Research Paper

Comparative Analysis of Natural Bioactive Metabolites of the Indigenous Host Plants of Muga Silkworm for Antioxidant and Antibacterial Properties against Swine Diseases

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The present study explores the natural bioactive metabolites, antioxidant and antibacterial activities present in the host plants of *Antheraea assamensis* (muga silkworm) *viz. Machilus bombycina* (som), *Litsea polyantha* (sualu), *Litsea salicifolia* (dighloti) and *Litsea citrata* (mejankari). The methanolic extracts of leaves of these plants were screened for their phytochemical analysis and free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl as the free radical. Phytochemical analysis revealed the presence of flavonoids, phenolic compounds, carotenoids, saponins, coumarins, terpenoids, tanins, cardiac glucosides and xanthoproteins in the said plants. The results of 2,2-diphenyl-1-picrylhydrazyl scavenging activity revealed *Litsea polyantha* to exhibit stronger antioxidant efficiency compared to the rest. Besides these, the plant extracts were investigated for their antibacterial assessment against Gram-positive *Staphylococcus aureus* and *Streptococcus suis* and Gram-negative *Pasteurella multocida* and *Escherichia coli* bacteria found in pig. All the plant extracts were validated to possess antibacterial activity with variable potency. *Litsea citrata* was the most effective extract retarding microbial growth of both Gram-positive and Gram-negative bacteria, followed by *Litsea salicifolia*, *Litsea polyantha* and *Machilus bombycina*. The present findings underscore the fact that indigenous food plants of muga silkworm are fortified with antioxidant and antibacterial efficiency, which might pave to the development of novel herbal pharmacological compounds for the medication of pig diseases.

Key words: Antibacterial property, 2,2-diphenyl-1-picrylhydrazyl, *Litsea citrata*, *Litsea polyantha*, *Litsea salicifolia*, *Machilius bombycina*, phytochemicals

Concerns involving food safety and food control are mounting in today's market place over the globe. The threat posed by drug-resistant pathogens has resulted in the increasing momentum in research and development of effective alternative medications. The primary cause behind refers to many commonly used antibiotics that have become less and less proficient against certain illnesses due to their frequent use ultimately forcing for the emergence of drug-resistant bacteria. Even after repeated efforts from pharmaceuticals to produce many new antibacterials over the years, resistance to these drugs has become a global menace[1]. The emergence and spread of multidrug-resistant bacterial pathogens have substantially endangered the current antibacterial prophylaxis[2]. Such sort of antimicrobial resistance is becoming a fatal cause of mortality in humans and other livestock. In India, pig husbandry is very much predominant and northeast India is hailed as one of the major hotspots of pig domestication. This ground for pork as an important component of the food basket for consumers in Eastern and Northeastern states of India. *Streptococcus suis* (*S. suis*), *Staphylococcus aureus* (*S. aureus*), *Pasteurella multocida* (*P. multocida*) and *Escherichia coli* (*E. coli*) are some of the major pathogens affecting porcine animals, triggering economic loss in the pig industry. The exploitation of antibiotics in the treatment of bacterial diseases in swine causes side effects to the health of the animal as well as hampers

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its meat quality. The present scenario necessitates innovation of alternative herbal therapies with strong remedial properties[2]. Plant-based products have constantly engrossed attention in humankind owing to their versatile applications. Scientific investigations focusing screening of antibacterial and antioxidant activities of plants have shown plants as a representative potential source of new anti-infective agents[2-8] for the treatment of several ill-fated diseases. The innumerable therapeutic and natural phytochemicals contained in them proved to be beneficial in the treatment of diverse diseases namely diabetes, heart disorders, chronic inflammatory disorders, tumorigenesis disorders etc[9]. Antioxidants are molecules, which inhibit or quench free radical reactions and avert oxidative cellular damage[10]. Intake of dietary antioxidants is the simplest way to reduce the development of induced oxidative stress pathologies[8,11]. A variety of synthetic antioxidants is commonly available in the food industry for food safety purposes. Nevertheless, the use of such compounds has been restricted by the regulatory authorities owing to their long-term toxicity and carcinogenicity[12]. Probe concerning phytochemical repertoire of varied plants and their parts have been executed in enormous in the past in response to its low side effects and cost effectiveness[6-10]. Identification of phytochemical essentials namely flavonoids, alkaloids, carotenoids, tannins, phenolic compounds etc.[6] is of paramount importance to discover and develop herbal therapeutic drugs with enhanced efficacy to replace synthetic antibacterials and antioxidants for use in food and medical purposes.

