Pharmacogn J. 2021; 13(1): 37-43
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Ethnomedicinal Knowledge Verification for the Antidiarrheal and Antioxidant Effects of *Rhus chinensis* Mill. Fruits with Identification of Thirty Constituents

Chandra Mohini Nemkul[1], Gan B Bajracharya[2,*], Hayato Maeda[3], Ila Shrestha[4]

ABSTRACT

Background: Ethnobotanical survey in the rural villages in Nepal revealed that the fruits of *Rhus chinensis* Mill. have been using for the treatment of diarrhea and dysentery. **Objective:** To evaluate antimicrobial and antioxidant effects, and identification of chemical constituents in the fruits of *R. chinensis*. **Materials and Methods:** Phytochemical screening was performed on the hexane and 70% methanolic extracts of the sample followed by gas chromatography-mass spectrometry (GC-MS). Total phenolic content (TPC) was estimated using Folin-Ciocalteu method. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical and hydrogen peroxide scavenging assays were used to evaluate the antioxidant capacity. Antibacterial effect was estabilished by the Agar well diffusion assay. **Results:** A total of 30 compounds belonging to phenolics, anhydrides, aldehydes, fatty acids and hydrocarbons were identified in the extracts. The TPC value of 123.52±1.29 mg GAE/g dry extract was estimated. IC_{50} value of 135.54±0.82 µg/mL was calculated in DPPH free radical scavenging assay. Scavenging of 42.69±0.1% DPPH free radical and 63.20±1.48% hydrogen peroxide at 100 µg/mL concentration of 70% methanolic extract were estimated. The maximum zone of inhibition (ZOI) observed was 23.00±0.57 mm against *Escherichia coli* at loading dose of 5 mg of the extract. **Conclusion:** All together 30 compounds were identified in the fruits. The extracts efficiently inhibited the growth of *E. coli* and *Shigella dysenteriae* verifying the rural knowledge. At the same time, the extracts displayed efficient antioxidant activity. The phytochemicals identified were responsible for these activities.

Key Words: Antibacterial susceptibility assay, DPPH radical scavenging assay, GC-MS, Hydrogen peroxide scavenging activity, Total phenolic content.

Chandra Mohini Nemkul[1], Gan B Bajracharya[2,*], Hayato Maeda[3], Ila Shrestha[4]

[1]Departement of Botany, Tri-Chandra Multiple Campus, Tribhuvan University, Ghantaghar, Kathmandu, NEPAL.
[2]Faculty of Science, Nepal Academy of Science and Technology, Khumaltar, Lalitpur, NEPAL.
[3]Faculty of Agriculture and Life Science, Hirosaki University, 3-Bunkyo-cho, Hirosaki, Aomori 036-8561, JAPAN.
[4]Departement of Botany, Patan Multiple Campus, Tribhuvan University, Patandhoka, Lalitpur, NEPAL.

Correspondence

Dr. Gan B. Bajracharya

Faculty of Science, Nepal Academy of Science and Technology, Khumaltar, Lalitpur, NEPAL.

Phone no: +977-1-5547368; +977-9849636069

E-mail: ganbajracharya@yahoo.com; gan.bajracharya@nast.gov.np

History

- Submission Date: 10-09-2020;
- Review completed: 03-11-2020;
- Accepted Date: 10-11-2020.

DOI : 10.5530/pj.2021.13.6

Article Available online

http://www.phcogj.com/v13/i1

INTRODUCTION

Belonging to the family Anacardiaceae, over 250 species of genus *Rhus* are distributed worldwide.[1] Species *Rhus chinensis* Mill. (synonyms: *R. javanica* var. *chinensis* (Mill.) T. Yamaz., *R. semialata* Murray) is known as Chinese sumac.[2] In Nepal, it is called as Bhaki-amilo (in Nepali) and Muruk (in Magar language). *R. chinensis* has been used by folk medicine practitioners for long time in Asia.[3] Fruits are used in stomachache, profuse bleeding in menstruation, bloody dysentery, diarrhea, gastrointestinal disorders, and foot and mouth diseases of animals.[4-8] Roots have been used in folk medicines as antitussive, and for the treatments of anasarca, jaundice and snake bite.[9] Gallarhois on the leaf of *R. chinensis* has been used for treating diarrhea, seminal emission, excessive sweating, bleeding, chronic cough and polyuria; and possesses anti-thrombotic and anti-anaphylactic effects.[3,10-13]

Although *R. chinensis* has been consumed since ancient times, the responsible phytoconstituents for the health benefits are remain to be identified.[14] Isolation of gallic acid, gallicin, betulin, betulonic acid, moronic acid, rhuscholide A, benzofuranones, phenolics, etc. has been reported from different parts (root, stem, gallarhois) of *R. chinensis*.[9,15-16] Recently, antibacterial activity of *R. chinensis* against methicillin-resistant *Staphylococcus aureus*,[6] *Shigella* species[17] and *Streptococcus iniae*[18] is reported. *R. chinensis* extract was identified as the most effective anti-inflammatory and anti-photoaging agent.[19] Gu *et al.* have reported significant anti-HIV-1 activity of some constituents isolated from the stems of *R. chinensis*.[15] Hot water extract of the galls showed therapeutic efficacy in mouse HSV-I infection models.[20] In several reports, anti-anaphylactic, anti-thrombotic, antiviral, antibacterial and anti-plague effects of the galls have been reported.[12-13,21-23] Though gall of *R. chinensis* leaf has been much investigated, fruit of it is rarely explored. Zhang *et al.* have reported that fruits of *R. chinensis* are enriched with phenolics and the extracts have exhibited strong antioxidant and pancreatic lipase inhibitory activities.[14]

