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

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**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 established 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 123.5±1.98 mg GAE/g dry extract was estimated. IC₅₀ 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.

**INTRODUCTION**

Belonging to the family Anacardiaceae, over 250 species of genus *Rhus* are distributed worldwide.¹ Species *Rhus chinensis* Mill. (synonyms: *R. javonica 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.⁴-⁶ Roots have been used in folk medicines as antitussive, and for the treatments of anasarca, jaundice and snake bite.⁷ 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.⁸-¹³

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*.¹⁵-¹⁶ Recently, antibacterial activity of *R. chinensis* against methicillin-resistant *Staphylococcus aureus,*² Shigella species¹⁷ and *Streptococcus iniae*²⁰ is reported. *R. chinensis* extract was identified as the most effective anti-inflammatory and anti-phoatoaging agent.²¹ Gu et al. have reported significant anti-HIV-1 activity of some constituents isolated from the stems of *R. chinensis.*²² Hot water extract of the galls showed therapeutic efficacy in mouse HSV-1 infection models.²³ In several reports, anti-anaphylactic, anti-thrombotic, antiviral, antibacterial and anti-plague effects of the galls have been reported.²⁴-²⁶-²⁷ 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.²⁸

In Nepal, some ethnobotanical studies have been made on *R. chinensis* considering different ethnic groups.²⁹ 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.*
Antibacterial activity of the fruits has never been reported though the leaves had displayed antimicrobial activity. 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 4ºC 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. 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 x 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. 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 αq. Na₂CO₃ 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, 6.25, 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: $C = \frac{C_v}{V}$, 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-picylhydrazyl (DPPH) radical scavenging assay**

The 70% methanolic extract was used to evaluate antioxidant capacity using the DPPH radical scavenging assay. 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:

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

**Hydrogen peroxide scavenging activity**

Hydrogen peroxide scavenging activity was measured according to the instructions for a commercial kit (Radical catch; Hitachi Ltd., Tokyo, Japan). 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:

$$\frac{\text{Luminescence (control)} - \text{Luminescence (sample)}}{\text{Luminescence (control)}} \times 100$$

**Antibacterial susceptibility assay**

The bacterial strains *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 25922), *Salmonella enterica* sub-sp. *Entericerovar typhi*, *Klebsiella pneumonia* (ATCC 708063), *Pseudomonas aeruginosa* (ATCC 27283) and *Shigella dysenteriae* (ATCC 13313) were used.

**Agar well diffusion assay**

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)**: 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 1×10⁶ 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. 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. 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. Hexadecanoic acid methyl ester and 3-pentadecylphenol possess antibacterial activity. Antioxidant activity of hexadecanoic acid ethyl ester and n-hexadecanoic acid is known. Oleic acid are reported as anti-inflammatory, anti-androgenic, cancer preventive, etc. 5-(Hydroxymethyl)furans-2-carbaldehyde acts as an antimicrobial, antioxidant and anticancer agents, and inhibits the formation of sickled cells in the blood.

### 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_{2} | 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_{19}H_{34}O_{2} | Fatty acid             | 4.86         |
| 7   | 43.973        | Octadecanoic acid  | C_{18}H_{34}O_{2} | Fatty acid             | 5.86         |
| 8   | 48.141        | Eicosanoic acid     | C_{20}H_{34}O_{2} | Fatty acid             | 0.38         |
| 9   | 50.454        | (Z)-3-(Pentadec-8-en-1-yl)phenol | C_{20}H_{34}O_{2} | Phenol                 | 26.7         |
| 10  | 50.623        | 3-Pentadecylphenol  | C_{21}H_{36}O_{2} | Phenol                 | 4.67         |
| 11  | 53.896        | 1-Heptatriacotanol  | C_{22}H_{38}O_{2} | Alcohol                | 4.66         |
| 12  | 54.043        | Butyl 6,9,12,15-octadecatetraenoate | C_{23}H_{46}O_{2} | Fatty acid             | 1.74         |
| 13  | 54.349        | (Z)-3-(Heptadec-10-en-1-yl)phenol | C_{24}H_{46}O_{2} | Phenol                 | 15.93        |
| 14  | 54.458        | Heptacosane         | C_{25}H_{46}O_{2} | Hydrocarbon            | 6.55         |
| 15  | 56.444        | 17-Pentatriacotene  | C_{26}H_{48}O_{2} | Hydrocarbon            | 0.53         |
| 16  | 57.911        | Octacosane          | C_{28}H_{50}O_{2} | 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_{5}H_{4}O        | Aldehyde               | 0.43         |
| 2   | 5.502         | Maleic anhydride     | C_{4}H_{2}O        | Anhydride              | 18.16        |
| 3   | 8.224         | 3-Methylfuran-2,5-dione | C_{5}H_{4}O        | Anhydride              | 17.37        |
| 4   | 10.815        | Succinic anhydride   | C_{5}H_{4}O        | Anhydride              | 0.68         |
| 5   | 13.908        | 2,3,6,7-Tetrahydroxepine-4-carboxylic acid, ethyl ester | C_{15}H_{10}O_{3} | Fatty acid             | 0.88         |
| 6   | 14.470        | Butanedioic acid, monomethyl ester | C_{10}H_{16}O_{2} | Fatty acid             | 1.13         |
| 7   | 14.830        | dl-Malic acid, dimethyl ester | C_{10}H_{16}O_{2} | Fatty acid             | 25.84        |
| 8   | 15.321        | 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one | C_{16}H_{16}O_{3} | Pyranone               | 0.32         |
| 9   | 18.485        | 5-(Hydroxymethyl)furans-2-carbaldehyde | C_{19}H_{34}O_{2} | Aldehyde               | 0.40         |
| 10  | 21.066        | Phthalic anhydride   | C_{4}H_{4}O        | Anhydride              | 16.17        |
| 11  | 26.117        | Citric acid, trimethyl ester | C_{5}H_{10}O_{3} | Fatty acid             | 0.79         |
| 12  | 38.446        | Hexadecanoic acid, methyl ester | C_{16}H_{32}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)-octadec-a-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_{32}O_{2} | Fatty acid             | 0.86         |
| 17  | 43.891        | Hexadecanoic acid, ethyl ester | C_{18}H_{34}O_{2} | Fatty acid             | 0.35         |
| 18  | 50.410        | 3-Pentadecylphenol  | C_{21}H_{36}O_{2} | Phenol                 | 1.47         |
Antioxidant property