Silkworm in northeast India occupies an imperative hold in socio-economic existence of the common people[13]. Indian golden silkmoth *Antheraea assamensis* Helfer (muga silkworm) is an indigenous, monotypic and semi-domesticated species geographically confined only to Brahmaputra valley of the northeastern biome of India, especially Assam. It is polyphagous and feeds on a wide variety of food plants out of which som (*Machilus bombycina* (*M. bombycina*)) and soalu (*Litsea polyantha* (*L. polyantha*)), synonyms (*Litsea monopetala*) function as the primary host plants while dighloti (*Litsea salicifolia* (*L. salicifolia*)) and mejankori (*Litsea citrate* (*L. citrate*)) serves as the secondary host plants. Geo-climatic conditions like high humid temperate, climate and forest vegetation of its primary and secondary host plants are the main factors accountable for its geographical isolation. Unlike mulberry silkworm, muga silkworm culture is an outdoor rearing tradition practiced by the livelihood of northeast particularly Assam throughout generations as a part of their ethnicity. Previously, investigations focusing *Litsea polyantha* have cited about its innumerable medicinal properties such as antioxidant, anti-microbial, anti-bacterial, anti-fungal, anti-hyperglycemic, anti-atherothrombosis and anti-diarrheal[14-17]. Mulberry, the host plant of mulberry silkworm (*Bombyx mori*) has been widely exploited and cited to have armoured with antimicrobial and antioxidant properties[18-20]. The current research is aimed to discover comparative screening of various phytochemical components and the antioxidant perspective present in leaves of muga food plants, which can be utilized for the formulation of potential medicines for the treatment of several diseases. The study also explores to uncover the antibacterial properties of the host plants against four important bacterial organisms of pig *viz*. Gram-positive *S. aureus* and *S. suis* and Gram-negative *P. multocida* and *E. coli*. The present findings might, therefore, be useful to devise new and innovative herbal drugs for the treatment of bacterial diseases affecting the swine industry and causing economic depredation to the same.

MATERIALS AND METHODS

Preparation of plant leaf extract:

Leaves of host plants of muga and mulberry were procured from different locations in and around Guwahati, Assam. Mulberry leaf has been used as a reference in the current investigation. The collected leaves were washed with tap water and subsequently sun-dried. The dried leaves were ground and extracted in 100 % methanol in the ratio 1:10, at 50° for 72 h. The extracts were filtered and later concentrated under reduced pressure to obtain a viscous semi-solid mass for further downstream applications.

Sample preparation for phytochemical screening:

A qualitative phytochemical screening of muga host plants to detect the presence of essential phytoconstituents, such as flavonoids, tannins, saponins, terpenoids, carotenoids, coumarins, phenolic compound, cardiac glycosides, xantho protein and quinones were carried out using standard biochemical procedures as reported previously[21].

2,2-diphenyl-1picrylhydrazyl (DPPH) method of total antioxidant capacity assessment:

Free radical scavenging activity of methanolic extract of plant samples was determined by the previously described method by Brand-Williams *et al.*[22]. The assay is based on the determination of the concentration of DPPH methanolic solution, after adding antioxidants. The solution of DPPH in methanol (6×10^{-5} M) was prepared just before Ultra-Violet (UV) measurements. The samples of different concentrations were added to DPPH solution in 1:1 ratio followed by vortexing. The reaction was allowed to take place in the dark at room temperature for 30 min. Absorbance at 517 nm was measured at different time intervals. Decreasing intensity of the purple colour was taken as increasing scavenging activity. The inhibition (%) of radical scavenging activity was calculated using the following equation.

Inhibition (%)=$[(A_0-A)/A_0]\times100$

Where A_0 is the absorbance of DPPH in the absence of the sample and A is the absorbance of DPPH in the presence of the sample

Collection and isolation of bacteria:

Tissue samples from pigs were collected from different pig farms, slaughter house and pork markets in and around Guwahati, Assam. The samples were collected aseptically in the collection container under the chilled condition for further processing. The bacterial samples were incubated in their respective broth, overnight at 37° and subsequently plated and streaked on nutrient agar, which was followed by staining, biochemical tests and polymerase chain reaction as per standard protocol.

Bacterial strains:

The antibacterial efficiency of each plant extract was evaluated against *S. aureus*, *S. suis*, *P. multocida* and *E. coli*. The bacterial strains were used from the repository of Animal Health Lab, ICAR-NRC on Pig, Rani, Assam.

***In vitro* evaluation of antibacterial activity by tube method:**

The antimicrobial activity of the plant extracts were studied *in vitro* using the macro dilution method[23]. Four different concentrations of each plant extract *viz*. 100, 200, 500 and 1000 mg/ml were prepared from the stock solution. 1 ml of broth culture of each of the four bacterial strains containing approximately 1×10^8 CFU/ml was transferred to five sterile tubes containing the plant extract in different concentrations as mentioned above and incubated at 37° for 24 h. Antimicrobial activity of all the five plant extracts along with positive (bacteria and broth) and negative control (only broth) against the four bacteria was determined by observing the turbidity of the broth.

Disc diffusion test:

Disc diffusion method[23] was performed to compare the bacterial growth inhibition by the plant extracts and other commercially available broad-spectrum antibiotics. In this method, sterile discs impregnated with 500 mg/ml concentration of the plant extracts was dried in incubator and used in the test. Broth culture of the four bacteria containing approximately 1×108 CFU/ml was uniformly spread separately over sterile Muller Hinton agar plates and discs containing the plant extract along with commercially available antibiotic discs namely amoxicillin and tetracycline. The samples and antibiotic discs were placed at a uniform distance and incubated for 24 h at 37°. After incubation, the zone of inhibition surrounding the discs was measured by using Hi zone antibiotic scale; (Hi-Media Laboratories Pvt. Ltd., Mumbai, India).