In Nepal, some ethnobotanical studies have been made on *R. chinensis* considering different ethnic groups.[4,8] During our ethnobotanical survey, we came to know that *R. chinensis* fruits have been used for the treatment of diarrhea and dysentery by the Magar community in Hupsekot rural municipality of Nawalpur district, Gandaki province, Nepal. To validate the ethnomedicinal knowledge, the present research was focused on evaluation of antibacterial along with antioxidant activities of the fruits of *R.*

Cite this article: Nemkul CM, Bajracharya GB, Maeda H, Shrestha I. Ethnomedicinal Knowledge Verification for the Antidiarrheal and Antioxidant Effects of *Rhus chinensis* Mill. Fruits with Identification of Thirty Constituents. Pharmacog J. 2021;13(1): 37-43.

chinensis. Antibacterial activity of the fruits has never been reported though the leaves had displayed antimicrobial activity.[6,17-18] The phytochemicals present in the plant material were also investigated by chemical tests and GC-MS analysis to identify the biologically active phytochemicals.

MATERIALS AND METHODS

Sampling site

The plant material was collected from Hupsekot rural municipality of Nawalpur district, Gandaki Province, Nepal during field visit in April 2017. Ethnomedicinal data of *R. chinensis* in the Magar community was collected through questionnaires, structural and un-structural interviews among healers and knowledgeable people. Fruits were collected from the study area. The fruits used for the studies were dried in shade at room temperature.

Preparation of the extracts

Air dried fruits of *R. chinensis* were ground. The ground material (100 g) was successively extracted with hexane (800 mL) and 70% methanol in water (800 mL) using a Soxhlet extractor until a clean solution was noticed. The extracts were concentrated using a rotary evaporator, vacuum dried and then stored in a refrigerator at 4°C until further use.

Phytochemical screening

Phytochemical screening of the hexane and 70% methanolic extracts was performed using different specific reagents to find out different phytoconstituents present in the fruit extracts.[24] Braymer, Dragendorff, Shinoda, Liebermann-Burchard, Salkowski tests were carried out to detect polyphenols, alkaloid, flavonoids, steroids and terpenoids, respectively.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses of the hexane and 70% methanolic extracts of *R. chinensis* fruits were performed using an Agilent 7890A GC system coupled with an Agilent 5975 C mass selective detector, equipped with a HP-5MS GC column (5% phenyl methyl siloxane, Agilent 19091S-433, 30 m × 250 µm internal diameter, 0.25 µm film thickness). Helium was used as a carrier gas at flow rate of 1.21 mL/min. The instrument was operated in the electron impact (EI) mode at 70 eV and ion source temperature 230°C in the scan range of 50-500 m/z. The initial column temperature was set at 40°C held for 2 min, ramped at a rate of 4°C/min to 270°C and held for 5.5 min (total run time 65 min). Dilute sample solutions of the extracts were prepared in HPLC grade hexane or methanol, and a volume of 2 µL was injected. The constituents were identified by comparing the mass spectra available in a MS database of National Institute Standard and Technology (NIST 08).

Total phenolic content (TPC)

TPC value was estimated by using the Folin-Ciocalteu method.[25] Briefly, a solution of 70% methanolic extract of concentration 0.4 mg/mL was prepared in distilled water. Thus prepared extract solution (50 µL) was treated with 25 µL of Folin-Ciocalteu reagent (Loba Chemie Pvt. Ltd) and 100 µL of aq. Na_2CO_3 solution (75 g/L). After 1 h, absorbance at 760 nm was measured using an Elisa microplate reader (EPOCH2, BioTek Instruments). Distilled water was taken as a blank. To obtain a calibration curve, standard gallic acid solutions of different concentrations 100, 50, 25, 12.5, 6.2, 3.1 and 1.6 µg/mL prepared in distilled water were used. TPC value was expressed as mg gallic acid equivalents (GAE) per g dry extract, which was calculated by using the formula: C = cV/m, where c = concentration of gallic acid obtained from the calibration curve in mg/mL, V = volume of the extract in mL, and m = mass of the extract in g.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The 70% methanolic extract was used to evaluate antioxidant capacity using the DPPH radical scavenging assay.[26] DPPH• radical solution of concentration 0.1 mM was prepared by overnight stirring of DPPH (3.94 mg; Sigma-Aldrich) in methanol (100 mL) at 0°C. A stock methanolic solution of the extract was prepared (concentration 2000 µg/mL). In a microplate, appropriate amounts of the stock solution were diluted with methanol to obtain 1500, 1000, 750, 500, 250, 100, 50 and 25 µg/mL concentrations (total volume = 50 µL), and then treated with 250 µL of DPPH• radical solution. To obtain linear curve of a positive control, 50 µL of gallic acid solutions of concentrations 20, 10 and 5 µg/mL were used. Methanol was used as a blank. A mixture of DPPH• radical solution (250 µL) and methanol (50 µL) was used as a control. After 30 min, the absorbance was determined at 517 nm using an Elisa microplate reader (EPOCH2, BioTek Instruments). The DPPH• radical scavenging ability was calculated according to the equation given below:

$$\text{DPPH}\bullet\ \text{scavenging rate}\ (\%) = 1 - \frac{\text{Absorbance (sample)} - \text{Absorbance (blank)}}{\text{Absorbance (control)} - \text{Absorbance (blank)}} \times 100$$

