by wind. Leaves are simple, alternate, entire, toothed, stipulate and petiolate. Mulberry plants are propagated by vegetative means. Locally cutting method of propagation is popular and is widely used but is restricted in a single season and usually done just prior to winter. In Bangladesh Mulberry is not only associated with sericulture, but is also useful as fodder, timber, and medicine (Vijayan *et al.* 2011).The seeds contain 25-30% oil (Md. Munsur Rahman 2014). Being a heavy leaf producing plant, it is also used as a shade tree (Perelman *et al.* 2010). Root, stem barks and leaves of mulberry have long been used in traditional medicine to treat fever, inflammation, hepatitis, cancer, diabetes, dislipidemia, diarrhoea, dyspepsia, hypertension, anthelmintic and to protect the liver, improve eyesight, strengthen joints, facilitate discharge of urine and lower blood pressure (Wang *et al.* 2012). Different parts of the mulberry have been extensively investigated for their various health benefits, including antioxidative, antibacterial, antiviral, and anti-inflammatory effects (Avci 2016). The plant is reported to contain the main active principles phytoconstituents like; tannins, phytosterols, sitosterols, saponins, triterpenes, flavanoids, benzofuran derivatives, morusimic acid, anthocyanins, anthroquinones, glycosides and oleanolic acid (Devi *et al.* 2013). Some phenolic compounds (flavonoids, stilbenes and 2-arylbenzofurans) have been reported from *M. Alba* and have been known to show antimicrobial action (Salem *et al.* 2013). In recent decades, many researchers have intensively investigated antimicrobial properties of plant extracts and natural products as the demand for safe and new pharmaceuticals which has increased due to the misuse of antibiotics and an increase in immuno-deficiency (Wang *et al.* 2012). On the other hand, food-borne diseases caused by microorganisms are major dilemma in the third world and developing countries and even in developed nations. The Consumption of foods contaminated with some microorganisms represents a serious health risk to humans. The subsistence and growth of microorganisms in foods may lead to spoilage, formation of toxins and quality deterioration of food products. Because of the resistance that pathogenic build against antibiotics, there is a great interest in the search for new antimicrobial drugs also from nature (Hajji *et al.* 2010). In recent years, numerous studies have been published on the antimicrobial activities of plant extracts against different types of microorganisms, including food-borne pathogens. J.H Kim *et al.* established a method to determine 12 marker compounds in four medicinal parts of *M. alba* and found that the marker compounds content varied from different medicinal parts (Kim, Doh, and Lee 2020). Some of these studies claim that the phenolic compounds present in plant extracts might also pay a major role in their antimicrobial effects. The aims of this study was to evaluate the chemical composition of methanol extracts of leaves, stem and root bark of *M. alba* by GC-MS as well as the in-vitro antimicrobial activities.

Material and methods

Chemicals

Methanol was purchased from Sigma Chemical Co. USA. All other chemicals and other solvents were of analytical grade. All chemicals were used without further purification.

Plant materials

Fresh plants with roots, stem and leaves of *M. alba* were collected from Rajshahi, Bangladesh. Stem and root barks were peeled and washed along with leaves using distilled water. The raw materials were then grounded and dried separately at 50°C for at least 24hrs to obtain leaf powder (LP), stem barks powder (SBP) and root barks powder (RBP). A fine powder was obtained by further grounding and then stored separately in glass bottles at room temperature.

Preparation of methanol extracts of LP, SBP and RBP

The dried fine powder of LP (50g) was extracted with methanol by sonication. The extract was filtered through Whatman No. 1 filter paper in a Buchner funnel. The filtrate was evaporated to dryness under reduced pressure in a rotatory vacuum evaporator. The remaining residues were successively extracted with methanol under the same conditions. The dried extracts were kept in dark at 4°C until further analysis. Methanol extracts of SBP and RBP were also obtained by following the same procedure.

GC-MS analysis

GC Ms analysis of the respective plant extract was carried out by GCMS-QP2020. The chromatography method was carried out by capillary column that is characterized by 30m in length and 0.25 mm in inner diameter. The column is prepared by 5% diphenyl and 95% dimethyl poly-siloxane. Injection temperature was set at 220°C. Initially the oven temperature was set at 80°C and it remained isothermal for 2 minutes, then programmed to 150°C at the rate of 5°C/min and finally held at 280°C. The ion source temperature was 280°C. Helium was used as a carrier gas with the flow rate of 1.72ml/min. 4 micro-liter of the sample was injected in the split-less mode in the ratio of 1:100. For GC-MS detection, the ionizing energy gained by the detector was 45 m/z. A scan interval of 0.30 second. Total GC running time was 50 minutes. Interpretation of mass spectrum of GC-MS was done by using database of National Institute Standard and Technology (NIST). The mass spectrum of unknown component was compared with the spectrum of the known component stored in the NIST library 2008 and 2014 edition.