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis:

The extracts were filtered through a 0.2 µm filter for GC-MS analysis performed in a Perkin Elmer Clarus 680/600 unit fitted with Elite 5MS column (30 m×0.25 mm, 0.25 µm film thickness). The injector and detector temperatures were maintained at 300°. The samples were injected in the split mode, using pressure-controlled helium as carrier gas at a linear velocity 37.2 cm/s (at 50°). The oven temperature was programmed from 50° (after 2.5 min) to 150° at 15°/min. Again temperature was increased up to 200° at 3°/min. The final temperature was programmed to 300° at 8°/min and held for 8 min. The mass spectrometer (Clarus 600; single quad) was operated in the Electron Ionization (EI) mode at 70 eV with a source temperature of 200° and a continuous scan from mass to charge ratio (m/z) 50 to 600. The peaks were identified by matching the mass spectra with the National Institute of Standards and Technology (NIST) library, USA.

RESULTS AND DISCUSSION

Preliminary phytochemical screening as shown in Table 1 confirms the presence or absence of arrays of phytochemicals in our plants of interest. Bioactive

metabolites specifically flavonoids, tannins, terpenoids, carotenoids and phenolic compounds were discovered to be present in all the extracts. Whereas, the evidence of quinone in the extracts haven't been witnessed in this study. On the other hand, metabolites like coumarin, cardiac glycoside and xanthoprotein were found to be absent only in dighloti extract.

The DPPH analysis is a widely used method to evaluate the free radical scavenging action of plant extracts. The method is based on the reduction of methanolic DPPH solution in the presence of antioxidants resulting in the formation of non-radical DPPH-H by the reaction. The findings of this study ascertained the antioxidant efficiency of muga host plants by inhibiting DPPH radical activity. Percentage inhibition of DPPH is presented in Table 2. DPPH quenching activity of sualu leaf extract was found to be highest among all followed by dighloti and som extract. The inhibition produced by mejankari extract was significantly less compared to the rest.

The results of antibacterial activity of the plant extracts against Gram-positive *S. aureus* and *S. suis* and Gram-negative *P. multocida* and *E. coli* by macro dilution method in four different concentrations of the extract is given in Table 3. The plant extracts at lower concentrations *viz*. 100 and 200 mg/ml were unable to inhibit the growth of all the four bacteria whereas the plant extracts at 500 and 1000 mg/ml could inhibit the growth of bacteria as indicated by the absence of turbidity in the tubes (fig. 1).

The extracts were investigated to assess their anti-bacterial dominance against Gram-positive *S. aureus* and Gram-negative *E. coli* using zone of inhibition method. The result validated that the crude extract possesses antimicrobial activity in comparison with standard drug amoxicillin and tetracycline. As per the observations, all plant extracts were potentially effective in suppressing bacterial growth with variable efficiency. This antimicrobial activity of the crude extract might be attributed due to the presence of phytochemical essentials mainly flavonoids, tannins and phenolic compounds in the plant leaves[24]. The extract of *Litsea citrata* (*L. citrata*) was the most effective retarding microbial growth of both Gram-positive and Gram-negative bacteria, followed by *L. salicifolia*, *L. polyantha* and *M. bombycina*. Inhibitory effect of *L. citrata* was found to be more compared to extracts of *Morus alba* (*M. alba*). The results of the disc diffusion test showing the zone of inhibition produced by the antibiotics and the plant extracts against two bacterial species are shown in fig. 2. In the present study, amoxicillin showed slightly higher zone of inhibition against the Gram-negative bacteria than the zones produced by the plant extracts of mejankari and mulberry at 1000 mg/ml (fig. 3). Whereas tetracycline which is the most commonly used antibiotic against respiratory tract infection in pigs was found to be resistant showing no zone of inhibition. Similarly, in Gram-positive bacteria amoxicillin showed a higher zone of inhibition compared to the extracts of mejankari and mulberry while tetracycline showed no zone of inhibition.

TABLE 1: LIST OF PHYTOCHEMICAL CONSTITUENTS PRESENT IN SOM, SUALU, DIGHLOTI, MEJANKARI AND MULBERRY LEAF EXTRACT SOLUTION

S No.	Phytochemicals	*M. bombycina* (som)	*L. polyantha* (sualu)	*L. salicifolia* (dighloti)	*L. citrate* (mejankari)	*M. alba*
(mulberry)	8 (8 %)	8 (8 %)	8 (8 %)	8 (8 %)	8 (8 %)	8 (8 %)
1	Flavonoid	+	+	+	+	+
2	Tannin	+	+	+	+	+
3	Saponin	+	+	+	+	+
4	Terpenoid	+	+	+	+	+
5	Carotenoid	+	+	+	+	+
6	Coumarin	+	+	-	+	-
7	Phenolic compounds	+	+	+	+	+
8	Cardiac glycoside	+	+	-	+	+
9	Xantho Protein	+	+	-	+	+
10	Quinone	-	-	-	-	-