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was measured according to the instructions for a commercial kit (Radical catch; Hitachi Ltd., Tokyo, Japan).[27] Briefly, 5 mM of cobalt chloride solution (Reagent A; 25 µL) and luminol solution (Reagent B; 25 µL) were mixed. Then 10 µL of a methanolic solution of 70% methanolic extract of 100 µg/mL concentration was added. Subsequently, the content was incubated at 37°C for 5 min in an incubator (Varioskan LUX Multimode Microplate Reader, Thermo Fisher Scientific, Waltham, MA, USA). Thereafter, the mixture was reacted with hydrogen peroxide solution (Reagent C; 25 µL) and then luminescence of light for 120 sec was measured. The luminescence was observed to subtract an amount of 120 to 80 sec. Control was measured using methanol. Hydrogen peroxide scavenging activity was calculated following the equation below:

$$\text{Hydrogen scavenging activity}\ (\%) = \frac{\text{Luminescence (control)} - \text{Luminescence (sample)}}{\text{Luminescence (control)}} \times 100$$

Antibacterial susceptibility assay

The bacterial strains *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6051), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Salmonella enterica* sub-sp. *Entericaserovar typhi*, *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27263) and *Shigella dysenteriae* (ATCC 13313) were used.

Agar well diffusion assay[28]: Inoculum was prepared and standardized. The bacterial inoculums were swabbed on sterile Mueller-Hinton agar (MHA) plates. Both the hexane and 70% methanolic extracts were dissolved in dimethyl sulfoxide (DMSO) to prepare sample solutions of 0.1 g/mL concentration. Wells of 6 mm diameter were bored on the MHA plates and were loaded with 50 µL of the samples prepared. Ampicillin and gentamicin of 10 µg per disc (Mast Diagnostics) were used as standards. DMSO was used as a negative control. The loaded MHA plates were incubated at 37°C for 24 h. Zone of inhibition (ZOI) was measured in mm.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)[29]: In a microplate, the extract solutions of 0.1 g/mL concentration prepared above in DMSO (50 µL) were mixed with Mueller-Hinton broth (MHB) (50 µL) and then the content was serially double diluted. The bacterial suspension adjusted to 1×10^8 cfu mL^{-1} (equivalent of McFarland 0.5) was further diluted to 1:100 using MHB and then 50 µL of the suspension was inoculated. After incubation for 24 h at 37°C, the MIC value was taken

as the lowest concentration that inhibited the visible growth of the tested bacteria. The MBC value was determined by plating directly the content of wells in the MHA plate.

Statistical analysis

Statistical analysis was done using Microsoft excel program. Experiments were performed in triplicates (n = 3) and the results are presented as mean ± standard error mean (SEM).

RESULTS AND DISCUSSION

Phytoconstituents

Successive Soxhlet extractions of the fruits of *R. chinensis* (100 g) yielded the hexane extract (3.67 g, 3.67%, light color) and 70% methanolic extract (11.54 g, 11.54%, reddish brown). Phytochemical screening of the extracts revealed that the fruits of *R. chinensis* constituted terpenoids, polyphenols and flavonoids.

Next, the extracts were used for the GC-MS analyses. Phytoconstituents identified in the hexane extract by GC-MS analysis are presented in Table 1. Sixteen compounds (accounting 94.68%) were identified in the hexane extract with a higher percentage of phenols (47.30%) and then fatty acids (25.25%) followed by hydrocarbons (16.90%). It has been reported that many fatty acids possess antibacterial and antifungal properties.[30] Antibacterial potentiality of hexadecanoic acid methyl ester, 1-heptatriacotanol and 3-pentadecylphenol that identified in the hexane extract of the *R. chinensis* fruits has been reported elsewhere.[31-34]

All together 18 compounds, accounting 94.09%, were identified in the 70% methanolic extract of the fruits of *R. chinensis* by GC-MS analysis (Table 2). The extract constituted abundance of anhydrides (52.38%) and fatty acids (39.09%). As a flavonoid fragment, the extract constituted of 0.32% of 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, which was reported as an antimicrobial and anti-inflammatory ingredient.[35] Hexadecanoic acid methyl ester and 3-pentadecylphenol possess antibacterial activity.[31,33-34] Antioxidant activity of hexadecanoic acid ethyl ester and n-hexadecanoic acid is known.[32,35] Linoleic acid and oleic acid are reported as anti-inflammatory, anti-androgenic, cancer preventive, etc.[32] 5-(Hydroxymethyl)furan-2-carbaldehyde acts as an antimicrobial, antioxidant and anticancer agents, and inhibits the formation of sickled cells in the blood.[36-37]

Table 1: Phytoconstituents identified by GC-MS in the hexane extract of *R. chinensis* fruit.

