Comparative assessment of ethnobotany and antibacterial activity of *Moringa oleifera* Lam. in Nepal

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Research

Abstract

Background: *Moringa oleifera* Lam. (Miracle tree) is traditionally used as food, vegetable and medicine in different parts of Nepal, to be precise in lowland Tarai. This study aimed at documenting the ethnobotanical knowledge regarding the use of *M. oleifera*, screening and testing the phytochemicals obtained from different parts (root, bark, leaves, and seeds) of the species and comparing the traditional and lab-based information for advancement in bioprospecting.

Methods: Assessment of ethnobotanical use of *M. oleifera* was carried out using questionnaire survey and informal meetings while the laboratory experiments were performed to appraise the chemical constituents and their activities. The crude methanolic extract of different plant parts of *M. oleifera* was prepared by cold percolation method and then qualitative phytochemical screening was done following standard protocols. The antibacterial activities of different plant parts were tested using agar-well diffusion method against five different human pathogenic bacteria namely *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Results: The plant is being used in 16 districts of lowland Tarai of Nepal for the treatment of 22 ailments including six potentially bacterial ailments: inflammation, tuberculosis, hysteria, diabetes, piles and tumors. Of the five useful plant parts, leaf, root, fruit/seed and bark were frequently harvested while the flower was least used. Analogous to the ethnomedicinal uses, phytochemical compounds of the plant, flavonoids, tannins, phenols, and glycosides exhibited the strong antibacterial activities. The extract from bark showed the higher zone of inhibition followed

by leaf and seed, revealing their high potentials for pharmacology. Bark showed the high antibacterial activity against *B. subtilis* followed by leaf, whereas seed shows its best against *S. aureus* and root against *E. coli*.

Conclusions: M. oleifera is a promising medicinal plant based on our ethnobotanical survey and laboratory assessment. More research on its ethnomedicinal and biochemical capabilities is needed. Documentation and comparative assessment of traditional knowledge and phytochemical findings might lead a consented and conscientious avenue for bio-prospecting and novel drug discovery.

Keywords: Miracle tree, crude extracts, agar-well diffusion method, phytochemicals, ethnomedicine, bioprospecting.

Background

Moringa oleifera is a species of the monogeneric family Moringaceae and is one of the 13 species of genus *Moringa* (order: Brassicales) (Olson & Fahey 2011; Minerva *et al.* 2016), which is a fast-growing evergreen medium-sized tree measured up to 10-12m high, can show its best in poor dry sandy soil can tolerate pH up to 9 and requires rainfall about 250-2000 mm dependent upon soil form (Azad *et al.* 2015; Amabye *et al.* 2016) native to India, Bangladesh, Pakistan, Afghanistan, West Africa, Arabia, South Asia, South America and the Pacific and Caribbean Islands areas and is known by names around the globe such as drumstick or **shobhanjana** in India, **malunggay** in Philippines, horseradish tree in Africa and America, **sitalchini** or **sajiwan** in Nepal. It is also naturalized in Southern Florida to Argentina and the islands of Caribbean and West Indies (Fahey *et al.* 2005; Olson 2010; Imohiosen *et al.* 2014; Amabye & Tadesse 2016). It grows best in dry sandy soil and has pH tolerance level from 5 to 9 (Morton 1991; Azad *et al.* 2015). It has a wide and open crown of droopy, delicate branches, tri-pinnate leaves, and thick corky whitish bark (Nikkon *et al.* 2003).

M. oleifera is considered as the world's most useful tree due to its nutritional and medicinal values (Elangovan *et al.* 2014). Leaf and fruit of *M. oleifera* are used as vegetables which is reported to contain more protein than eggs and milk, 4 times higher vitamin A than carrot, 7 times higher vitamin C than oranges, and high amount of several minerals such as calcium, iron, potassium etc. (Ashfaq *et al.* 2012; Fahey 2005; Fugile 2001). Similarly, root, bark, stems bark, exudates, leaves, flowers, and seeds of *M. oleifera* are also used traditionally to treat several ailments (Fahey 2005; Ramchandran *et al.* 1980). Bark is frequently used to treat rheumatism in Nepal (Acharya-Siwakoti & Pokharel 2006). Other ethnomedicinal records of *M. oleifera* in Nepal are meant to use for treatment of constipation (DPR, 2007), blood pressure (Mandar & Chaudhary 1993; Chaudhary & Rai 2017), hair growth (Singh 2017), tuberculosis (Siwakoti & Siwakoti 2000; Rai 2004; Uprety *et al.* 2012); liver disorders (Kunwar & Bussmann 2009; Kunwar *et al.* 2009), diabetes (Manandhar 1986; Siwakoti *et al.* 2005), chocked voice and glandular swelling (Singh *et al.* 2018), articular pain, headaches, sore throat (pharyngitis), and piles (Thakur & Bajagain 2020). Seed decoction with other ingredients are used as vermifuge (Bhattarai 1992; Pathak *et al.* 2020; Dev Das *et al.* 2021); purgative (Manandhar 2002), heart tonic and coolant (Siwakoti *et al.* 2005; Singh *et al.* 2011; Dhakal 2019), antipyretic, anti-inflammatory (Uprety *et al.* 2012; Pokhrel *et al.* 2016); tonic (Devkota & Bhusal 2020), and anti-tumors (KC *et al.* 2022).

