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Phytochemical analysis of *Simarouba glauca* DC and its antibacterial activity against MDR *Salmonella Typhi*

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Abstract:

The plant, *Simarouba glauca* DC (SG), has a rich source of pharmaceuticals and thus is one of the important herbal sources to treat certain infectious and non-infectious diseases, like diarrhea, malaria, edema, fever hemorrhages, intestinal parasites, and colitis. Especially, the leaves and bark of this plant in the form of tonic are specifically used to treat malaria, fever, dysentery, and to stop bleeding. Typhoid is a disease caused by *Salmonella* serotype Typhi bacteria that are responsible for a huge burden on developing nations for generations. Typhoid fever is more common in children and young adults and is associated with areas where poor sanitation is prevalent. The study was aimed at the phytochemical investigation, metal analysis, and antibacterial activity of aqueous extract of *Simarouba glauca* DC leaves and stem bark samples. Preliminary screening indicated the presence of phytochemicals viz., alkaloids, flavonoids, tannins, terpenoids, carbohydrates. The essential metals such as Cu^{2+}, Fe^{2+}, Mg^{2+}, and Zn^{2+} were found to be present in the SG aqueous extract. The antibacterial and antibiogram activities were evaluated and the MIC and MBC values were determined for *Salmonella enterica* serovar Typhi and *Escherichia coli* which were in the range of 5 to 10mg/ml. Overall, the results of our study demonstrated that the extracts have an array of phytochemical components which showed moderate sensitivity as compared to standard antibiotics. However, our findings showed that the aqueous leaves and stem bark extract of *Simarouba glauca* DC., possesses good antimicrobial activity which could further be evaluated for the development of potential novel nutraceuticals.

Keywords: *Simarouba glauca* DC., *Salmonella enterica* serovar Typhi, Antibiogram, Multi drug resistance, MIC and MBC.

INTRODUCTION:

The newly emerging and re-emerging diseases are due to inadequate vaccination which continues to pose a substantial threat throughout the world. The antibiotic resistance has been referred to as "*the unvoiced tsunami facing modern medicine*" which is one of the main reasons leading to prolonged illness, higher expenditures for health care and an immense risk of death[1]. Continuous deployment of antimicrobial drugs in treating infections has led to the emergence of drug resistance among the various strains of microorganisms well known as 'Super bugs' resulting in spreading of infections[2]. The rise of multi drug resistance (MDR) has become a particularly serious challenge for healthcare professionals. One of the most prime examples of disease prone to MDRis typhoid caused by *Salmonella enterica* serovar Typhi, a major public health threat at a terrifying rate in developing countries. The infectivity spreads through fecal oral route and asymptomatic carrier individuals[3]. According to the WHO's most recent estimates, between 11 and 21 million cases and 128,000 to 161,000 typhoid related deaths occur annually worldwide. The science behind emerging and re-emerging infectious diseases remain largely unexplored but holds an extremely rich potential for innovation and discovery. As medicines could be traced back from human civilization, the medicinal plants with a rich source of bioactive phytocomponents and nutrients would be the greatest source to obtain an array of drugs. The monitoring of trace metals in the therapeutic plants is of immense importance for shielding the public from the hazardous toxic effects and besides informing the community in relation to the nutritional assessments of the plants [4, 5]. The trace metal ions are very vital for living organisms to carry out their various metabolic processes. In modern check, pharmacopoeia contains at least 25% drugs derived from plants secondary metabolites which have immunostimulation and disease preventive properties. In this direction, the present study has focused on *Simarouba glauca* DC., also known as laxmitaru or paradise tree, belongs to *Simaroubaceae* family, consists of six subfamilies with 32 genera and more than 170 species. The crude aqueous extract contains phytocomponents for instance glaucarubin, quassinoids, ailanthinone, melianone, simaroubidin, simarubin, simarubolide, sistosterol along with essential metal ions. It has shown wide range of potency that are essentially leading to pharmacological activities and we are assessing the possibility of combating the MDR *S.typhi* infection by immune stimulation to overcome the problem of MDR[6].

MATERIAL AND METHODS

Collection of plant materials:

The plant samples, leaf and stem bark of *Simarouba glauca* DC. was collected from the botanical garden of Kuvempu university, Shankaraghatta. The samples (leaf and stem bark) were thoroughly rinsed with tap water and dried at room temperature for 7 to 8 days and stored in air tight container.