No.	Retention time	Name of the compound	Molecular formula	Nature of the compound	Peak area (%)
1	38.446	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Fatty acid	0.61
2	39.445	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Fatty acid	8.22
3	40.661	Octadecanal	$C_{18}H_{36}O$	Aldehyde	0.57
4	42.347	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	Fatty acid	2.03
5	43.411	Oleic acid	$C_{18}H_{34}O_2$	Fatty acid	3.93
6	43.716	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	Fatty acid	2.48
7	43.973	Octadecanoic acid	$C_{18}H_{36}O_2$	Fatty acid	5.86
8	48.141	Eicosanoic acid	$C_{20}H_{40}O_2$	Fatty acid	0.38
9	50.454	(Z)-3-(Pentadec-8-en-1-yl)phenol	$C_{21}H_{34}O$	Phenol	26.7
10	50.623	3-Pentadecylphenol	$C_{21}H_{36}O$	Phenol	4.67
11	53.896	1-Heptatriacotanol	$C_{37}H_{76}O$	Alcohol	4.66
12	54.043	Butyl 6,9,12,15-octadecatetraenoate	$C_{22}H_{36}O_2$	Fatty acid	1.74
13	54.349	(Z)-3-(Heptadec-10-en-1-yl)phenol	$C_{23}H_{38}O$	Phenol	15.93
14	54.458	Heptacosane	$C_{27}H_{56}$	Hydrocarbon	6.55
15	56.444	17-Pentatriacontene	$C_{35}H_{70}$	Hydrocarbon	0.53
16	57.911	Octacosane	$C_{28}H_{58}$	Hydrocarbon	9.82

Table 2: Phytoconstituents identified by GC-MS in the 70% methanolic extract of *R. chinensis* fruit.

No.	Retention time	Name of the compound	Molecular formula	Nature of the compound	Peak area (%)
1	4.869	Furfural	$C_5H_4O_2$	Aldehyde	0.43
2	5.502	Maleic anhydride	$C_4H_2O_3$	Anhydride	18.16
3	8.224	3-Methylfuran-2,5-dione	$C_5H_2O_3$	Anhydride	17.37
4	10.815	Succinic anhydride	$C_4H_4O_3$	Anhydride	0.68
5	13.908	2,3,6,7-Tetrahydrooxepine-4-carboxylic acid, ethyl ester	$C_9H_{14}O_3$	Fatty acid	0.88
6	14.470	Butanedioic acid, monomethyl ester	$C_5H_8O_4$	Fatty acid	1.13
7	14.830	dl-Malic acid, dimethyl ester	$C_6H_{10}O_5$	Fatty acid	25.84
8	15.321	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	$C_6H_8O_4$	Pyranone	0.32
9	18.485	5-(Hydroxymethyl)furan-2-carbaldehyde	$C_6H_6O_3$	Aldehyde	0.40
10	21.066	Phthalic anhydride	$C_8H_4O_3$	Anhydride	16.17
11	26.117	Citric acid, trimethyl ester	$C_9H_{14}O_7$	Fatty acid	0.79
12	38.446	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Fatty acid	3.6
13	39.347	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Fatty acid	3.79
14	42.342	Methyl (9Z,11E)-octadeca-9,11-dienoate	$C_{19}H_{34}O_2$	Fatty acid	1.10
15	43.220	Linoleic acid	$C_{18}H_{32}O_2$	Fatty acid	0.75
16	43.351	Oleic acid	$C_{18}H_{34}O_2$	Fatty acid	0.86
17	43.891	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	Fatty acid	0.35
18	50.410	3-Pentadecylphenol	$C_{21}H_{36}O$	Phenol	1.47

Antioxidant property

Phenolics are potent antioxidants.[38] A large number of studies have proved that they are anti-cancer agents. And, several bioassay techniques have been developed to estimate antioxidant capacity of either pure compounds or plant extracts. Here, we have evaluated the antioxidant capacity of the 70% methanolic extract of *R. chinensis* fruit by estimating TPC, and measuring scavenging capacity of DPPH• radical and hydrogen peroxide.

To estimate TPC, a linear curve of standard gallic acid ($Y = 0.023x + 0.088$, $R^2 = 0.996$) was obtained from the measured absorbance values using different gallic acid concentrations. The TPC content in the 70% methanolic extract of the fruits of *R. chinensis* was calculated using the regression equation and was found to be 123.52±1.29 mg GAE/g dry extract. A few authors have reported TPC values in different extracts of the fruits of *R. chinensis*. Fu *et al.* reported TPC value of 17.2±1.49 mg GAE/g wet weight in the tetrahydrofuran extract[39]; Sharma *et al.* reported 172.84±15.33 mg GAE/g extract in the methanolic extract;[40] and Heirangkhongjam and Ngaseppam reported 7.25±0.03, 42.57±0.24, 28.37±0.46 and 35.76±1.71 mg GAE/g extract in the aqueous, acetone, ethanolic and methanolic extracts, respectively.[41] These data clearly indicate that the value of TPC varies with the extractive solvents and our sample constituted comparably a higher amount of phenolics.