Antimicrobial activity

Antimicrobial susceptibility pattern of the study compounds was measured in vitro by employing the modified Kirby-Bayer method (Bayer *et al.* 1966). It is frequently used to determine the drug sensitivity of microorganisms isolated from infectious process and to interpret their disease potential. This method allows for the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that results from diffusion of the agent into the medium surrounding the disc. Commercially available antimicrobial discs (ciprofloxacine) were used as positive control for bacteria whereas amphotericin B was used as control for fungus.

A single colony of respective culture of the organisms was inoculated in Mueller-Hinton broth (for bacterial culture) and PDB (for fungal culture) to match the equivalent turbidity standard to that of McFarland 0.5 Standard. A sterile cotton swab was dipped into the suspension of these culture and the medium of choice was Mueller-Hinton agar for bacteria and PDA for fungus, with a pH of 7.3, which was poured into plates to a uniform depth of 5mm.

The swab (inoculums) was then heavily inoculated over the entire surface of the plate to obtain a confluent growth of the organism. Antibiotic control discs and the discs impregnated with study compounds (1, 2, and 3) were applied aseptically to the surface of the inoculated plates and at an appropriate special arrangement with the help of a sterile forceps. The plates were then inverted and incubated at 37˚C for 24h for bacterial growth and 28˚C for 48h for fungal growth.

Results

GC-MS analysis of the methanol extracts

The methanol extracts from LP, SBP and RBP were analyzed by GC-MS and summarized in Table 1- 3 and their GC-MS chromatograph in Figure 1-3 respectively.

Table 1. Suggested chemical compounds present in methanol extracts of LP from *M. alba*.

ID#	Compound Name	R.T	m/z	Area	Conc. (%)
1	Ethanol, 2,2'-oxybis-	3.538	45.00	99404	5.113
2	Hexanoic acid	3.675	60.00	1178	0.061
3	Glycerin	3.698	61.00	636944	32.76
4	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-o	3.815	101.00	3586	0.184
5	Oxirane, [(2-propenyloxy)methyl]-	3.950	57.00	238997	12.29
	Thymine	5.488	126.00	67241	3.459
7	1,4-Dioxaspiro[2.4]heptan-5-one	6.529	56.00	19587	1.008
8	Ethanamine, N-ethyl-N-nitroso-	6.724	102.00	27447	1.412
9	2(R),3(S)-1,2,3,4-Butanetetrol	6.886	61.00	90704	4.666
10	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-	7.013	144.00	34311	1.765
11	Butanedioic acid, dimethyl ester	7.481	115.00	10569	0.544
12	(S)-5-Hydroxymethyl-2[5H]-furanone	7.688	84.00	138762	7.138
13	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	9.179	67.00	10519	0.541
14	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclop	13.446	191.00	6961	0.358
15	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,	16.840	111.00	25496	1.312
16	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahyd	25.250	111.00	10067	0.518
17	9-Eicosene, (E)-	26.295	55.00	4192	0.216
18	2-Pentadecanone, 6,10,14-trimethyl-	27.663	58.00	47814	2.460
19	1,2-Benzenedicarboxylic acid, bis(2-methylp	28.072	149.00	247539	12.73
20	Hexadecanoic acid, methyl ester	29.889	74.00	29167	1.500
21	9,12-Octadecadienoic acid, methyl ester	33.661	67.00	3075	0.158
22	9-Octadecenoic acid (Z)-, methyl ester	33.814	55.00	10277	0.529
23	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	34.021	71.00	28781	1.481
24	Methyl stearate	34.396	74.00	6158	0.317
25	Undec-10-ynoic acid, tetradecyl ester	36.915	57.00	3424	0.176
26	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-e	41.335	57.00	6041	0.311
27	1,2-Benzenedicarboxylic acid, diisooctyl este	41.670	149.00	67664	3.481
28	Hexacosane	44.355	57.00	15796	0.813
29	9-Octadecenamide	45.501	59.00	44603	2.294
30	Pentatriacontane	47.281	57.00	8875	0.457

The relative retention times (Rt) and mass spectra of the extract components were compared with those of authentic samples and with mass spectra from a data library. GC-MS based phyto-chemical profiling revealed the presence of potent bioactive components in methanolic extracts of different parts (leaves, stem and root) of *M. alba*. Occurrence of twenty to thirty bioactive constituents ranging from 0.06 to 32% based on their peak area was observed in the methanolic extract of *M. alba*. Among different methanolic extract of *M. alba* maximum number of phytocompounds were observed in leaves extract with glycerin (32.77%) as predominant compound and lowest in root bark (SBP) extract which is dominated hexadecanoic acid methyl ester (30.55%) respectively. GC-MS analysis of the LP methanol extract resulted in the identification of 30 chemical compounds (Table-1, Figure-1) representing the highest yield (32.77%) of glycerin depending on relative area whereas, in stem and root bark about 20 and 25 bioactive compounds having hexadecanoic acid methyl ester was found to be maximum. Some bioactive compound such as 1,2-Benzenedicarboxylic acid, bis(2-methylp (12.73%), Oxirane, [(2-propenyloxy)methyl]- (12.29%), (S)-5-Hydroxymethyl-2[5H]-furanone (7.14%), Ethanol, 2,2'-oxybis- (5.11%) respectively were also observed with relatively low concentration.