A variety of medicinal plants used in traditional medicines are being assessed for their antibacterial properties (Olatunde *et al.* 2022; Rahman *et al.* 2009). *M. oleifera* Lam. is one of such well-known plants of subtropical and tropical regions that is valued for its significant nutraceutical (Gopalakrishnan *et al.* 2016; Moyo *et al.* 2011), antimicrobial (Oluduro 2012; Moyo *et al.* 2012; Prasad *et al.* 2014; Bukar *et al.* 2010), anti-inflammatory (Cheenpracha *et al.* 2010; Sashidhara *et al.* 2009), and antifungal values (Singh *et al.* 2013; Alhakmani *et al.* 2013). Because *M. oleifera* contains unique combination of isothiocyanate and glucosinolates, it also shows antitumor activities (Jung 2014; Farooq *et al.* 2012).

Regarding antibacterial properties, ethyl acetate and acetone extract of *M. oleifera* bark showed significant activities against *S. aureus* and *P. aeruginosa* (Dewangan *et al.* 2010), whereas the n-hexane extract of plant seeds was found to show a higher inhibition on *S. typhi, V. cholerae* and *E. coli* (Peter *et al.* 2011). Similarly, leaf extract was found to inhibit *S. aureus* and *E. coli* and *P. aeruginosa* (Singh & Tafida 2014). Shailemo *et al.* (2016) found antibacterial activities of n-hexane extract of *M. oleifera* bark against *E. coli*, *E. faecalis*, and *B. cereus*. Despite the studies, a comparative assessment of efficacies of all plant parts of *M. oleifera* was missing. Despite its availability and common usage in folklore in Nepal as vegetable, food and medicine, *M. oleifera* is underutilized and understudied with its potential (Devkota & Bhusal 2020). In this regard this study aims at documenting traditional uses of different

parts of *M. oleifera* and comparing the uses with the pharmacological findings in order to use the plant with greater confidence and to help advance the possible bioprospecting.

Materials and Methods

Study area and ethnobotanical surveys

Three field surveys were carried out between 2017 and 2018, where based on study species availability we selected 16 districts representing each seven provinces of Nepal (Figure 1, yellow asterisks). In each district, 12 people belonging to low income with different ethnic group, gender, caste and who have at least one *Moringa* tree in their house and who consume it and local healers were selected as the key informants. Local healers were consulted to acquire pharmacological uses of study species and to validate the results of the questionnaire survey.