In DPPH free radical scavenging activity assay, a linear curve of standard gallic acid ($Y = 3.043x + 12.03$, $R^2 = 0.998$) was obtained from the values of inhibition and the concentrations of gallic acid. The IC_{50} value of gallic acid in the assay was found to be 12.47 µg/mL. We found that the 70% methanolic extract of the fruits of *R. chinensis* scavenged 42.69±0.1% DPPH• radical at 100 µg/mL concentration and maximum of 87.24±0.14% of DPPH• radical scavenged at 750 µg/mL concentration, and the IC_{50} value calculated was 135.54±0.82 µg/mL. Heirangkhongjam and Ngaseppam also reported IC_{50} values of 86.54±0.64, 10.35±0.13, 11.19±0.22 and 12.27±0.04 µg/mL using the aqueous, acetone, ethanolic and methanolic extracts of *R. chinensis* fruits respectively in the DPPH assay.[41] Similar antioxidant activity was also reported by Sharma *et al.*[40]

Next, we found that the 70% methanolic extract scavenged 63.20±1.48% of hydrogen peroxide at 100 µg/mL concentration. From these results, it was considered that the fruits of *R. chinensis* contained phenolic compounds abundantly hence suitable for consumption to affect cancer chemo-prevention.

Antidiarrheal (antibacterial) activity

Results of the antibacterial susceptibility assay of both the hexane and 70% methanolic extracts are given in Table 3 showing ZOI. The 70% methanolic extract showed strong antibacterial activity against resistant strain of *P. aeruginosa, K. pneumoniae* and *B. subtilis*. This extract displayed similar antibacterial potentiality as standard antibiotics used against *E. faecalis*, a causal agent of urinary tract infection. The 70% methanolic extract was found more effective against *S. aureus* than gentamicin. The growth of *E. coli* and *S. dysenteriae* was also effectively inhibited, which are casual agents of diarrhea and dysentery. The hexane extract was also found effective to inhibit the growth of *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. Perhaps the antibacterial activity observed was mainly due to the presence of fatty acids. Noteworthy is that both the extracts were ineffective towards *S. typhi*. You *et al.* have reported antimicrobial activity of the ethanolic extract of *R. chinensis* leaf against methicillin-resistant *S. aureus*, and mentioned its bactericidal effect on the bacterial strain.[6] Yang *et al.* have also reported antimicrobial activity of the ethanolic extract of *R. chinensis* against S. *dysenteriae*;[17] however, to the best of our knowledge, antimicrobial activity of the fruits of *R. chinensis* is not evaluated yet.

MIC and MBC values obtained are tabulated in Table 4. The 70% methanolic extract displayed bactericidal effect on *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa* while it was bacteriostatic to *B. subtilis*, *K. pneumoniae* and *S. dysenteriae*. The bacteria were killed at higher concentrations of the extract in a dose-dependent manner. The hexane extract was found significantly effective against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*.

The result of the antimicrobial susceptibility assay presented above supports the ethnomedicinal use of *R. chinensis* fruit by magars of

Table 3: Antibacterial activity of the extracts of *R. chinensis* fruit.

Sample	Diameter of ZOI ± SEM (in mm)							
	Gram positive bacteria				Gram negative bacteria			
	Sa	Bs	Ef	Ec	St	Kp	Pa	Sd
Hexane extract	14.00 ± 0.57	15.66 ± 0.33	–	16.00 ± 0.33	–	–	12.33 ± 0.33	–
70% Methanolic extract	20.25 ± 0.75	20.66 ± 0.33	17.83 ± 0.16	23.00 ± 0.57	–	14.66 ± 0.33	16.33 ± 0.33	14.66 ± 0.33
Ampicillin	32.50 ± 0.50	8.50 ± 0.50	17.75 ± 0.25	25.00 ± 1.00	15.50 ± 0.50	8.50 ± 0.50	–	23.75 ± 0.25
Gentamicin	16.75 ± 0.25	15.50 ± 0.50	18.5 ± 0.50	17.50 ± 0.50	12.66 ± 0.33	11.33 ± 0.88	14.66 ± 0.33	18.66 ± 0.66
DMSO	–	–	–	–	–	–	–	–

Sa = *S. aureus*, Bs = *B. subtilis*, Ef = *E. faecalis*, Ec = *E. coli*, St = *S. typhi*, Kp = *K. pneumoniae*, Pa = *P. aeruginosa*, Sd = *S. dysenteriae*. (–) sign indicates no ZOI was observed.

Table 4: MIC and MBC values of the extracts of *R. chinensis* fruit.

No.	Bacterial strain	Hexane extract		70% Methanolic extract	
		MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
1	*S. aureus*	6.25	50	3.12	3.12
2	*B. subtilis*	12.5	25	25	50
3	*E. faecalis*	–	–	25	25
4	*E. coli*	1.56	6.25	25	25
5	*S. typhi*	–	–	–	–
6	*K. pneumoniae*	–	–	6.25	25
7	*P. aeruginosa*	12.5	50	12.5	12.5
8	*S. dysenteriae*	–	–	12.5	25

the study site for curing of diarrhea and dysentery. From the result, it can also be concluded that the fruits of *R. chinensis* are efficacious against other infectious diseases, such as urinary tract infection and pneumonia, as the fruit extracts exhibited antimicrobial activity against the related bacteria of the diseases.

CONCLUSION

The Magar community of Hupsekot rural municipality, Nawalpur district, Gandaki Province, Nepal uses fruits of *R. chinensis* (with yogurt) for the treatment of diarrhea and dysentery. This work showed an efficient antibacterial activity of the fruits of *R. chinensis* against *E.coli* and *S. dysenteriae* in the support of the traditional knowledge. The growth of *S. aureus*, *B. subtilis* and *P. aeruginosa* were also inhibited by the extracts indicating the antibacterial efficacy of the plant material in the treatment of other infectious diseases. Evaluation of TPC, and DPPH• radical and hydrogen peroxide scavenging activities has indicated that the fruits of *R. chinensis* constituted potential antioxidants.