Table 2. Suggested chemical compounds present in methanol extracts of SBP from *M. alba*.

ID#	Name	R.T	m/z	Area	Conc. (%)
1	2,5-Cyclooctadien-1-ol	3.220	55.00	1833	0.128
2	2,3-Anhydro-d-mannosan	3.273	45.00	5703	0.396
3	2-Heptenal, (E)-	3.358	55.00	5579	0.389
4	Ethanol, 2,2'-oxybis-	3.427	45.00	69943	4.878
5	Glycerin	3.481	61.00	243555	16.985
6	Methyl 4-oxo-2-pentenoate	3.586	113.00	4064	0.283
7	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-o	3.691	101.00	1639	0.114
8	Oxirane, [(2-propenyloxy)methyl]-	3.820	57.00	56486	3.939
9	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-	6.931	144.00	7034	0.491
10	Phenol, 2,4-bis(1,1-dimethylethyl)-	16.27	191.00	15155	1.057
11	1,2-Benzenedicarboxylic acid, bis(2-methylp	28.06	149.00	170228	11.871
12	13-Tetradecenal	28.87	55.00	7599	0.530
13	9-Hexadecenoic acid, methyl ester, (Z)-	29.29	55.00	16724	1.166
14	Hexadecanoic acid, methyl ester	29.88	74.00	438752	30.598
15	9,12-Octadecadienoic acid, methyl ester	33.65	67.00	39731	2.771
16	11-Octadecenoic acid, methyl ester	33.81	55.00	172394	12.022
17	Methyl stearate	34.39	74.00	21424	1.494
18	4,8,12,16-Tetramethylheptadecan-4-olide	38.54	99.00	7970	0.556
19	Diisooctyl phthalate	41.67	149.00	43665	3.045
20	13-Docosenamide, (Z)-	45.49	59.00	110170	7.683

On the other hand, GC-MS analysis of methanol extracts of SBP resulted in the identification of 20 compounds (Table-2, Figure-2). The extract was predominated by hexadecanoic acid methyl ester (30.60%) and other compounds including glycerin (16.99%), 1,2-Benzenedicarboxylic acid, bis(2-methylp (11.87%), 11-Octadecenoic acid methyl ester (12.02%), 13-Docosenamide, (Z)-(7.68%) etc.

Table 3. Suggested chemical compounds present in methanol extracts of SBP from *M. alba*.

ID#	Name	R.T	m/z	Area	Conc. (%)
1	Glycerin	3.538	61.00	290157	15.95
2	Thymine	5.439	126.00	42740	2.796
3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-	6.968	144.00	23413	1.532
4	2-Pentanol, 3-ethyl-2-methyl-	8.754	59.00	11714	0.766
5	Dodecanoic acid, methyl ester	16.72	74.00	117229	6.523
6	Benzoic acid, 2,4-dihydroxy-, methyl ester	17.503	136.00	27226	1.781
7	Methyl tetradecanoate	24.057	74.00	112727	6.264
8	1,2-Benzenedicarboxylic acid, bis(2-methylp	28.084	149.00	21134	1.383
9	9-Hexadecenoic acid, methyl ester, (Z)-	29.319	55.00	19409	1.270
10	Hexadecanoic acid, methyl ester	29.909	74.00	556019	30.55
11	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13	30.100	73.00	15631	1.023
12	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-t	33.326	73.00	15104	0.988
13	9,12-Octadecadienoic acid, methyl ester	33.672	67.00	78246	5.120
14	9-Octadecenoic acid (Z)-, methyl ester	33.831	55.00	211303	11.71
15	11-Octadecenoic acid, methyl ester	33.950	55.00	12265	0.802
16	Methyl stearate	34.409	74.00	111549	6.187
17	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-t	36.078	73.00	18890	1.236
18	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(t	38.542	73.00	16406	1.073
19	Heptasiloxane, hexadecamethyl-	40.821	73.00	14299	0.936
20	Di-n-octyl phthalate	41.683	149.00	12050	0.788
21	Heptasiloxane, hexadecamethyl-	42.946	73.00	11317	0.740
22	9-Octadecenamide	45.510	55.00	7029	0.460
23	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15	46.028	69.00	13506	0.884
24	24-Norursa-3,12-diene	47.923	218.00	16283	1.065
25	3,4-Dihydro-3,5,8-trimethyl-3-(4,8,12-trimet	49.397	151.00	21619	1.415

The suggested chemical compounds found in methanol extracts of RBP had 25 compounds (Figure 3, Table 3) and highest yield showed by hexadecanoic acid methyl ester(30.55%) and it was also found that there was some compounds in lower concentration including Glycerine (15.96%), 9-Octadecenoic acid (Z)- methyl ester (11.72%), Dodecanoic acid methyl ester (6.52%), Methyl tetradecanoate (6.26%) etc.