Note: (+): Present and (-): Absent

TABLE 2: PERCENTAGE INHIBITION OF DPPH BY THE MUGA HOST PLANT EXTRACTS

Extract material	Percentage Inhibition (%)				
	40 (µl/ml)	80 (µl/ml)	120 (µl/ml)	160 (µl/ml)	200 (µl/ml)
Som	64.61±0.48	67.82±1.02	70.50±0.41	71.84±0.84	75.87±0.91
Sualu	76±0.52	80.83±0.61	95.84±1.16	96.21±0.53	96.64±0.30
Dighloti	72.11±1.04	89.72±0.64	92.67±0.82	93.26±0.27	93.85±0.61
Mejankari	11.12±0.67	12.69±0.30	14.20±0.44	17.04±0.29	18.63±0.15
Mulberry	70.23±0.55	78.86±0.36	84.75±0.63	90.46±0.61	93.45±0.38
Ascorbic acid	77.44±0.61	82.03±1.09	96.32±0.53	97.18±0.32	97.72±0.27

Note: (SD=n±3)

TABLE 3: LIST OF PLANT EXTRACTS SHOWING ANTIBACTERIAL ACTION AGAINST *S. aureus*, *S. suis*, *P. multocida* AND *E. coli* AT DIFFERENT DOSES

Plant extracts Concentration (mg/ml)	Growth of the bacterial species in broth			
	P. multocida	*E. coli*	*S. suis*	*S. aureus*
100	Turbidity present	Turbidity present	Turbidity present	Turbidity present
200	Turbidity present	Turbidity present	Turbidity present	Turbidity present
500	Turbidity present	Turbidity present	No turbidity	No turbidity
1000	No turbidity	No turbidity	No turbidity	No turbidity
Broth with bacterial culture (Positive control)	Turbidity present	Turbidity present	Turbidity present	Turbidity present
Only Broth (Negative control)	No turbidity	No turbidity	No turbidity	No turbidity

Note: (-ve means no growth in the broth)

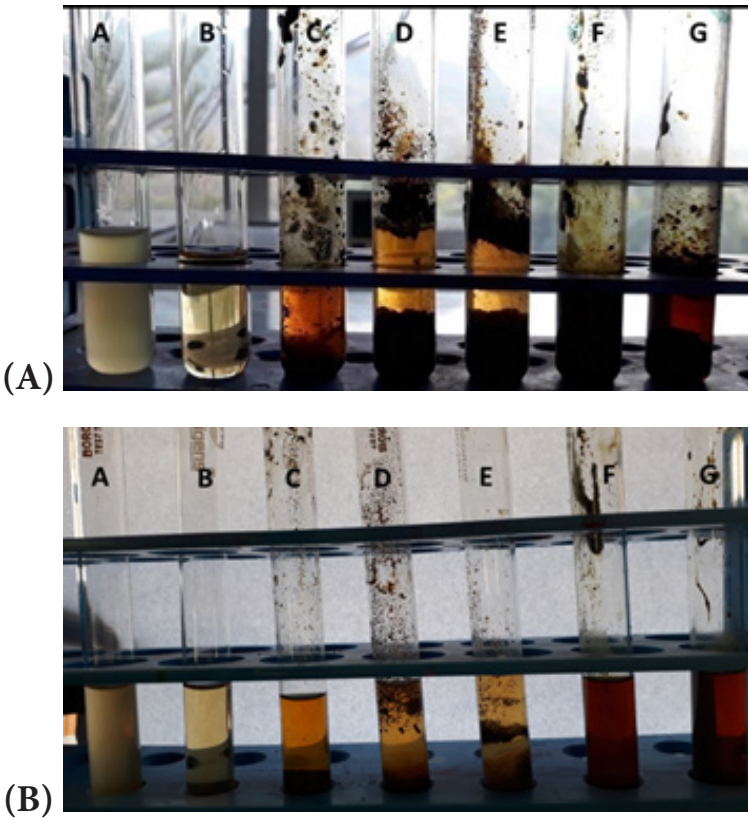

Fig. 1: *In vitro* antibacterial analysis of the plant extracts against gram positive and gram negative bacteria
Note: (a): *S. suis* (500 mg/ml); (b): *P. multocida* (1000 mg/ml); (A): Positive control; (B): Negative control; (C): Mejankari extract; (D): Dighloti extract; (E): Sualu extract; (F): Som extract and (G): Mulberry extract

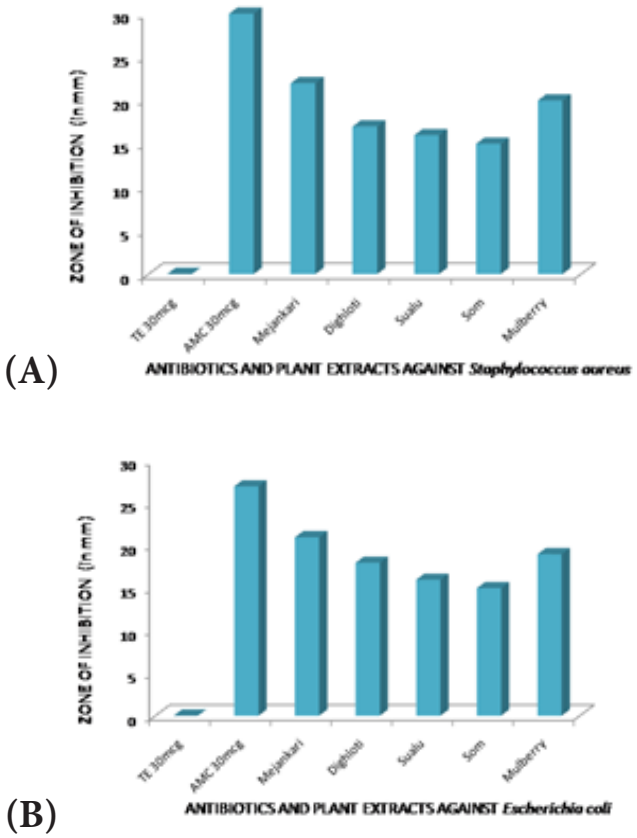

Fig. 2: Graphical representation of the zone of inhibition (mm) by specific antibiotics and the plant extracts against bacteria
Note: (a): *S. aureus*; (b): *E. coli*; TE: Tetracyclie; AMC: Amoxicillin and (■): Zone of inhibition