ACKNOWLEDGEMENT

We thank the University Grants Commission (UGC), Nepal for providing a research grant. For laboratories facilities, Nepal Academy of Science and Technology (NAST) is highly acknowledged. We thank local villagers, healers and informants of Hupsekot rural municipality of Nawalpur district for their cooperation. Thanks to the National Herbarium and Plant Laboratories, Godavari, Lalitpur for the plant identification.

CONFLICTS OF INTEREST

No conflicts of interest has been declared by any of the authors.

ABBREVIATIONS

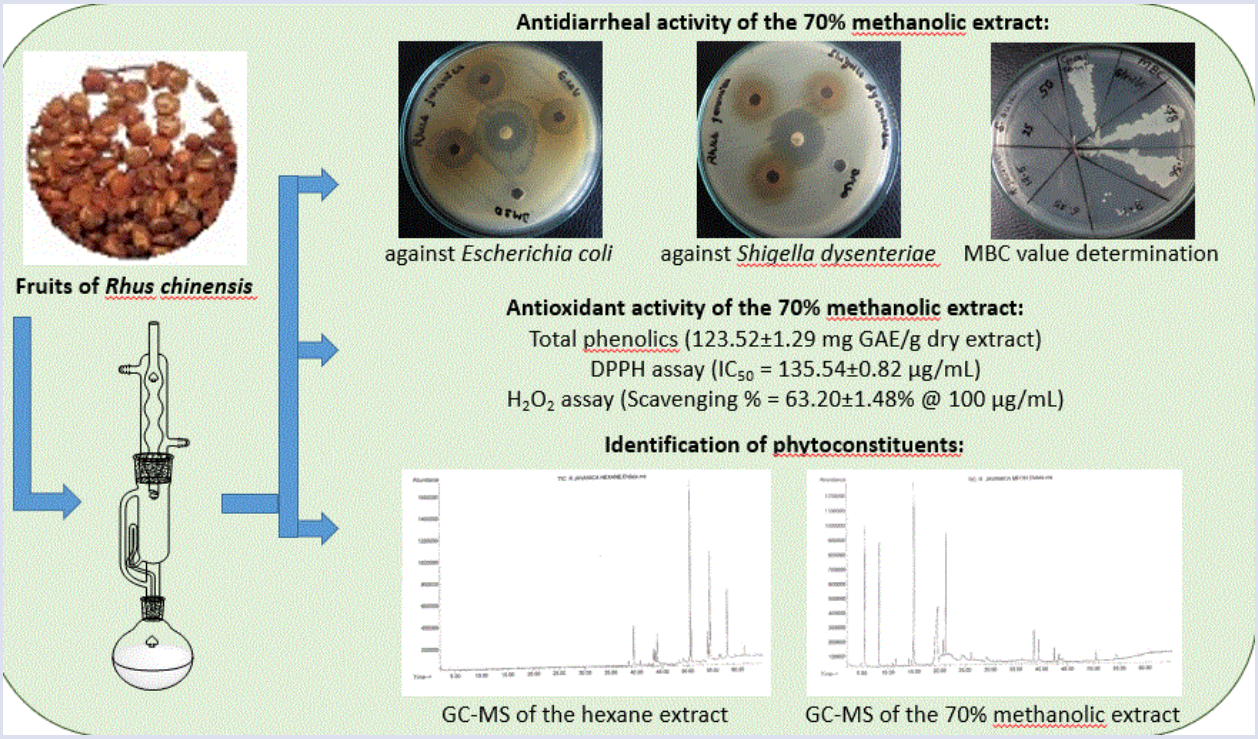
DPPH: 2,2-diphenyl-1-picrylhydrazyl; GAE = gallic acid equivalents; GC-MS: gas chromatography-mass spectrometry; HPLC = high performance liquid chromatography; IC_{50}: 50% inhibition concentration; MBC: minimum bactericidal concentration; MHA = Mueller-Hinton agar; MHB = Mueller-Hinton broth; MIC: minimum inhibitory concentration; TPC = total phenolic content; ZOI: zone of inhibition.