Table 4. Antibiotic susceptibility pattern of study compounds.

Culture Type		Zone of inhibition of study compound (mm)			Control (CIP-1µg for bacteria), Amphotericine B-25mg/kg for fungus) (mm)
		LP	SBP	RBP	
Gram positive bacteria	*Listeria spp*	0	25	0	26
	Bacillus spp	0	16	8	20
Gram negative bacteria	*Salmonella spp*	0	12	0	30
	Pseudomonas spp	0	0	0	23
Fungus	*Trichoderma spp*	0	0	0	20

High level of glycerin was found in LP (32.77%) comparing with SBP and RBP. 2-Benzenedicarboxylic acid, bis (2-methylp was found in both LP and SBP but high content found in LP (12.73%). In the contrary, methyl ester was found in both SBP and RBP but maximum level found in SBP (12.02%).

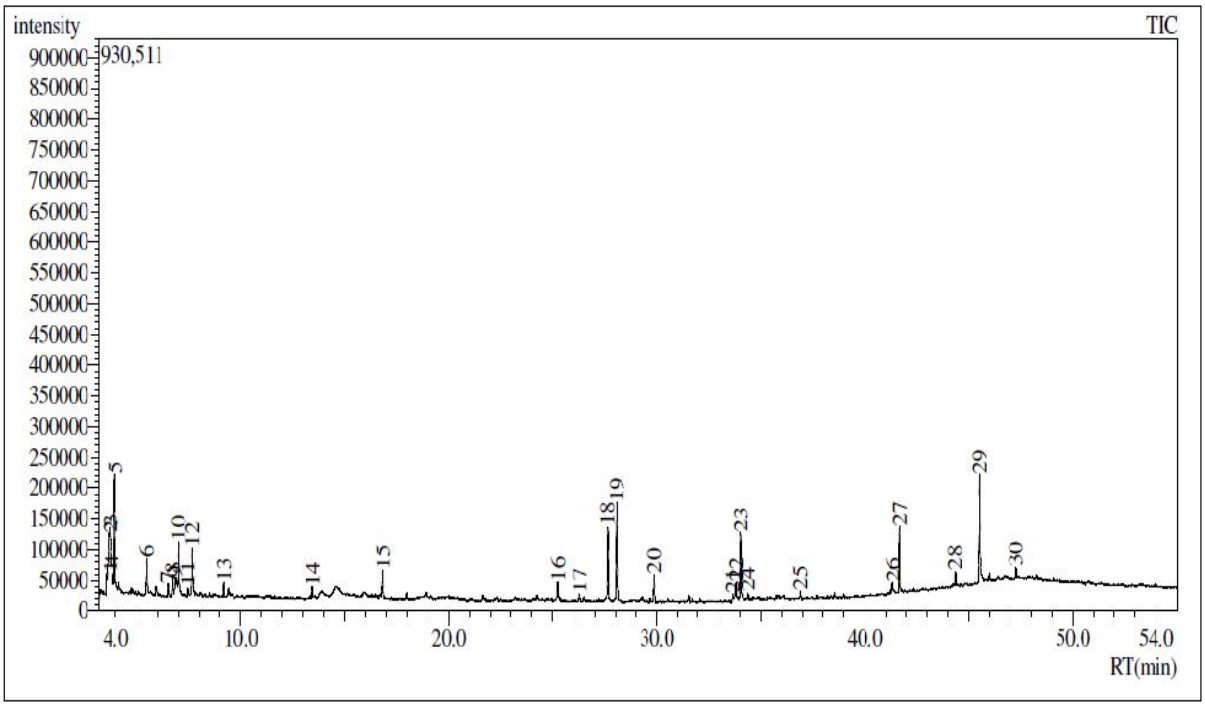

Fig. 1. GC-MS chromatogram of compounds presents in methanol extracts of LP from *M. alba*.

Antimicrobial activity

The study compounds were tested for evaluation of their antibacterial and antifungal activities against both Gram positive and Gram negative bacteria along with fungus according to Clinical and Laboratory Standard Institute (CLSI) guidelines (Figure 4). *Listeria spp*. and *Bacillus spp*. were used as Gram positive bacteria whereas *Salmonella spp*. and *Pseudomonas spp*. were used as Gram negative bacteria and ciprofloxacine was used as control. Moreover, *Trichoderma spp* was used as fungus for evaluation of antifungal activity where amphotericin B was used as control. Among the study extracts only stem bark extract (SBP) were found to active against both gram positive and gram negative bacteria with zone of inhibition from 12 to 25 mm although root

bark extract showed activity against *Bacillus spp*. with 8 mm zone of inhibition but all are inactive against fungal strain (Table 4).