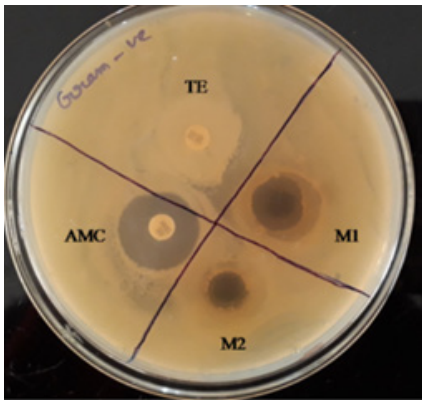

Fig. 3: Disc diffusion test against *E. coli* showing zone of inhibition
Note: TE: Tetracycline; AMC: Amoxicillin; M1: Mejankari and M2: Mulberry

The GC-MS analysis of methanolic extracts of the host plants as listed in Table 4-Table 7, discovered the presence of diverse medicinal compounds of volatile nature belonging to different chemical families. Fig. 4 shows the GC-MS spectrum of the investigated plant extracts. Nine major compounds were discovered from *M. bombycina*, among which the compounds possessing pharmaceutical importance are caryophyllene (1.75 %), 1,6,10-dodecatrien-3-ol,3,7,11-trimethyl (6.51 %), furan,2,5-bis(3,4-dimethoxyphenyl)tetrahydro-3,4-dimethyl (1.12 %), cardenolide (1.11 %), phenol 4,4'-methylenebis[2-(1,1-dimethylethyl)-6-methyl (33.82 %), d-Mannitol, 1-decylsulfonyland thiazolo[3,2-a]benzimidazol-3(2H)-1,2-(2-fluorobenzylideno)-7,8-dimethyl (40.34 %). Caryophyllene (1.75 %), 1,6,10-dodecatrien-3-ol,3,7,11-trimethyl (6.51 %) and furan, 2,5-bis(3,4-dimethoxyphenyl) tetrahydro-3,4-dimethyl (1.12 %) were also identified from *L. polyantha* but in different quantities. Other compounds with medicinal potency identified from *L. polyantha* were beta carotene (0.11 %), phenol, 2,2'-methylenebis[6-methoxy-3-(2-propenyl) (33.30 %), coniferyl aldehyde (40.41 %), 5-formyl-2,3,3',4'-tetramethoxystilbene (1.51 %), benzoic acid,

2,6-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester (3.02 %) and t-Butylphosphonic acid, di-TMS derivative (0.30 %). From *Litsea salisifolia* identified compounds having medicinal significance are 1,3-benzoxazol-5-amine, 4,6-dibromo-2-phenyl (1.42 %), methyl-4-thio.alpha.d-arabinofuranoside (1.61%), benzyl benzoate (83.15 %), 4'-Apo-.beta.psi.-carotenoic acid- carotene (1.43 %) and oxymetholone (1.65 %). Adding to the same, constituents with therapeutic value screened from *L. citrata* leaf extract are mainly eremophilane (0.19 %), thiazolo[3,2-a]benzimidazol-3(2H)-one, 2-(2-fluorobenzylideno)-7,8-dimethyl (2.18 %), caryophyllene (3.96 %) digitoxin (1.09 %) and benzenepropanoic acid (1.56 %).

As cited by Varadarajan *et al.*[25] the secondary metabolites and other chemical constituents of medicinal plants account for their medicinal value. They possess various biological therapeutics which provide the scientific base for the use of herbs in traditional medicine in many ancient communities[26]. The important bioactive essentials investigated in our research might be the key drivers responsible for the anticipated pharmacological properties of the muga food plants.

The absorbance of DPPH solution with methanolic extracts of muga food plants was found to be decreased with increased concentration confirming the free radical scavenging activity of the test extracts. Flavonoids, tannins, phenolics, terpenoids and carotenoids are a major group of compounds that act as primary antioxidants or free radical scavengers[27,28]. The flavonoids and phenolic compounds are known to hold antioxidant power due to the presence of hydroxyl groups in their structures and their contribution to defence system against the oxidative damage due to endogenous free radicals is extremely important[29,30]. The antioxidant activity of the studied extracts can be credited to the incidence of identified phytochemicals detected in the samples. Scavenging activity is found to be directly proportional to the concentrations of extracts. The results unveiled that food plants of muga silkworm mainly sualu and dighloti have better antioxidant potency compared to other plants including mulberry.