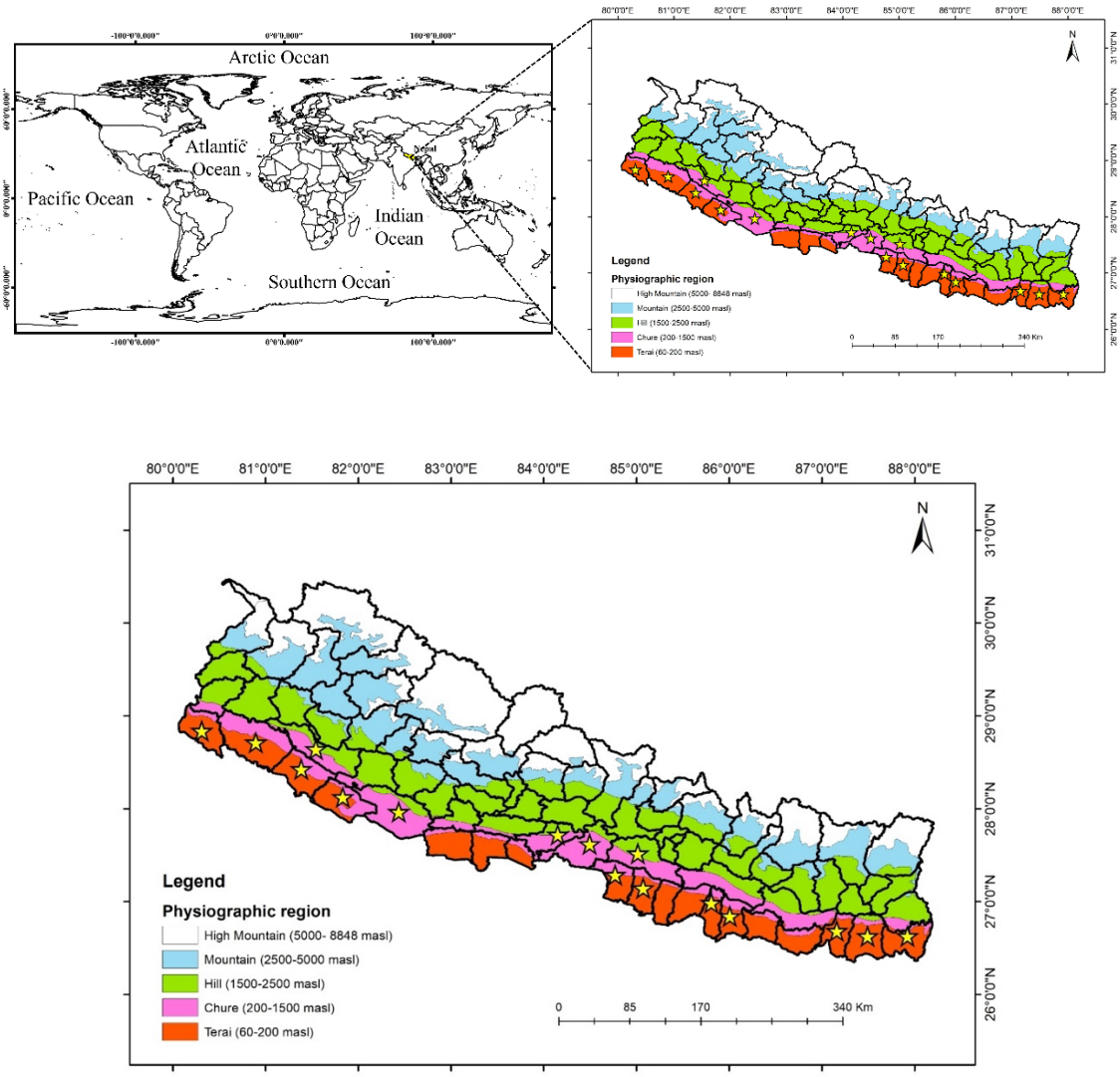

Figure 1. Key map including political map of Nepal, where small, yellow-colored stars show 16 survey districts (Kanchanpur, Kailali, Bardiya, Banke, Dang, Surkhet, Nawalpur, Chitwan, Makwanpur, Bara, Parsa, Mahottari, Dhanusa, Sunsari, Morang and Jhapa) with the presence of *Moringa oleifera.*

Altogether 192 people (12 from each district) belong to different occupation; caste, income, gender and ethnicity of the age range between 25 and 70 years were interviewed using questionnaires. There were 30 questions we assessed the purpose and level of harvest of *M. oleifera* by asking questions: (a) Do you harvest *M. oleifera*? (b) If yes, then for what purpose? (c) Which parts and season do you prefer for collection? Most participants were men (67.18%), fewer women (32.81%) and most of them were illiterate and belonging to different ethnic group. Among the participants, 81.77% stated they were agriculture background, 8.85% were small business owners, 6.77% local healers and 2.60% hold other jobs.

Sampling and extraction

For biochemical analysis, three field visits (March, July and December 2016) were made in Attariya, Godawori municipality, Kailali district (28° 22' to 29° 05' N and 80° 30' to 81° 18' E), Far-west (Sudurpaschim) province of Nepal to harvest the samples. Four different parts (leaves, seeds, bark and root) of *M. oleifera* tree were collected from the same tree and it was also ensured that the plant was healthy and uninfected; their herbarium was prepared following the protocol of Martin (1995). The plants were identified by comparing with herbarium specimens housed in the Central Department of Botany, Tribhuvan University (TUCH) Nepal. Screening and testing of the phytochemicals were done at the laboratory of Central Department of Botany, Tribhuvan University.