Discussion

GC-MS analysis is a preliminary technique to suggest the presence of compounds in any sample according to the respective library. In our present study GC-MS analysis was carried out to reveal the methanolic extract of the three parts i.e. leaves, root bark and stem bark of the plant of *M. alba*. According to our present study 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-o, 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4, are identically present in the leaf extract. 4,8,12,16-Tetramethylheptadecan-4-olide, Diisooctyl phthalate, 13-Docosenamide, (Z)- are the three suggested compounds that are present in the stem bark of the plant rather than the methanolic extract of root and leave. Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13; 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-t; 24-Norursa-3,12-diene are suggested to be present in the only stem bark extract.

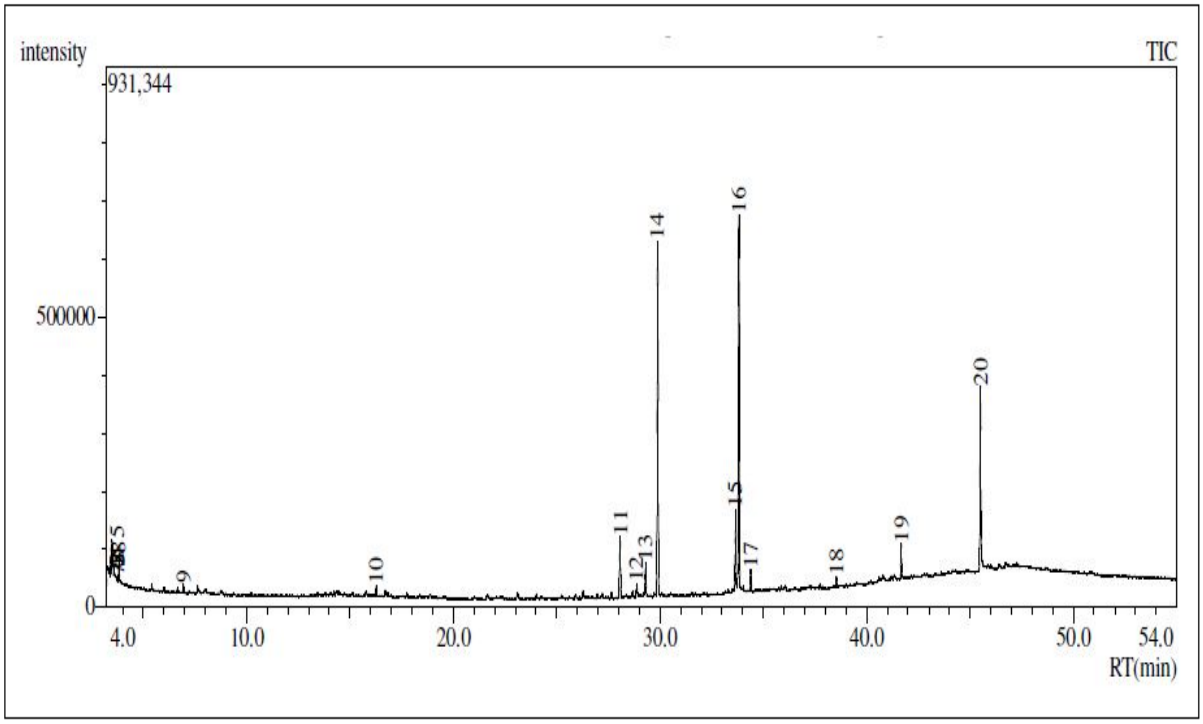

Fig. 2. GC-MS chromatogram of compounds presents in methanol extracts of SBP from *M. alba*.

The ethanolic extract of bark of *Ficus religinosa Linn*, which is also a member of Moraceae family is suggested to contain phenol,4-methoxy phenol, 2-methoxy phenol that have antimicrobial activity (Saravanan *et al.* 2014) Similar compound Phenol, 2,4-bis(1,1-dimethylethyl)- is suggested in our study of GC-MS of stem bark extract. Phenol, 2,4-bis(1,1-dimethylethyl)- is a naturally synthesized compound within both plant and bacterial cell that impart antioxidant (Choi and Lee 2009), antibacterial (Abdullah *et al.* 2011) and antifungal activity (Zhou *et al.* 2011) also. Asiye Aslı Emniyet *et al* reported that the leaves of Moras alba contain 9,12,15-octadecatrienoic acid, ethyl ester (Avci 2016). In our study stem bark extract contain hexadecanoic acid, methyl ester; 11-Octadecanoic acid, methyl ester, Diisooctyl phthalate, 13- Docosenamide prominently. hexadecanoic acid, methyl ester possesses antioxidant and anti-fungal property (Pinto *et al.* 2017). Rahman M.M *et al* reported that 11-octadecenoic acid, methyl ester is responsible for antioxidant and antimicrobial activity(M M Rahman *et al.* 2014). GC-MS analysis of *Clerodendrum phlomidis* revealed the presence of 13-Docosonamide that impart antioxidant activity (Kumaradevan *et al.* 2015). 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester was reported to possess antibacterial property (Joshi *et al.* 2011).