Phenolics are potent antioxidants. 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, R² = 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, Sharma et al. reported 172.84±15.33 mg GAE/g extract in the methanolic extract, 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. 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, R² = 0.998) was obtained from the values of inhibition and the concentrations of gallic acid. The IC₅₀ value of gallic acid in the assay was found to be 12.47 µg/mL. The IC₅₀ values of gallic acid concentrations. The TPC content in 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. The IC₅₀ value calculated was 135.54±0.82 µg/mL. Heirangkhongjam and Ngaseppam also reported IC₅₀ 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. Similar antioxidant activity was also reported by Sharma et al.

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 causal 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 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 mitchellin-resistant S. aureus, and mentioned its bactericidal effect on the bacterial strain. Yang et al. have also reported antimicrobial activity of the ethanolic extract of R. chinensis against S. dysenteriae; 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          | Gram positive bacteria | Diameter of ZOI ± SEM (in mm) | Gram negative bacteria |
|-----------------|------------------------|-------------------------------|------------------------|
|                 | Sa                     | B.s                        | 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  | 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           |
| extract         | 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           |
| Gentamycin      | 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, B.s = 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 | MIC (mg/mL) | MBC (mg/mL) | 70% Methanolic extract | MIC (mg/mL) | MBC (mg/mL) |
|-----|-----------------|----------------|-------------|-------------|-------------------------|-------------|-------------|
| 1   | S. aureus       | 6.25           | 50          | 3.12        | 3.12                    | 3.12        | 3.12        |
| 2   | B. subtilis     | 12.5           | 25          | 25          | 25                      | 25          | 25          |
| 3   | E. faecalis     | –              | –           | 25          | 25                      | 25          | 25          |
| 4   | E. coli         | 1.56           | 6.25        | 25          | 25                      | 25          | 25          |
| 5   | S. typhi        | –              | –           | –           | –                       | –           | –           |
| 6   | K. pneumoniae   | –              | 6.25        | –           | 25                      | 25          | 25          |
| 7   | P. aeruginosa   | 12.5           | 50          | –           | 12.5                    | 12.5        | 12.5        |
| 8   | S. dysenteriae  | –              | 12.5        | –           | 25                      | 25          | 25          |

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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.

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**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<sub>50</sub>: 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* Mill. Fruits with Identification of Thirty Constituents. Pharmacognosy Journal, 2014;6:150-4.
4. Parajuli SP. Ethnobotanical study at Khandbari municipality of Sankhuwasabha district, Nepal. Banko Janakari. 2000;10:29-34. Available from: https://www.nejol.info/index.php/BANKO/article/view/17600
5. Pradhan BK, Badola HK. Ethnomedicinal plant use by Lepcha tribe of Dzongu valley, bordering Khandchendzonga, Biosphere Reserve, in north Sikkim, India. J Ethnobiol Ethnomed. 2006;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. Tanguo Y, Yadav AK. Antidiarrhoeal activity of *Rhus verniciflua* fruit in albino mice. Fitoterapia. 2004;75:39-44.
8. Manandhar NP. Plants and people of Nepal. Portland Oregon, USA: Timber Press; 2002.
9. Li TH, Chiu JL, Lee CK, Kuo YH. Separation and determination of chemical constituents in the roots of *Rhus javanica* 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 Entomol. 1997;36:83-7.
11. Song GY, Park BJ, Kim SH. Antithrombotic effect of galla rhos. Korean J Pharmacoc. 2002;32:125-3.
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37. Al-Marzoqi AH, Hadi MY, Hameed IH. Determination of metabolites products by Cassia angustifolia and evaluate antimicrobial activity. J Pharmacog Phytother. 2016;8:26-48.
38. Zeb A. Concept, mechanism, and applications of phenolic antioxidants in foods. J Food Biochem. 2020; e13394. Doi: 10.1111/jfb.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. (Heimangl) and development of value added products. Indian J Tradit Knowledge. 2019;18:16-24.

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.

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