All the collected plant parts (roots, barks, leaves, and seeds) were first air-dried and then oven dried at 60°C until they were completely dried. The plant parts were then pulverized to obtain a fine powder. Phytochemical screening of crude plant parts extracts was done by following standard protocols (Todkar *et al.* 2010; Harbour & Baxtre 1993). A crude extract of all plant parts was prepared by cold percolation method using methanol (Merck HPCL grade) as solvent. For this 50 g of each powdered plant parts were soaked in 450 mL of methanol for 72 hrs. Afterward, filtrations were conducted first by muslin cloth and then by Whatman No. 1 filter paper (Whatman Ltd, Kent, and UK). The filtrates were kept in conical flask and allowed to evaporate methanol for 48 hrs. The crude extracts were then dried using rotary evaporator at room temperature and then kept at 4°C. The stock solution of each extract was prepared by dissolving 100 mg of dried crude extract in 1 mL of Dimethyl sulphoxide (DMSO).

Phytochemical screening:

The protein test was performed using crude extract mixed with 2 mL of Millon's reagent. In this test, the occurrence of white precipitates that turn red upon mild heating indicates the presence of protein. For glycosides, 2 mL chloroform and 2 mL acetic acid were added to the crude extract. After cooling, H_2SO_4 was gently added to the mixture. A change in color from violet to blue to green indicates glycoside presence.

To test saponins, crude extracts were mixed with 5 mL of distilled water and stirred vigorously. The formation of steady foam indicates the presence of saponins. Similarly, for phenol, crude extract was mixed with 2 mL of 2% $FeCl_3$ solution. A blue-green or black coloration indicates the presence of phenols.

The flavonoids test was performed using crude extract mixed with 2 mL of 2% NaOH solution. An intense yellow color that turns colorless after a few drops of diluted acid indicates flavonoids. For the phytosterols test, the crude extract was combined with 2 mL chloroform and then 2 mL of conc. H_2SO_4 was added and thoroughly mixed. The presence of a reddish-brown color indicates the presence of a steroidal ring (phytosterols). To test terpenoids, the crude extract was dissolved in 2 mL of chloroform and dried again. To this, 2 mL of conc. H_2SO_4 was added and heated for about 2 min. The presence of a grayish color indicates the presence of terpenoids. Similarly, for tannins, crude extracts were treated with 5 mL of 1% gelatin solution containing NaCl. The formation of a white precipitate indicates the presence of tannins.

Antibacterial activities test:

Five different human pathogenic bacteria, *B. subtilis* and *S. aureus* 25923, both gram positive whereas *K. pneumonia*, *E. coli* 25922 and *P. aeruginosa* 27853, three-gram negative bacteria were selected for the antibacterial test. All strains of bacteria were collected from Central Department of Biotechnology, Tribhuvan University, Nepal. The collections were maintained in Nutrient Agar (NA) and Muller-Hilton agar (MHA). For streaking, bacterial inoculum was cultured in Nutrient broth (NB).

Antibacterial activities test was done by agar-well diffusion method with modification (Onsare *et al.* 2013). Blank DMSO (*Dimethyl sulfoxide*) solution was used as negative control and Gentamicin disc of 10 mg/mL was used as positive control. The plates were incubated for 24 hrs. at 37°C and then zone of inhibition (ZOI) was measured. All the experiments were made in three sets.

Statistical Analysis:

Zone of inhibition in antibacterial tests was summarized as mean value ± standard deviation using packages *ggplot2* and *ggpubr* in R statistical programming (R Core Team 2021). Significant difference between ZOI at each concentration of different extract types for each bacterium was evaluated using one-way Kruskal-Wallis test. Tukey HSD or pairwise Wilcoxon test was done as post hoc test for one-way ANOVA and Kruskal-Wallis test respectively using package *Car* in R v.4.1.1 (2021).

Results

Present survey revealed that, *M. oleifera* is mainly confined to tropical and lowland Tarai region of Nepal (Kanchanpur, Kailali, Bardiya, Banke, Dang, Surkhet, Nawalpur, Chitwan, Makwanpur, Bara, Parsa, Mahottari, Dhanusa, Sunsari, Morang and Jhapa districts). Distribution recorded in all seven provinces from east to west with yellow asterisk (Figure 1). All the plants were recorded in cultivated form and most of them were as planted in eastern Nepal for vegetable and medicinal uses (Thapa *et al.* 2019). We found *M. oleifera* population is rare and limited in use in Surkhet district of Karnali province.