Extraction of Sample:

According to standard protocol, the decoction was prepared. 10g of samples (leaf and bark) were taken and soaked in 100ml of water (w/v,1:10) and subjected to boiling for 15 to 20 minutes at 121°C. After cooling, the extracts were filtered by using Whatman No. 1 filter paper.

The extracts were further subjected to lyophilisation and the obtained powder was stored in air tight container until further use[7,8].

Chemicals:

Alcohol, ammonia, chloroform, copper acetate, ferric chloride, Folin Ciocalteu (FC) reagent, concentrated HNO_3, $HClO_4$,gallic acid, glacial acetic acid, hydrochloric acid, lead acetate, Mayer's reagent, petroleum ether, sodium carbonate, sodium hydroxide and sulphuric acid (All chemicals were analytical grade and brought from Himedia).

Test Organisms:

Bacterial organisms used were Enterotoxigenic *Escherichia coli* (ETEC) (MTCC 723) and*Salmonella enterica*serovar Typhi (MTCC 733). TheStock cultures were maintained on nutrient agar slants at 4°C and sub cultured in nutrient broth at 37°C prior to each test.

Culture media:

Nutrient agar was used for the maintenance of bacterial cultures.Mueller Hinton Agar (MHA) and Muller Hinton Broth (MHB) were used to determineminimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), and also antimicrobial activity.

Qualitative Phytochemical Analysis

Preliminary qualitative phytochemical screening for the freshly prepared leaf and stem bark aqueous crude extract was carried out for alkaloids [Mayer's test], cardiac glycosides [keller kelliani's test], flavonoids [Alkaline reagent test, Lead acetate test], phenols [Ferric chloride test], tannins [Precipitate test, Braymer's test], saponins [Foam test], terpenoids [Salkowki's test] anthraquinines [Borntrager's test], diterpenes [Copper acetate test] by following the standard protocols which were identified by distinctive colour changes [9,10].

Quantitative Phytochemical Analysis

Freshly prepared leaf and stem bark aqueous extract was analysed for phenols, flavonoids and terpenoids content quantitatively.

Phenols

Total phenolic content was determined by Folin Ciocalteu (FC) reagent method. Plant samples extract (1ml) was mixed with FC reagent (2ml) and sodium carbonate Na_2CO_3 (2ml). The mixture was allowed to stand for 90min at room temperature and the total phenol was determined spectrophotometrically at 750nm. Gallic acid was used as standard. The values of total phenolic content were expressed in terms of Gallic acid equivalent (mg/g) [11].

Flavonoids Content

10g of plant leaf and stem bark powder repeatedly extracted with 100mL of 50% alcohol at room temperature and filtered through Whatman filter paper no. 42. The filtrate obtained was transferred to a crucible and evaporated till dryness over water bath, weighed until constant weight was obtained. Percentage of flavonoids was calculated by using formula[12].

Percentage of Flavonoids (%)

= Weight of flavonoid content (g)/ Weight of the Sample (g) X 100

Terpenoids

10g of plant powder was taken in conical flask and soaked in alcohol overnight. It was then filtered and filtrate was extracted with petroleum ether. This ether extract was treated as total terpenoids. The residue obtained was dried and weighed. Percentage of terpenoids was calculated by using the following formula[13].

Percentage of Flavonoids (%)

= Weight of Terpenoid content (g)/ Weight of the Sample (g) X 100

Determination of metal ions through ICP-OES analysis

Extraction procedure:

The ICP-OES (inductively coupled plasma – optical emission spectrometry) is a technique in which the composition of elements in mostly water dissolved samples will be determined using plasma and a spectrometer. To determine the trace metal elements in the sample, 0.25g of SGL and SGB plant samples was taken in digestion tube and 5ml of concentrated HNO_3 was added. After the pre-digestion for 12hrs,the samples were digested at 180°C on hot plate until the nitric acid is about to evaporate. A mixture of di-acids (HNO_3 and $HClO_4$) was added and the samples were allowed to digest until it becomes transparent. The samples were cooled at room temperature and filtered into the volumetric flask (25ml) and the volume was made with 2% HNO_3. The blank was also processed similarly. The elements were analyzed through (ICP-OES, Perkin Elemer – 5300v) [14, 15].