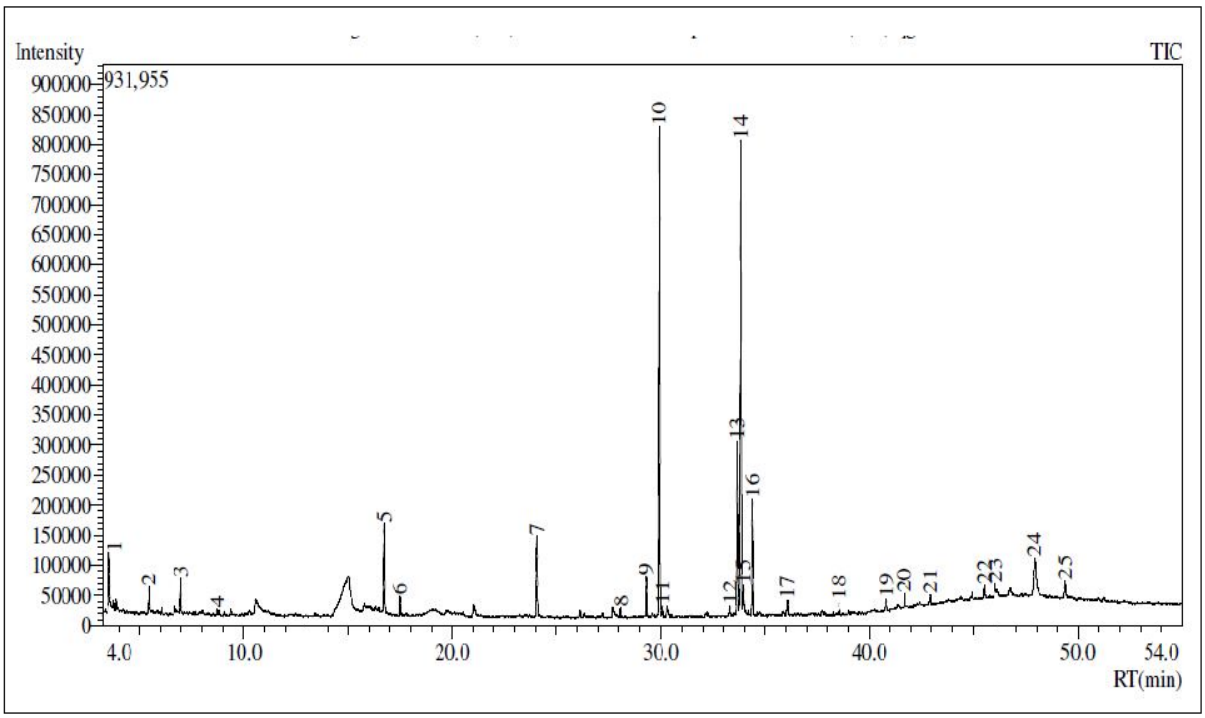

Fig. 3. GC-MS chromatogram of compounds presents in methanol extracts of RBP from *M. alba*.