The results also discovered that all plant extracts were potentially effective in suppressing bacterial growth with variable potency. This antimicrobial activity of the crude extract might be attributed due to the presence of phytochemical essentials primarily flavonoids, tannins and phenolic compounds in the leaves[24]. The extract of mejankari leaf was the most effective retarding microbial growth of both Gram-positive and Gram-negative bacteria, followed by dighloti, sualu and som. Inhibitory effect of mejankari extract was found to be more compared to extracts of mulberry. The study also disclosed and confirmed that the Gram positive and Gram negative bacteria used in this study has become drug-resistant against tetracycline, the most commonly used drug against respiratory disorders in swine.

TABLE 4: LIST OF ACTIVE COMPOUNDS PRESENT IN SOM LEAF EXTRACT

Peak	Retention time (min)	Area %	Compound name	Activity
1	8.021	1.03	Benzene, 1,3-dichloro-	Organic compound
2	15.775	1.75	Caryophyllene	Antibacterial, Antifungal
3	17.474	6.51	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	Antimicrobial, Antioxidant, Antifungal, Anticancer
4	18.828	1.11	Cardenolide	Cardiac glycosides, Anticancer
5	24.03	0.29	d-Mannitol, 1-decylsulfonyl-	Antimicrobial
6	29.235	40.34	Thiazolo[3,2-a]benzimidazol-3(2H)-one, 2-(2-fluorobenzylideno)-7,8-dimethyl	Antimicrobial
7	29.422	33.82	Phenol, 4,4'-methylenebis[2-(1,1-dimethylethyl)-6-methyl-	Phenolic
8	29.48	0.22	Benzoic acid, 2,3-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	Antibacterial, Antifungal
9	29.879	1.23	2-(2-Acetoxy-3-methoxyphenyl)-3-methoxy-4H-chromen-4-one	Not specific activity reported
10	30.043	1.41	5,12d-Ethano(furo[2,3,4-mn] oxepino[2,3,4-ed]anthracen-9-ol-2-one)	Dye
11	30.824	1.12	Furan, 2,5-bis(3,4-dimethoxyphenyl) tetrahydro-3,4-dimethyl-	Anticancer, Antifungal, Antimicrobial etc

TABLE 5: LIST OF ACTIVE COMPOUNDS PRESENT IN SUALU LEAF EXTRACT

Peak	Retention time (min)	Area %	Compound name	Activity
1	17.35	2.9	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	Antimicrobial, Antifungal, Anticancer
2	17.719	0.99	Diethyl Phthalate	Plasticizer
3	17.89	0.48	Caryophyllene oxide	Antibacterial, Antifungal
4	18.774	0.11	Beta Carotene	Antioxidant
5	28.978	40.41	Coniferyl aldehyde	Phenylpropanoid compound
6	29.153	33.3	Phenol, 2,2'-methylenebis[6-methoxy-3-(2-propenyl)	Phenolic compound
7	29.238	1.51	5-formyl-2,3,3',4'-tetramethoxystilbene	Anticancer
8	29.63	3.82	9-Oxabicyclo[4.3.0]non-2-ene-4,5-dicarboxylic acid, 8-hydroxy-1,2,7,7-tetramethyl-, dimethyl ester	No specific activity reported yet
9	29.785	3.02	Benzoic acid, 2,6-bis[(trimethylsilyl) oxy]-, trimethylsilyl ester	Antifungal, Antiseptic
10	30.2	0.3	t-Butylphosphonic acid, di-TMS derivative	Antiretroviral, antimalarial, Antihypertensive etc
11	30.516	0.85	Furan, 2,5-bis(3,4-dimethoxyphenyl) tetrahydro-3,4-dimethyl-,	Antifungal, Antimicrobial, Disinfectant, etc

TABLE 6: LIST OF ACTIVE COMPOUNDS PRESENT IN DIGHLOTI LEAF EXTRACT

Peak	Retention time (min)	Area %	Compound name	Activity
1	8.854	1.16	Propene-1,2,3-tricarboxylic acid	Organic compound
2	12.289	1.42	1,3-Benzoxazol-5-amine, 4,6-dibromo-2-phenyl-	Antibacterial, Antifungal, Anticancer, Anti-inflammatory etc.
3	17.455	1.48	Diethyl Phthalate	Plasticizer
4	18.926	1.43	4'-Apo-.beta.psi.-carotenoic acid-carotene	Antioxidant
5	19.46	1.61	Methyl-4-thio.alpha.d-arabinofuranoside	Antimycobacterial
6	20.016	83.15	Benzyl Benzoate	Used against scrabies, insect repellent
7	21.545	1.65	Oxymetholone	Used in treatment of anaemia

TABLE 7: LIST OF ACTIVE COMPOUNDS PRESENT IN MEJANKARI LEAF EXTRACT

Peak	Retention time (min)	Area %	Compound name	Activity
1	27.31	0.19	Eremophilane	Antimicrobial, Anticancer, Immunomodulatory
2	29.217	2.18	Thiazolo[3,2-a]benzimidazol-3(2H)-one, 2-(2-fluorobenzylideno)-7,8-dimethyl	Antimicrobial
3	23.402	1.65	9,12-Octadecadienoic acid, methyl ester	Used in paints, oils
4	21.815	1.56	Benzenepropanoic acid	Phenylpropanoid compound
5	20.321	1.47	Heneicosane	Pheromone
6	15.778	3.96	Caryophyllene	Antibacterial, Antifungal
7	15.048	0.62	2-Propenoic acid	Used in textile industry
8	11.82	0.57	Azulene	Naphthalene
9	26.825	1.09	Digitoxin	Cardiac glycoside