Anti-typhoidal study by well diffusion method

Preparation of standard inoculum *Salmonella enterica* serovar *typhi* and *Escherichia coli*for *in-vitro* assay was prepared according to 0.5 Mcfarland turbidity standards. The anti-typhoidal activity of the plant extract against test organism was studied by agar well diffusion method by varied concentrations (25, 50, 75, 100, 200, 300, 400 and 500mg/ml) in triplicates (n=3). Aliquot of 100µl bacterial culture, which corresponded to 105 colony forming unit (CFU) was spread on the agar plate. Wells of 6mm diameter were then punched into agar plate and vehicle as control and different standard antibiotics (Co-Trimaxazole, Amphicillin 1mg/ml) were transferred into the respective wells. Plates were left for pre-diffusion aseptically and incubated at 37°C for 24h. After overnight incubation, the plates were observed for the zone of inhibition (ZI). At the end of incubation, diameter of zone of inhibition was measured against each test organism[9,16,17].

Minimum Inhibitory Concentration (MIC) by microdilution method

The MIC value of the extract was determined as the lowest concentration of the extract that do not permit the growth of test organisms after 24 h of incubation at 37º C by using 96 well microtiter plate. Test samples extract was dissolved in sterile distilled water and two fold serial dilutions (10mg/ml to 1.25mg/ml) were made with Muller Hinton broth to yield volumes of 100µl/well. 100µl bacterial inoculums (10^5 CFU/ml) were added to respective wells containing the test plant extract except negative control and mixed well. Then microtiter plate was covered and incubated for 24h. After incubation, 50µl MTT dye (3-(4, 5 –Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide; yellow dye; 0.020mg/ml) was added to each well and incubated for 30 minutes at 37°C. The viable bacterial cells in the well, changes the

yellow MTT dye to blue colour. The lowest concentration at which no visible colour change was observed was considered as MIC value[18-20].

Determination of minimum bactericidal concentration (MBC) by broth dilution from minimum inhibitory concentration

On agar, the lowest concentration of extract showing no growth represents the MBC. To determine MBC, bark extract was diluted by two-fold serial dilution (10mg/ml to 1.25mg/ml) with freshly prepared Muller Hinton broth and 100µl of the standard bacterial inoculum (10^5 CFU/ml) added to each well containing plant extract except the negative control. Micro titer plate was covered and incubated for 24h. After incubation, samples were cultured on fresh Muller Hinton agar separately and MBC was determined that shows no growth[21, 22].

Antibiogram Studies

The antibiotic susceptibility patterns of the test isolate were determined by the Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines and interpretative criteria [National Committee for Clinical Laboratory Standards (NCCLS) 2000] using antibiotic discs (Hi Media Lab.Pvt.Ltd., Mumbai, India), viz.,Ampicillin, Chloramphenicol, Gentamycin, Ciprofloxacin, Tetracyclin and varied concentrations of *Simarouba glauca* DC. leaves and stem bark samples viz., 100, 200, 300, 400 and 500mg were studied.Briefly, 24hr old test culture of *Salmonella enterica* serovar Typhistrain (MTTCC 733, Chandigarh) was taken and the growth was adjusted to match 0.5 MacFarland Standards (NCCLS 2000). The adjusted inoculum was applied over nutrient agar media and the various antimicrobial discs were placed over the inoculated plates in equidistance and incubated overnight at 37° C. Subsequently, the plates were read and the results were interpreted as sensitive, intermediate or resistant. *Escherichia Coli* strain (MTCC 723) was used as the control[23-25].

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 6.0 (GraphPad Software, La Jolla, CA). Data for each analysis represents a minimum of 3 repetitions. Data sets were analyzed using standard error mean (SEM).

RESULTS

Qualitative Analysis

The qualitative phytochemical analysis of *Simarouba glauca* DC., leaf and stem bark aqueous extract revealed the presence of flavonoids, alkaloids, phenols, terpenoids, tannins, carbohydrates, amino acids, proteins, anthraquinies, diterpenoids,whereas alkaloids and saponins were absent.The qualitative phytochemical data obtained in this study were resolute and shown in Table 1.