Ethnobotanical uses

M. oleifera is frequently used as a medicine, however the use varied for different therapeutic uses. Roots are used to care anti-lithic, anti-fertility, rheumatism, inflammations, lower back or kidney pain, and constipation. Similarly, stem barks are used to relieve earaches, placed in tooth cavity as a pain killer, and anti-tubercular activity, leaves were for headaches, piles, fevers, diabetic, sore throat, bronchitis, eye and ear infections, scurvy and catarrh. Fruits/Pods (Seeds) for heart disease and malaria and flowers used for inflammations, muscle pain, hysteria, tumors and enlargement of the spleen by the indigenous people. We found that, different parts of *M. oleifera* used by local people to treat 22 diseases including the six antibacterial diseases. Hence, we analyzed the phytochemical composition of the frequently used plant parts, such as roots, bark, leaves, and seeds, based on the consumption of plants by local people.

Distribution of Knowledge

The bubble and asterisk presentations in the Figure 2 shows the area where the *M. oleifera* is utilized for medical reason. While the violet-colored bubble and asterisk (specimens collection district) suggested that *M. oleifera* is widely used as seen in Provine One, Madhesh, Lumbini and Sudur Pashchim. Similarly, the green-colored bubble showed that it is moderately used, as apparent in Province Bagmati and Gandaki. The yellow-color in Figure 2 indicated that the plant is less used and it is recorded in Karnali province. The familiarity of use of *Moringa* are transferred to new generations either orally or by individual practice or understanding. It is realized that people in close interactions with the plants in daily basis, with their expertise shifted from generation about use of plant, know best about the value of such resources or by observing the traditional practices. Our survey also revealed that other neighborhood districts (Sarlahi, Rauthat, Udaypur, Siraha, Illam, Rupandehi, Kapilbastu, Parasi, Salyan and Siraha) of study districts were also used *M. oleifera*, traditionally as medicine to care different diseases.

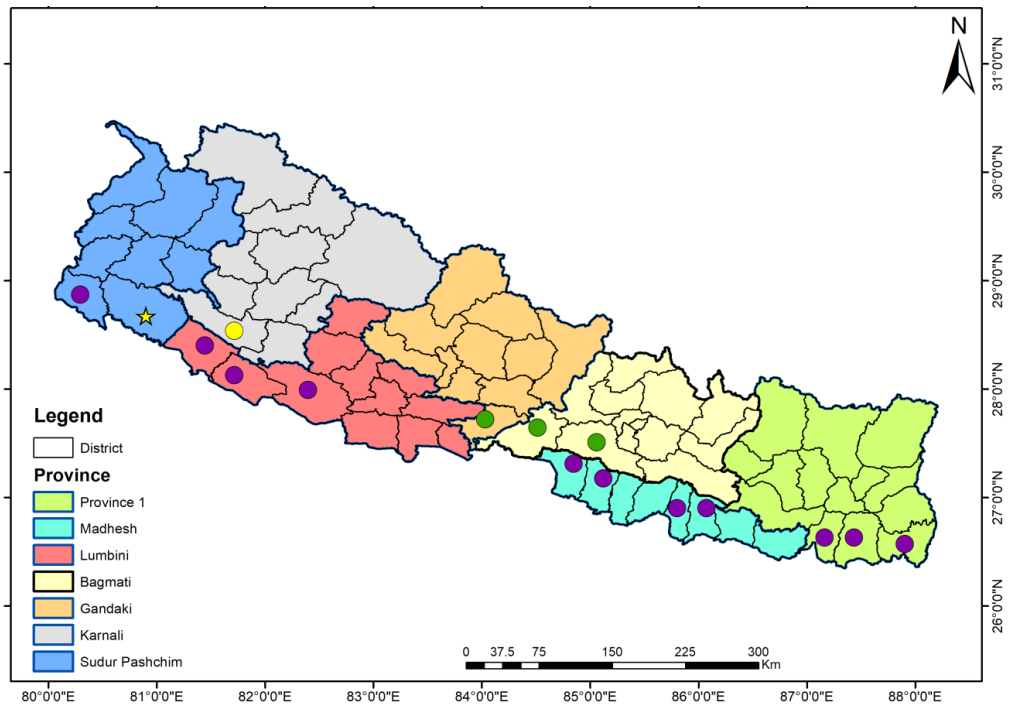

Figure 2. Political map of Nepal showing seven different provinces. The bubble and asterisk in the map indicate the area where *M. oleifera* was utilized for medicinal reasons. The violet-colored bubble and yellow-colored asterisk suggests that highly used, whereas the green-colored bubble shows moderate used and yellow-colored bubble represent low used. The yellow-colored asterisk also denotes that specimen collection for phytochemical screening.

Phytochemical Screening and Antibacterial activities

The percentage yield and characteristics of methanol crude extract (Table 1) was found to be different depending upon the plant parts. Where, the higher yield was from leaf (10.46%) with consistency greasy and dark green color, followed by seed (8.36%), oily consistency and shiny white color, bark (7.74%), powdery consistency and off green color and root (6.96%), powdery consistency and reddish in color.