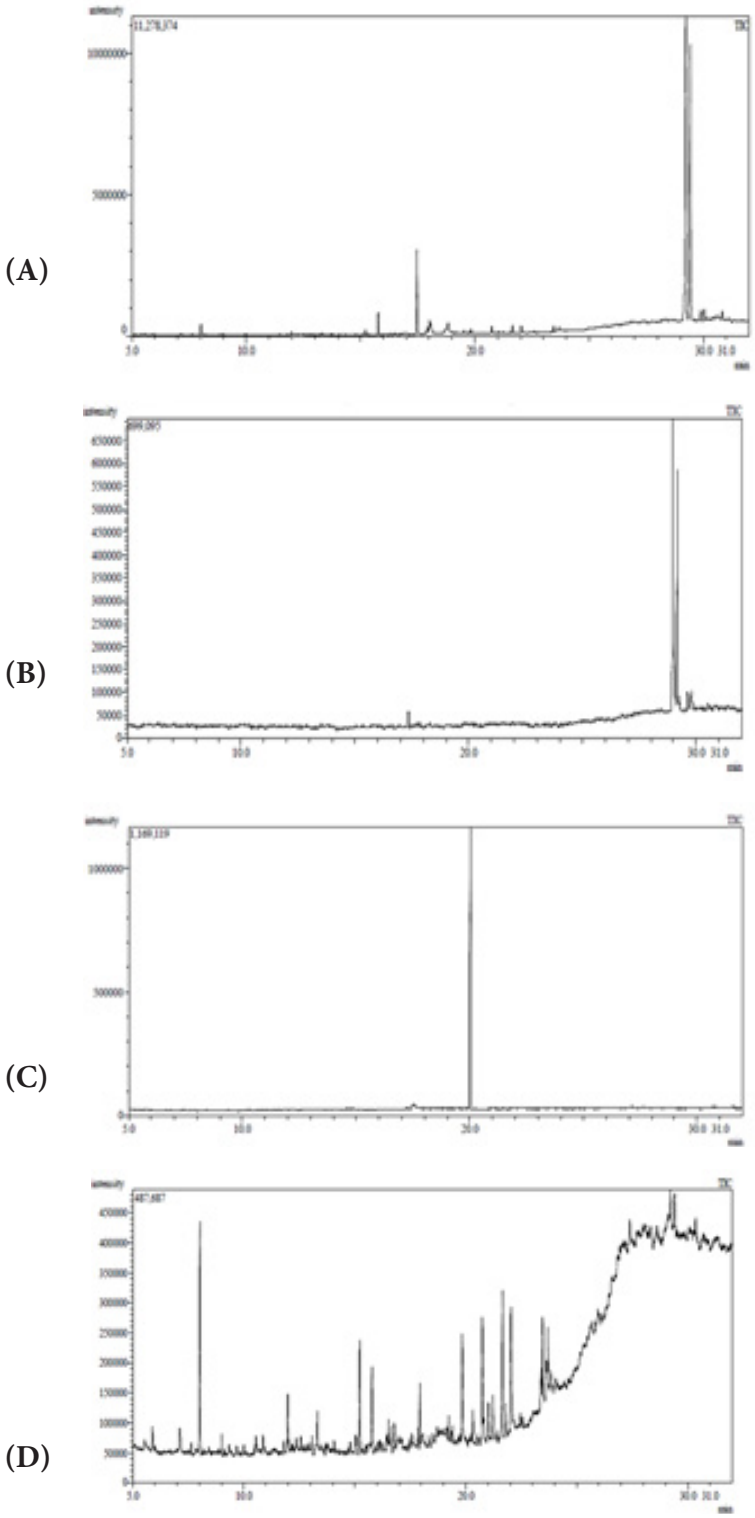

Fig. 4: Gas chromatogram of leaf extracts
Note: (a): Som; (b): Sualu; (c): Dighloti and (d): Mejankari

The GC-MS analysis authenticated profuse presence of compounds with potent medicinal supremacy in the investigated food plants. Based on prior literature the compounds identified are reported to be manifested with pharmaceutical values like antimicrobial, anti-fungal, anti-viral, anti-oxidant, anti-cancer, anti-retroviral etc, which may contribute to the healing potential of muga host plants. Caryophyllene is reported to have antibacterial and antifungal activity by Sabulal *et al.*[31] 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl also termed as nerolidol is proposed to have multi-facet pharmacological properties such as anti-microbial, anti-biofilm, anti-oxidant, antifungal, anti-parasitic, skin-penetration enhancer,

skin-repellent, anti-nociceptive, anti-inflammatory and anti-cancer[32]. Cardenolide compounds are evidenced to have anticancer activity by Krishna *et al.*[33] Diverse biological properties have been associated with thiazolo[3,2-a]benzimidazole derivatives in the passing decades, including antibacterial, antifungal, anti-inflammatory, anti-ulcer, anti-viral, anthelmintic and anti-cancer activity. Moreover, thiazolo[3,2-a] benzimidazole derivatives are well known as platelet-activating factor antagonists and neoplasm inhibitors. Some thiazolo[3,2-a]benzimidazole derivatives inhibit H^+/K^+-ATPase and gastric secretion and are thus useful as anti-ulcer agents. Furthermore, thiazolo[3,2-a] benzimidazol-1-oxide shows gastric antisecretory activity[34]. Importance of furan based compounds as studied by Chandrashekarachar *et al.*[35], have cited to have loaded with innumerable biological activities like anti-cancer, anti-bacterial, anti-fungal etc. Countless studies throughout the years have confirmed carotenoids and their derivatives as an established source of rich antioxidants[36]. Correspondingly phenolic compounds are too regarded for their potent antioxidant nature. Antioxidant significance of phenolic compounds can be attributed to their ability to chelate metal ions involved in the production of free radicals[37,38].