Quantitative Analysis

The quantitative phytochemical analysis of *Simarouba glauca* DC., leaf aqueous extract revealed the presence of 25.4% terpenoids,12% flavonoids and 0.019% phenols, where as in the stem bark extract, 9.6% terpenoids, 4.2% flavonoids, 0.014% phenols werefound (Table 2).

Determination of metal ions through ICP-OES analysis

The average total extractable metalspresent in*S. glauca* DC. leaf were found to be copper (8.3 µg/g), Iron (559.4 µg/g), Magnesium (14230.6 µg/g) and Zinc (1.6 µg/g), respectively. In the case of *S. glauca* DC. stem bark,the presence of copper (102.3µg/g), Iron (413.4 µg/g), Magnesium (9880.6 µg/g) and Zinc (11.1 µg/g), have been recorded (Table 3).

Antibacterial activity by agar well diffusion method

We evaluated the antibacterial activity of *S. glauca* DC., leaf (SGL) and stem bark (SGB)crude aqueous extract against theinvestigated bacterial strains (Table 4 and 5). The activity was based on varied dose of the test material.As expected, the increased concentration of SGL and SGB extracts also resulted in an increase in thesusceptibility of test organism. The antibacterial activity of SGL aqueous extract exhibited significant zone of inhibition (ZI), which was ~21mm at 500mg and ~20mm at200mg, 300mg and 400mg,as compared to control, against*Salmonella enterica* serovar Typhi.In comparison, for *E.coli*, SGL extract as showed a ZI of ~28mm at 500mg and ~25mm at 300mg and 400mg. On the other hand,SGB crude aqueous extractshoweda ZIof ~18mm at 500mg and ~17mm at 400mg against *Salmonella enterica* serovar Typhi. In the case of *E.coli*,SGB crudeaqueous extract, the ZIwas found to be ~21mm at 500mg and ~20mm at 400mg. These data in comparison with standard antibiotics (Amphicilin and Bacterin), has clearly indicated the corresponding extracts have decreased the load of *S.typhi* and *E.coli* at 500mg/mlveryefficiently. However, resistance at low concentration doesn't mean the absence of bioactive constituents, but it may be due to insufficient quantities of active compounds.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

The broth micro dilution method was used to determine the MIC according to The Clinical and Laboratory Standards Institute (CLSI) protocol. Based on this, the efficiency of extracts on the test pathogens was determined (Table 6). According to the standard norm, if the ratio of MBC/MIC is '1' or '2', the extract is said to be bactetericidal in nature. Further, if the ratio of MBC/MIC is 4 or 16, it is assumed to be bacteriostatic in nature. *S. glauca* DC., aqueous leaf extract exhibitedhigh MBC and MIC at 10mg/mL and the ratio is 1. While, stem bark extract has themaximum MBC and MIC values at 5mg/mL and the ratio is also 1.

Antibiogram Studies

We also measured the sensitivity of organism to different antibiotics and plant samples and the results are presented in Table 7.The standard antibiotics usedfor antibiogram studies were Ampicillin, Chloramphenicol, Gentamycin, Ciprofloxacin, Tetracyclin)(10µg/discs). The results were analyzed on the basis of extent of definite zone of inhibition according to CLSI standards. The outcome of our antibiogram studies indicates that theorder of resistance to the test organism is Am>Ge>Te>Chl>Cip, whereas the leaf and bark extract of *S. glauca* DC. showed moderate sensitivity.

Table no 1 - Qualitative Phytochemical Screening of *Simarouba glauca* DC. leaf and Stem bark aqueous extract.

Phytochemical Tests	Results	
	SGL	SGB
Carbohydrates	+	+
Alkoloid	-	-
Cardiac Glycoside	+	+
Flavonoid	+	+
Phenol	+	_
Tannins	+	+
Amino acids and Proteins	+	+
Saponins	-	-
Terpenoid	+	-
Quinones	+	-
Anthraquinies	+	-
Diterpenoids	+	+

Table no 2 - Quantitative phytochemical analysis of *Simarouba glauca* DC. leaf and stem bark aqueous extract.