REFERENCES

1. Rayne S, Mazza G. Biological activities of extracts from sumac (*Rhus spp.*): a review. Plant Foods Human Nutr. 2007;62:165-75.
2. Wagstaff DJ. International poisonous plants checklist: an evidence-based reference. Boca Raton: CRC Press, Taylor and Francis; 2008.
3. Djakpo O, Yao W. *Rhus chinensis* and *Galla chinensis* - folklore to modern evidence: review. Phytother Res. 2010;24:1739-47.
4. Parajuli SP. Ethnobotanical study at Khandbari municipality of Sankhuwasabha district, Nepal. Banko Janakari. 2000;10:29-34. Available from: https://www.nepjol.info/index.php/BANKO/article/view/17600
5. Pradhan BK, Badola HK. Ethnomedicinal plant use by Lepcha tribe of Dzongu valley, bordering Khangchendzonga, Biosphere Reserve, in north Sikkim, India. J Ethnobiol Ethnomed. 2008;4:22. Doi: 10.1186/1746-4269-4-22.
6. You YO, Choi NY, Kang SY, Kim KJ. Antibacterial activity of *Rhus javanica* against methicillin-resistant *Staphylococcus aureus*. Evid Based Complement Alternat Med. 2013. http://dx.doi.org/10.1155/2013/549207.
7. Tangpu V, Yadav AK. Antidiarrhoeal activity of *Rhus javanica* ripen fruit extract in albino mice. Fitopterapia. 2004;75:39-44.
8. Manandhar NP. Plants and people of Nepal. Portland Oregon, USA: Timber Press; 2002.
9. Lee TH, Chiou JL, Lee CK, Kuo YH. Separation and determination of chemical constituents in the roots of *Rhus javanica* L. var. *roxburghiana*. J Chinese Chem Soc. 2005;52:833-41.
10. Lee SM, Lee DW, Park JD, Kim JI. Study on formation and development of gall in *Rhus javanica*. Korean J Appl Entomology. 1997;36:83-7.
11. Song GY, Park BJ, Kim SH. Antithrombotic effect of galla rhois. Korean J Pharmacog. 2002;33:120-3.
12. Kim SH, Park HH, Lee S, Jun CD, Choi BJ, Kim SY, *et al.* The anti-anaphylactic effect of the gall of *Rhus javanica* is mediated through inhibition of histamine release and inflammatory cytokine secretion. Int Immunopharmacol. 2005;5:1820-9.
13. Shim YJ, Doo HK, Ahn SY, Kim YS, Seong JK, Park IS, *et al.* Inhibitory effect of aqueous extract from the gall of *Rhus chinensis* on alpha-glucosidase activity and postprandial blood glucose. J Ethnopharmacol. 2003;85:283-7.
14. Zhang C, Ma Y, Zhao Y, Hong Y, Cai S, Pang M. Phenolic composition, antioxidant and pancreatic lipase inhibitory activities of Chinese sumac (*Rhus chinensis* Mill.) fruits extracted by different solvents and interaction between myricetin - 3 - O - rhamnoside and quercetin - 3 - O - rhamnoside. Int J Food Sci Technol. 2018;53:1045-53.
15. Gu Q, Wang RR, Zhang XM, Wang YH, Zheng YT, Zhou J, *et al.* A new benzofuranone and anti-HIV constituents from the stems of *Rhus chinensis*. Planta Med. 2007;73:279-82.
16. Wang RR, Gu Q, Wang YH, Zhang XM, Yang LM, Zhou J, *et al.* Anti-HIV-1 activities of compounds isolated from the medicinal plant *Rhus chinensis*. J Ethnopharm. 2008;117:249-56.
17. Yang SK, Kim HJ, Oh SW. Antimicrobial activities of ethanol extracts of *Rhus javanica* and *Terminalia chebula* against *Shigella* species. J Food Safety. 2014;34:95-102.
18. Choi HS, Kim JS, Jang DS, Yu YB, Kim YC, Lee JS. Antibacterial activities of galla rhois extracts against fish pathogenic bacteria. J Fish Pathol. 2005;18:239-45.
19. Ha JS, Lee J, Kim H, Song KM, Lee NH, Kim YE, *et al.* Preventive effect of *Rhus javanica* extract on UVB-induced skin inflammation and photoaging. J Functional Foods. 2016;27:589-99.
20. Kurokawa M, Ochiai H, Nagasaka K, Neki M, Xu H, Kadota S, *et al.* Antiviral traditional medicines against herpes simplex virus (HSV-1), poliovirus, and measles virus in vitro and their therapeutic efficacies for HSV-1 infection in mice. Antiviral Res. 1993;22:175-88.
21. Kurokawa M, Sato H, Ohyama H, Hozumi T, Namba T, Kawana T, *et al.* Effects of traditional herbal medicines against herpes simplex virus (HSV) type 2 and acyclovir-resistant HSV type1 *in vitro* and *in vivo*. J Tradit Med. 1995;12:187-94.
22. Nakano M, Kurokawa M, Hozumi T, Saito A, Ida M, Morohashi M, *et al.* Suppression of recurrent genital herpes simplex virus type 2 infection by *Rhus javanica* in guinea pigs. Antiviral Res. 1998;39:25-33.
23. Cho MJ, Hong SJ, Choi CH, Jeong SS. Effects of dentifrice containing extract of galla rhois or *Psoralea corylifolia* on inhibition of plaque formation. J Korean Acad Dental Health. 2005;29:141-52.
24. Ciulei I. Practical manual on the industrial utilisation of medicinal and aromatic plants 1. Methodology for analysis of vegetable drugs. Bucharest, Romania; 1982.
25. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999;299:152-78.
26. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci Technol. 1995;28:25-30.
27. Maeda H, Fukuda S, Izumi H, Saga N. Anti-oxidant and fucoxanthin contents of brown alga Ishimozuku (*Sphaerotrichia divaricata*) from the west coast of Aomori, Japan. Marine Drugs. 2018;16:255. Doi: 10.3390/md16080255.
28. Perez C, Pauli M, Bazerque P. An antibiotic assay by the agar-well diffusion method. Acta Biologiae et Medecine Experimentalis. 1990;15:113-5.
29. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature protocols. 2008;3:163-75.
30. Knapp HR, Melly MA. Bactericidal effects of polyunsaturated fatty acids. J Infectious Diseases. 1986;154:84-94.
31. Rukshana MS, Doss A, Kumari PRTP. Phytochemical screening and GC-MS analysis of leaf extract of *Pergularia daemia* (Forssk) Chiov. Asian J Plant Sci Res. 2017;7:9-15.
32. Alagammal M, Tresina SP, Mohan VR. Chemical investigations of *Polygala chinensis* L. by GC-MS. Sci Res Reporter. 2011;1:49-52.
33. Nemkul CM, Bajracharya GB, Shrestha I. Phytochemical, antibacterial and DPPH free radical scavenging evaluations of the barks of *Aegle marmelos* (L.) Correa. J Pharmacog Phytochem. 2018;7:1637-41.
34. Devakumar J, Keerthana V, Sudha SS. Identification of bioactive compounds by gas chromatography-mass spectrometry analysis of *Syzygium jambos* (L) collected from Western Ghats region Coimbatore, Tamil Nadu. Asian J Pharma Clin Res. 2017;10:364-9.
35. Kumar PP, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. African J Biochem Res. 2010;4:191-5.
36. Jadhav V, Kalase V, Patil P. GC-MS analysis of bioactive compounds in methanolic extract of *Holigarnagrahamii* (wight) Kurz. Int J Herbal Med. 2014;2:35-9.