Coniferyl aldehyde and benzenepropanoic acid belong to the family of phenylpropanoid compound. Phenylpropanoids form a large class of phenolic compounds. They are derived from cinnamic acid/p-coumaric acid, which on the other hand is obtained from the amino acid phenylalanine catalyzed from the enzyme phenylalanine ammonia-lyase. This enzyme is the branch-point enzyme between the shikimate pathway and phenylpropanoid metabolisms[39]. The phenylpropanoid pathway serves as a rich source of metabolites in plants, is required for the biosynthesis of lignin, and serving as a starting point for the production of many other important compounds, such as the flavonoids, coumarins and lignans[40].

According to Xu[41] 5-formyl-2,3,3',4'-tetramethoxystilbene holds significant anticancer efficiency and has been investigated to induce apoptosis and reduce the viability of paclitaxel and cisplatin-resistant osteosarcoma cells. Benzoic acid has long been used as a strong antimicrobial agent in food preservatives[42]. Furthermore, benzoic acid also possesses antibacterial effectiveness against *Bacillus subtilis* I, *Bacillus megaterium* I, *Bacillus sphaericus*, *Bacillus polymyxa*, *S. aureus* I, *S. aureus* II, *S. aureus* III (Gram-positive), *E. coli* I, *E. coli* II and *E. coli* III (Gram-negative)[43,44]. Benzoic acid in its undissociated form exhibits various antibacterial and antifungal activities[45]. It is also investigated to be powerful against another Gram-negative bacterium, *Listeria monocytogenes* at 1000 µl/ml concentration[46]. Phosphonic acid and its derivatives are well known to have actions like antiretroviral, anti-malarial, anti-hypertensive etc. Benzoxazol compounds and benzoic acid are benzamide analogues that are established to possess a countless number of pharmacological activities like antibacterial, antifungal, antidepressant, antiseptic etc.[47]

Arabinofuranoside compounds carry mycobacterial growth inhibition activities[48]. Glycolipids containing an arabinofuranoside trisaccharide inhibited the growth of *Mycobacterium smegmatis* (*M. smegmatis*). Both arabinofuranoside trisaccharide and lipidic portion were essential for the biological activity[48]. It was shown further that arabinomannan glycolipids inhibited the sliding motility and biofilm formation of *M. smegmatis*[49]. Benzyl benzoate identified from *L. salicifolia* in our present study is an established drug used against scabies in animals and is also exploited as an insect repellant[50,51]. Oxymetholone extract is conventionally used for the treatment of anaemia[52]. Eremophilane identified in *L. citrata* is probed to possess antimicrobial, anticancer and immunomodulatory function by Yuyama *et al.*[53].

Digitoxin is a cardiac glycoside widely known for treating congestive heart failure[54]. It is also evidenced to function as a promising anticancer agent when used at therapeutic concentrations[55]. Thiourea, 1-[2-(2-benzylphenoxy)ethyl]-3-(O-totyl) are volatile organic compounds with antifungal property[56]. Pregn-4-ene-3,20-dione, 17,21-dihydroxy-, bis(O-methyloxime) is inspected to have anti-inflammatory function[57]. d-Mannitol, 1-decylsulfonyl compound is examined to have anti-microbial activity by Alagammal *et al.*[58]. In addition to the abovementioned, compounds with various industrial importances like anthracene, diethyl phthalate, 2-propenoic acid etc. have also been detected in the current study.

The use of herbal therapeutics in replace of synthetic drugs for treatment of diverse diseases and disorders has become a bourgeoning area of research. The present work is the first study reporting the comparative screening of primary and secondary host plants of muga silkworm and identification of their rich phytochemical inventory. The phytoconstituents of profound interest discovered in this study suggest the fortified antioxidant

and antibacterial potentiality of muga food plants. The results of DPPH scavenging activity uncovered *L. polyantha* to exhibit strong antioxidant efficiency compared to other food plants. Besides these, the host plants have also revealed anti-bacterial efficacy against Gram-positive and Gram-negative bacteria. Among the food, plants investigated *L. citrata* was evidenced to possess greater antibacterial inhibition compared to the rest in opposition to both Gram-positive *S. aureus* and Gram-negative *E. coli*. Compiling the above findings it can be accomplished that food plants of muga silkworm are equipped with antioxidant and antibacterial competence, which can be developed as natural alternative therapeutics against a plethora of diseases. The study does indicate that further exploration for isolation, characterization and structure elucidation of bioactive compoundsin the mentioned plants may contribute to the discovery of noble drug initiatives.

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Conflict of interests:

The authors declare that there is no conflict of interest.

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