Phytoconstituents	Test	Results	
		SGL(%)	SGB(%)
Total Phenol	FC Method	0.019	0.014
Total Flavonoids	General Method	12	4.2
Total Terpenoids	Ferguson's Method	25.4	9.6

Table no 3 – Determination of nutritional elements/metal ions through ICP-OES analysis

Test Samples	Nutritional elements/Metal ions (µg/g)			
	Cu^{2+}	Fe^{2+}	Mg^{2+}	Zn^{2+}
SGL	8.3	559.4	14230.6	1.6
SGB	102.3	413.4	9880.6	11.1

SGL: *Simarouba glauca* DC., leaf, SGB : *Simarouba glauca* DC., stem bark, Cu^{2+} : Copper,Fe^{2+}:Iron,Mg^{2+}: Magnesium, Zn^{2+}: Zinc.

Table no 4 – Antibacterial activity of *Simarouba glauca* DC. aqueous leaf extract by well diffusion method.

Test Organism	Zone of Inhibition (mm) SGL Concentration (mg)									
	25	50	75	100	200	300	400	500	Am	Bac
S.typhi	11.33± 0.33	13.0± 0.0	13.66± 0.33	16.0± 0.0	18.33± 1.66	18.0± 1.0	19.33± 0.66	21.0± 0.0	21.33± 0.33	
E.coli	12.33± 0.33	12.66± 0.33	18.0± 0.0	20.0± 0.0	24.66± 1.33	25.66± 0.33	25.0± 0.0	28.33± 0.33		25.66± 0.88

'0': No inhibition zone was observed, SGL: *Simarouba glauca* DC., leaves,Am: Amphicilin, Bac: Bacterin.

Table no 5– Antibacterial activity of *Simarouba glauca* DC. aqueous stem bark extract by well diffusion method.

Test Organism	Zone of Inhibition (mm) SGB Concentrations (mg)							
	25	**50**	**75**	**100**	**200**	**300**	**400**	**500**
S. typhi	6.66±	7.0±	7.0±	12.33±	13.66±	14.66±	17.33±	18.0±
	0.33	0.0	0.0	0.66	0.33	0.33	0.33	0.0
E.coli	0.0±	0.0±	0.0±	17.33±	18.66±	18.33±	21.0±	21.0±
	0.0	0.0	0.0	0.33	0.33	0.33	0.0s	0.57

'0': No inhibition zone was observed, SGB: *Simarouba glauca* DC., stem bark.

Table 6- MIC and MBC values of *S. glauca* DC., leaves and stem bark extract against contagious bacterial pathogens.

Test Organisms	SGL MIC (mg/ml)	MBC (mg/ml)	SGB MIC (mg/ml)	MBC (mg/ml)	MIC/MBC Ratio	Activity
S.Typhi	10000	10000	5000	5000	1:1	Bactericidal
E.coli	10000	10000	5000	5000	1:1	Bactericidal

ETEC: Enterotoxigenic *E.coli* H10407, *Salmonella typhi;* MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration.

Table 7 - Antibiogram studies of *Simarouba glauca* DC., leaf and stem bark extract against test pathogen.

Test Organisms	Plant Parts	Solvents	Standard Antibiotics					SG Concentrations				
			Am	Chl	Ge	Cip	Te	100	200	300	400	500
S. typhi	Leaf	Aqueous	R	S	I	S	S	I	I	I	I	I
	Bark	Aqueous	R	S	I	S	S	I	I	I	I	I
E.coli	Leaf	Aqueous	R	S	I	S	S	I	I	I	I	I
	Leaf	Aqueous	R	S	I	S	S	I	I	I	I	I

SG(B) : *Simarouba glauca* DC. bark, Am : Ampicillin, Chl: Chloramphenicol, Ge: Gentamycin, Cip : Ciprofloxacin, Te : Tetracycline, R: Resistance, S: Sensitive, I: Intermediately sensitive.