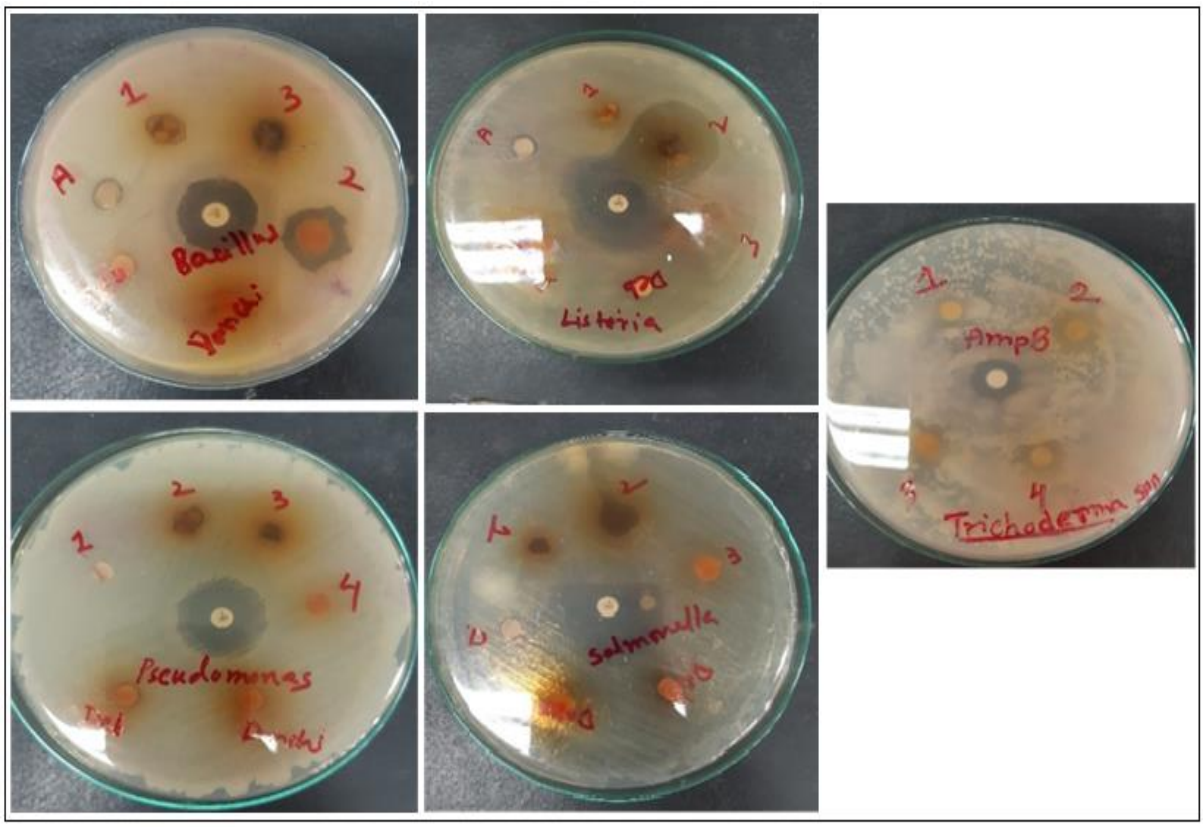

Fig. 4. Antimicrobial activities of the compound against Gram positive (*Listeria spp*. and *Bacillus spp*) and Gram negative bacteria (*Salmonella spp*. and *Pseudomonas spp*) along with fungus (*Trichoderma spp*).

Our study demonstrated that the methanol extract of stem bark of *Morus alba* is able to inhibit Gram positive bacterial growth at an extent of similar to the positive control, but we did not conduct the study in a dose dependent manner. Although GC-MS analysis of stem bark extract revealed the presence of some bioactive compounds that have been reported as antifungal activity earlier, the respective extract did

not show any antifungal activity in our study. This may due to the responsible compounds are in too low concentration to impart the bioactivity. Our study carried out using GC-MS and didn't find any match compound comparing with J.H Kim *et al.* identified marker compounds in different medicinal parts of *M. alba* using high performance liquid chromatography-diode array detector with chemometric analysis (Kim, Doh, and Lee 2020). As the identified compounds are plant metabolites, the content of them can be vary with species, climate and geography of the cultivated region and the age of the collected plant also.

Conclusion

GCMS analysis and antimicrobial study of the methanolic extract of *Morus alba* revealed that the stem bark extract contains a vast array of bioactive constituents that may contribute to various pharmacological bioactivity. Although various compounds including free fatty acid ester, sterol, terpenoids are identified in the three samples, only stem bark extract showed potential antibacterial activity. The identified compounds in the respective plant extract were reported earlier to have antioxidant, antimicrobial, antifungal, antiproliferative, hemolytic activity and so on.

The inhibition of bacterial growth by the extract in our study support the property of the identified compounds present in other plants. We can conclude that *Morus alba* is a potential and promising natural resource in the Indian subcontinent containing bioactive constituents that support the traditional use of the plant in the field of ethno pharmacology. The work is in progress to ascertain its biological efficacy so that we can pave a way to remark our medicinal plants as source of therapeutically valued metabolites against various diseases.

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Competing interests

Authors have declared that no competing interests exist.

Authors' Contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

References

Abdullah, Al-shwyeh Hussah, Mohamed Elwathig, Saeed Mirghani, Parveen Jamal. 2011. "Antibacterial Activity of Malaysian Mango Kernel" **10(81),** 18739–48. https://doi.org/10.5897/AJB11.2746.

Avci, Emre. 2016. "Antioxidant and Antimicrobial Activities with GC/MS Analysis of the *Morus alba* L. Leaves," no. March. http://dx.doi.org/10.17350/HJSE19030000006

Bayer AW, Kirby WMM, Sherris JC, Turck M. 1966. "Antibiotic Susceptibility Testing by a Standardized Single Disc Method." Americal Journal of Clinical Pathology **45(4),** 493–96.

Choi Youngmin, Junsoo Lee. 2009. "Antioxidant and Antiproliferative Properties of a Tocotrienol-Rich Fraction from Grape Seeds." Food Chemistry **114(4),** 1386—1390. https://doi.org/10.1016/j.foodchem.2008.11.018.

Datta RK. 2000. "Mulberry Cultivation and Utilization in India." FAO Animal Production and Health Paper, 45–62.