37. Al-Marzoqi AH, Hadi MY, Hameed IH. Determination of metabolites products by *Cassia angustifolia* and evaluate antimicrobial activity. J Pharmacog Phytother. 2016;8:25-48.
38. Zeb A. Concept, mechanism, and applications of phenolic antioxidants in foods. *J Food Biochem.* 2020; e13394. Doi: 10.1111/jfbc.13394
39. Fu L, Xu BT, Xu XR, Qin XS, Gan RY, Li HB. Antioxidant capacities and total phenolic contents of 56 wild fruits from South China. Molecules. 2010;15:8602-17.
40. Sharma PB, Handique PJ, Devi HS. Antioxidant properties, physico-chemical characteristics and proximate composition of five wild fruits of Manipur, India. J Food Sci Technol. 2015;52:894-902.
41. Heirangkhongjam MD, Ngaseppam IS. Nutritional evaluation of *Rhus chinensis* Mill. (Heimang) and development of value added products. Indian J Tradit Knowledge. 2019;18:16-24.

GRAPHICAL ABSTRACT



SUMMARY

- Thirty phytoconstituents present in the fruit of *Rhus chinensis* Mill. are identified. (Z)-3-(Pentadec-8-en-1-yl)phenol, (Z)-3-(heptadec-10-en-1-yl)phenol, maleic anhydride, phthalic anhydride, 3-methylfuran-2,5-dione and dl-malic acid, dimethyl ester are the major constituents.
- The fruit possesses antidiarrheal property as the extracts could inhibit the growth of *Escherichia coli* and/or *Shigella dysenteriae*.
- The fruit contains abundant amounts of phenolics and antioxidants hence considered to be efficacious in cancer prevention.

ABOUT AUTHORS

Chandra Mohini Nemkul is currently working in the capacity of Associate professor at Department of Botany, Tri-Chandra Multiple Campus, Tribhuvan University, Nepal. She has been involving in research focusing on ethnomedicinal plants used by indigenous people, evaluating indigenous knowledge scientifically as well as isolation and identification of bioactive compounds. Previously, she had successfully conducted studies on ethnomedicinal knowledge of Newar communities residing in Nepal, a project granted by University Grants Commission (UGC), Nepal.

Dr. Gan B. Bajracharya is a Senior Scientific Officer at Nepal Academy of Science and Technology, Nepal. He received his Doctor of Science degree in 2004 from Tohoku University, Japan working under Prof. Dr. Yoshinori Yamamoto. He carried out post-doctoral researches at Osaka University, Japan (with Prof. Dr. Hiroaki Sasai); University of Houston, Texas (with Prof. Dr. Olafs Daugulis); and HEJ Research Institute of Chemistry, University of Karachi, Pakistan (with Prof. Dr. Muhammad Iqbal Choudhary). He has published about 50 research articles in international and national peer-reviewed journals. His research interest is focused on the development of catalytic reactions and natural product chemistry.

Dr. Hayato Maeda is Associate Professor in Faculty of Agriculture and Life Science at Hirosaki University, Japan. He analyzes chemicals in foods using high performance liquid chromatography and gas chromatography. He is affiliated with Japan Society for Bioscience, Biotechnology, and Agrochemistry; Japanese Society of Nutrition and Food Science; the Japanese Society for Food Science and Technology; Japanese Society for Food Factors; etc.

Dr. Ila Shrestha (Pradhan) is Professor at Department of Botany, Patan Multiple Campus, Tribhuvan University, Nepal. Her research is focused on ethnobotanical study of different geographic areas and social communities with application of Geographic Information System and Remote Sensing. She had involved in herbal farming in Dhading district, Nepal. She is affiliated with Ethnobotanical Society of Nepal.

Cite this article: Nemkul CM, Bajracharya GB, Maeda H, Shrestha I. Ethnomedicinal Knowledge Verification for the Antidiarrheal and Antioxidant Effects of *Rhus chinensis* Mill. Fruits with Identification of Thirty Constituents. Pharmacog J. 2021;13(1): 37-43.