DISCUSSION

The natural products such as phytochemicals serves as a blueprint for the development of novel and innovative drugs. Hence, the medicinal plants play a vital role in covering the health needs in developing countries[26]. The fact that water extracts portrayed the highest potency of the herbal extracts against the test microbes justifies the basis for use of water as medium for extracting herbal medicines from plants. In our present study, the findings of preliminary phytochemical analysis of *Simarouba glauca* DC., leaves (SGL) and *Simarouba glauca* DC., stem bark (SGB) plant aqueous extract showed the presence of phytoactive components likeflavonoids, phenols, terpenoids, tannins, carbohydrates, amino acids, proteins, anthraquinies, and diterpenoids. The absence of saponin andalkaloids in the extract may be due to the type of solvent used, climatic conditions of plant growth and the screening method[27]. The crude extracts from plants are always a mixture of active and non-active compounds. The active phytoconstituentssuch as flavonoids have shown to possessa potent water soluble antioxidant and free radical scavenger activity which prevent oxidative cell damage. The presence of tannins is known for its amazing stringent properties which heal the wounds and inflamed mucous membranes and the terpenoidspossess antimicrobial, antiallergic, anti-inflammatory and immunomodulatory properties. Even,the carbohydrates, glycosides are known to exert a beneficial action on immune system by increasing body strength[28,29]. Meanwhile, since ancient times, phytometal elements have been used during various treatment methods which favors to keep the body in a healthy condition[30]. It is clearly evident from our data that *S.glauca* DC., plant could be a very good source of elements such as Fe^{2+}, Zn^{2+}, Cu^{2+} and Mg^{2+}that are very essential[31,32].Many studies have described the use of copper to reduce inflammation, required to produce red and white blood cells, helps the brain development, immunity augmentation and essential for strengthening of bones. Iron, used to treat anemia, is essential for the formation of hemoglobin in red blood cells, which binds to oxygen for its transportation. The major function of zinc in human metabolism is as a cofactor for numerous enzymes, essential for cell division, for normal reproductive development, helps in tissue repair and heal wounds. Hence, the trace metals of medicinal plants origin occupy an important role in preventing chronic diseases[33,34]. Besides, SGL and SGB aqueous extracts at high concentrations against test pathogens,*S. typhi* and *E. coli*, have shown goodantibacterial activity in a dose dependent manner. Further, the resistance at low concentration doesn't mean the absence of bioactive constituents, butit may be due to insufficient quantities of active compounds[36]. The fact that both SGL and SGB extracts have shown antibacterial activity against Gram negative pathogenic bacteria, it is very encouraging and important considering the role of Gram negative bacterial infections leading to increased morbidity and mortality rates[34, 36]. The MIC and MBC values for the test organisms,*S. typhi* and *E.coli*, was found to be 10mg/ml and 5mg/ml, respectively. The MIC values of less than100mg/L has been suggested as a good antimicrobial agent. Thus, the action of bactericidal drugs is most effective than bacteriostatic in order to control actively dividing cells which results in bacterial cell death. According to Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility testing (EUCAST) standards more recent reports suggested that *S. typhi* is still relatively sensitive to ciprofloxacin despite ongoing treatment failure and relapse which has consequently led to the production of β-lactamase by Extended Spectrum of β-lactamase producing Bacteria (ESBLs), an enzyme by Gram negative bacteria including *S. typhi*[37].Our findings are also in accordance with antibiogram profile for *S. typhi* which gives an overall picture of antimicrobial susceptibility results of a specific microorganism to a battery of antimicrobial drugs[38, 41]. Regarding antibiotic susceptibility, the highest resistance of test pathogen was found to Ampicillin, followed by Gentamycin, Tetracyclin, and Chloramphenicol,whereas the tested strains showed highest susceptibility to Ciprofloxacin. Overall, our current investigation showed that SGL and SGB aqueous extracts contain rich phytoconstituents, resulting in good antibacterial, antityphoidal, and bactericidal properties by probably interrupting the protein synthesis and membrane leakage of infectious human MDR pathogens.

CONCLUSION

Overall, it is evident from the obtained data that *Simarouba glauca* DC., plant extract could be a source of essential elements (Cu^{2+}, Fe^{2+}, Mg^{2+}, Zn^{2+}) andpossesses promising immunomodulatory components which can combat the *S.typhi* infection and couldreduce the emerging problem of MDR. In this study, the detected levels of phytocomponents are below the WHO permissible levels and may not lead to any adverse health hazard. Thus, furtherdetailed *in vivo* studiesare needed to confirm the mechanism and for the development of potential novel nutraceutical with least side effects and the data presented here is likely to lay the foundation for further detailed work in this direction.

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