Devi, Bandna, Neha Sharma, Dinesh Kumar, Kamal Jeet. 2013. "Morus Alba Linn: A Phytopharmacological Review." International Journal of Pharmacy and Pharmaceutical Sciences **5(2),** 14–18.

Hajji, Mohamed, Raoudha Jarraya, Imen Lassoued, Ons Masmoudi, Mohamed Damak, Moncef Nasri. 2010. "GC/MS and LC/MS Analysis,

and Antioxidant and Antimicrobial Activities of Various Solvent Extracts from Mirabilis Jalapa Tubers." Process Biochemistry **45(9),** 1486–93. https://doi.org/10.1016/j.procbio.2010.05.027.

Joshi, Shivani, Devendra Mishra, Ganga Bisht, Khadak Singh Khetwal. 2011. "Essential Oil Composition and Antimicrobial Activity of *Lobelia Pyramidalis* Wall." EXCLI Journal **10,** 274–79.

Kim, Jung-Hoon, Eui-Jeong Doh, Guemsan Lee. 2020. "Quantitative Comparison of the Marker Compounds in Different Medicinal Parts of Morus Alba L. Using High-Performance Liquid Chromatography-Diode Array Detector with Chemometric Analysis." Molecules **25(23),** 5592.

Kumaradevan G, Damodaran R, Mani P, Dineshkumar G, Jayaseelan T. 2015. "Phytochemical Screening and GC-MS Analysis of Bioactive Components of Ethanol Leaves Extract Of *Clerodendrum Phlomidis* (L.)". American Journal of Biological and Pharmaceutical Research **2,** 142–48.

Mahboubi, Mohaddese. 2019. "Morus Alba (Mulberry), a Natural Potent Compound in Management of Obesity." Pharmacological Research **146,** 104341. https://doi.org/10.1016/j.phrs.2019.104341.

Perelman, Patricia, Ana Faggi, Maria Castro, Eduardo Martinez Carretero. 2010. "Pollution Trends Using Bark of Morus Alba in the Cities of Buenos Aires and Mendoza (Argentina)." Revista Árvore **34(3),** 505–11. https://doi.org/10.1590/s01006762201000030014.

Pinto Maria EA, Sthéfane Araújo G, Marcela Morais I, Nívea Sá P, Caroline Lima M, Carlos Rosa A, Ezequias Siqueira P, Susana Johann, Luciana Lima ARS. 2017. "Antifungal and Antioxidant Activity of Fatty Acid Methyl Esters from Vegetable Oils." Anais Da Academia Brasileira de Ciencias **89(3),** 1671–81. https://doi.org/10.1590/0001-3765201720160908.

Rahman MM, Ahmad SH, Mohamed MTM, Ab Rahman MZ. 2014. "Antimicrobial Compounds from Leaf Extracts of *Jatropha Curcas*, *Psidium Guajava*, and *Andrographis Paniculata*." Edited by Prasat Kittakoop. The Scientific World Journal 635240. https://doi.org/10.1155/2014/635240.

Rahman Md. Munsur. 2014. "Investigation Some Physicochemical Properties, Lipids, Glycerides and Fatty Acid Composition of Mulberry (*Morus Alba* L) Seed Oil of Three Different Regions of Bangladesh." American Journal of Applied Chemistry **2(3),** 38. https://doi.org/10.11648/j.ajac.20140203.11.

Salem, Mohamed Zidan Mohamed, Hussein Aly, Yousry Gohar, Abdel-Wahab El-Sayed. 2013. "Biological Activity of Extracts from *Morus Alba* L., *Albizzia Lebbeck* (L.) Benth. and *Casuarina Glauca* Sieber Against the Growth of Some Pathogenic Bacteria." International Journal of Agricultural and Food Research **2(1),** 9–22. https://doi.org/10.24102/ijafr.v2i1.122.

Saravanan P, Chandramohan G, Mariajancyrani J, Shanmugasundaram P. 2014. "GC-MS Analysis of Phytochemical Constituents in Ethanolic Bark Extract of Ficus Religiosa Linn." International Journal of Pharmacy and Pharmaceutical Sciences 6 (January), 457–60.

Vijayan, Kunjupillai, A Tikader, Zhao Weiguo, Chirakkara Nair, Chi-Hua Tsou. 2011. "Morus," June. https://doi.org/10.1007/978-3-642-20447-0_5.

Wang, Wei, Yuangang Zu, Yujie FU, Thomas Efferth. 2012. "In Vitro Antioxidant and Antimicrobial Activity of Extracts from Morus Alba L. Leaves, Stems and Fruits." American Journal of Chinese Medicine **40(2),** 349–56. https://doi.org/10.1142/S0192415X12500279.

Zhou BL, Chen ZX, Du L, Xie YH, Zhang Q, Ye XL. 2011. "Allelopathy of Root Exudates from

Different Resistant Eggplants to Verticillium Dahliae and the Identification of Allelochemicals" **10(42),** 8284–90. https://doi.org/10.5897/AJB10.2300.