Table 1. Yield (%) and characteristics of methanol extract of different plant parts

Name of the plant	Parts used	Weight of the extract (gm)	(%) Yield	Characteristics of extract	
				Consistency	Color
Moringa oleifera Lam.	Leaf	5.23	10.46	Greasy	Dark green
	Seed	4.18	8.36	Oily	Shiny white
	Bark	3.87	7.74	Powdery	Off green
	Root	3.47	6.96	Powdery	Reddish

Screening data (Table 2) showed the presence of phenols, glycosides, Phytosterols, flavonoids, saponins, tannins, terpenoids and proteins in all four plant parts extracts. We consider presence of phytochemicals which showed distinctive characteristics clearly/sharply as highly present, and which showed little or less or blur characters as moderate to low presence. Where we find that, phenols were present in high amount in leaf extract, moderate in root and bark extract and slightly present in seed. Similarly, glycosides were present in high amount in leaf, followed by seed and root. Phytosterols were present in high amount in all four plant parts as it showed sharp reddish-brown color. Flavonoids were found only in trace amount in bark.

Table 2. Comparative phytochemical tests on the methanol extracts of 4 different plant parts

Plant parts	*Phenols*	*Glycosides*	*Phytosterols*	*Flavonoids*	*Saponins*	*Tannins*	*Terpenoids*	*Protein*
Leaf	++++	++++	++++	++++	+++	+++	++++	++++
Root	+++	+++	++++	++++	+++	++	++	+++
Seed	++	+++	++++	++++	++	++	+	++
Bark	+++	++	++++	+	+++	++	+	++

Note: '+' - presence in trace amount, '++' - slight presence, '+++' - moderate presence, and '++++' high presence.

Various parts of *M. oleifera* showed different antibacterial activities against tested bacteria in methanolic extract (Figure 3). It is observed that increase in concentration of plant extract, also increase in ZOI (6.25mg/mL>12.5mg/mL>25mg/mL>50mg/mL>100 mg/mL). Overall, leaf extract showed higher ZOI (25.67±0.577) against *B. subtilis* in 100 mg/mL followed by *P. aeruginosa* (24.33±1.155) in same concentration. Bark showed the highest ZOI (30.33±0.58) against *B. subtilis* in 100 mg/mL and least against *P. aeruginosa* (17±1) in same concentration. Root extract showed highest ZOI (18.33±0.58) against *E. coli* in 100 mg/mL concentration of extract followed by *K. pneumoniae* (17.67±0.58) in same concentration. Seed extract showed the highest ZOI (17.67±0.58) against *S. aureus* followed by *K. pneumoniae* (17.67±0.58) in 100 mg/mL concentration. All plant methanolic extracts used to test for antimicrobial potential were effective against tested bacteria in all concentration of extract (100 mg/mL to 6.25 mg/mL). The higher concentration of plant parts extract ZOI were comparable to the standard antibiotic Gentamicin, where average ZOI 20 mm shown by *B. subtilis* and *P. aeruginosa* (27853), followed by 19 mm *E. coli* (25922) and 18 mm *S. aureus* (25923).

In a separate analysis of effect of extract types on ZOI of each bacterium, it was found that plant part extract types showed significant differences in ZOI depending upon types of bacteria and the concentration of extract used (Table 3). For instance, ZOI of *B. subtilis* were significantly differed among plant part extract types at different concentrations at 100 mg/mL, 50 mg/mL, and 12.5 mg/mL. In case of *E. coli,* the ZOI significantly differed among extract types to as low as 12.5 mg/mL. Likewise, ZOI in *K. pneumoniae* significantly differed only at lower concentration of extract i.e., 6.25 mg/mL and in *P. aeruginosa* only at 12.5 mg/mL and 6.25 mg/mL. In case of *S. aureus,* ZOI significantly different at all concentration except for 25 mg/mL.

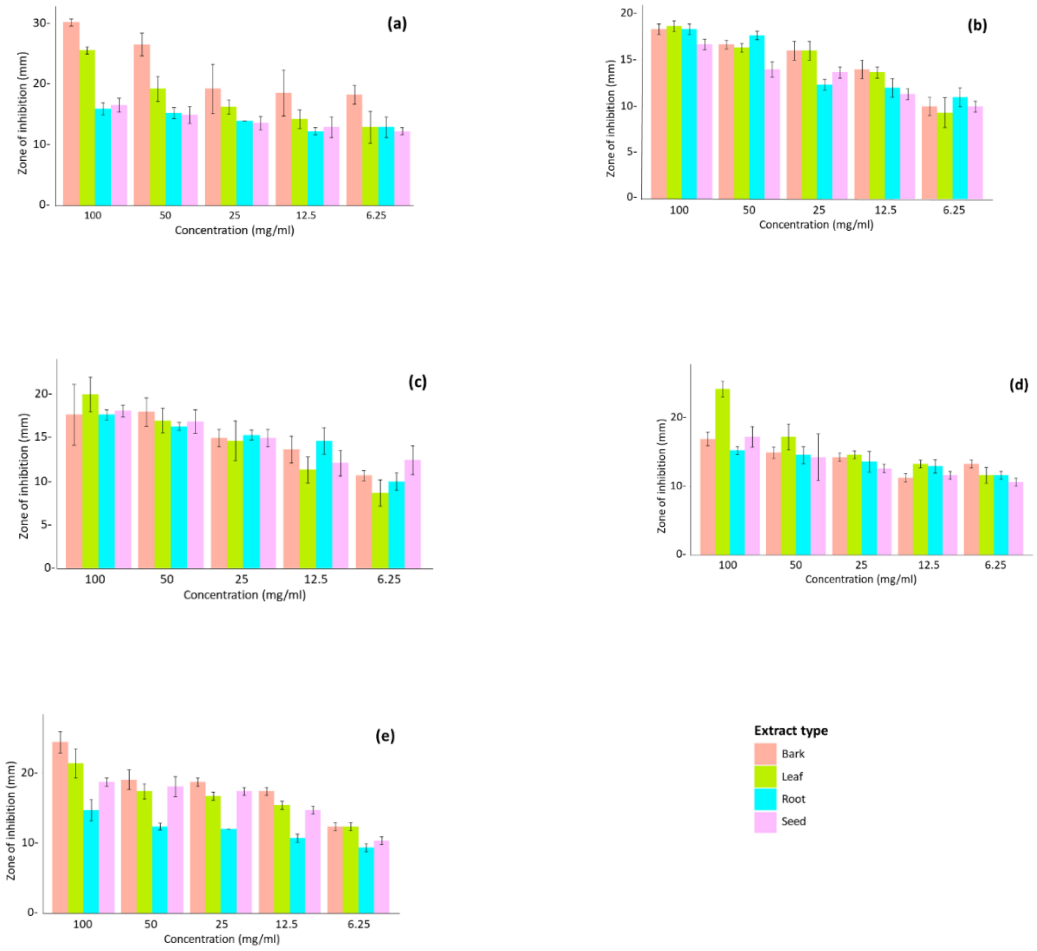

Figure 3. Mean zone of inhibition of with standard deviation (a) *B. subtilis*, (b*) E. coli*, (c) *K. pneumoniae*, (d) *P. aeruginosa*, and (e) *S. aureus.*

Table 3. Significance test (p value ≤ 0.05) of ZOI of different plant parts extract against tested bacteria

Concentration of extract	*B. subtilis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
100 mg/ml	**0.02**	**0.01**	0.49	0.44	**0.00**
50 mg/ml	**0.00**	**0.00**	0.49	0.34	**0.00**
25 mg/ml	0.27	**0.00**	0.94	0.11	0.27
12.5 mg/ml	0.44	**0.01**	0.09	**0.02**	**0.00**
6.25 mg/ml	**0.01**	0.27	**0.03**	**0.01**	**0.00**

Note: significant differences are in bold font.

Discussion

The use of *M. oleifera* species as a source of vegetable and medicine is a common phenomenon in the Tarai region of Nepal, substantiated by its abundance and year-round availability. It was substantiated by the fact that there were multiple uses of the species in the areas where the plant was abundantly found, whereas the limited use was recorded from the hilly areas where its population was restricted. *M. oleifera* is mostly in cultivated form; its wild patches are rarely found yet in tropical region of Nepal. People thought that this plant was imported from India because of open border. Consistent to our findings, Thakur & Bajagain (2020) reported the use of different parts of *Moringa* in Tarai region of Nepal for food and medicinal values. Local people used *M. oleifera* roots, leaf, bark, flower and seed to cure different human diseases as anti-lithic, anti-fertility, rheumatism, inflammations, lower back or kidney pain, constipation, relieve earaches, placed in tooth cavity as a pain killer, anti-tubercular, headaches, piles, fevers, sore throat (pharyngitis), bronchitis, eye and ear infections, scurvy, catarrh, heart disease, malaria,

muscle pain, hysteria, diabetic, and tumors. Other studies (Thapa *et al.* 2019; KC *et al.* 2022) carried out in different parts of the tropical region also revealed the similar accounts.

The plants parts were dried and stored for future need as well. The common use of fresh roots, leaf, bark, flower and seed could be due to the relative ease of collection, simplicity of preparation and more likely to have alkaloids with more medicinal value than older ones. Fresh parts were preferred if remedies contain essential oils, the concentration of which could be lost on drying (KC *et al.* 2022). The similarities in the uses and harvesting of plant parts with the findings of the previous researchers (Uprety *et al.* 2012; Pokhrel *et al.* 2016; Devkota & Bhusal 2020) from the same region indicate the highly reliable pharmacological effectiveness of the *M. oleifera*.

Different solvents such as water, methanol, ethanol, chloroform, ethyl acetate and acetone are recognized as suitable solvent for preparation of crude plant extracts (Dhanani *et al.* 2013). Consequently, in this study we used methanol, which is reported as most suitable solvents for *M. oleifera*, that gives higher yield of crude extract (Akinyeye *et al.* 2014; Anokwuru *et al.* 2011). But we found that methanol is not equally effective in extraction for all plant parts of *M. oleifera* because percentage yields are relatively low for barks and roots. This could be due to the woody tissues of roots and barks. The phytochemical screening results showed relatively lower abundance of phenolics in barks and roots this could be due to the choice of solvent. Kamarul *et al.* (2020) reported that methanol is not best solvent for phenolics. Thus, use of varieties of solvent is recommended. Interestingly, even if crude extract from seeds was oily only trace amount of terpenoids was found. But seeds contain high amount of phytosterols.

All tested phytochemicals are presented in all types of plant part extracts; however, abundance of these phytochemicals varies among plant parts extract types. Leaves showed abundant phytochemicals, which conforms to the previous observation (Natsir *et al.* 2019). In contrast to the findings of Patel *et al.* (2014) in ethanolic extract of leaf, we found moderate presence of glycosides in leaf extract. Likewise, Abdulkadir *et al.* (2015) did not report the presence of tannins and saponins in ethanolic extracts of seeds and roots respectively, but we reported these compounds in corresponding crude extract types. These differences could be due to the choice of solvent for extraction. Thus, successive re-extraction is highly recommended in phytochemical analysis (UC & Nair 2013). Presence of such phytochemicals illustrated several known medicinal and nutritional values of *M. oleifera*. For instance, phytochemicals like flavonoids, saponins and tannins are known for their antibacterial properties (Shojaemehr *et al.* 2020), and phenols and flavonoids are recognized for their antioxidant values (Verma *et al.* 2009).

The plant extracts exhibited effective antibacterial activities, as found in other results (Amabye & Tadesse 2016; Peter *et al.* 2011, Dewangan *et al.* 2010). Prasad *et al.* (2014) also reported the antibacterial action of methanolic extract of *M. oleifera* against *P. aeruginosa*, *S. aureus*, *E. coli*, and *K. pneumonia.* All the tested crude plant part extracts showed effective activities against gram-positive bacteria *B. subtilis* and *S. aureus*, whereas the least effective against gram-negative *K. pneumoniae* and *P. aeruginosa*. Less effective antibacterial activities against gram-negative *K. pneumoniae* and *P. aeruginosa* could be due to the presence of hydrophilic outer membrane (lipopolysaccharide) that did not allow diffusion of antibacterial phytochemicals into the bacterial cell. Interestingly, gram-negative *E. coli* also showed significant differences in ZOI among extract types. Bark shows the higher ZOI among all four extracts in all concentration which is against pathogenic microbe *B. subtilis* whereas root extract showed the least ZOI against *S. aureus*. Antibacterial activity shown by methanolic extracts of leaves and seed of *M. oleifera* against pathogenic germs *S. aureus* and *E. coli* was supported by previous study (Dodiya & Amin 2015). In all extract types, the lower concentrations did not show effective antibacterial activities as compared to standard Gentamicin antibiotic. But remarkably, *K. pneumonia* showed no inhibition in Gentamicin but with all extract types, notable ZOI were observed. Since, many intrinsic and extrinsic factors like temperature, pH of culture medium, growth phase, incubation period, concentration of the plant extracts, diffusing properties, humidity, number of microorganisms, volume of extract poured in wells may affect the antibacterial test and zone of inhibition (Langsrud & Sundheim 1998; Anderson & Yu 2005; Fraise *et al.* 2008). More the ZOI more the potential as antibacterial drug (Onsare *et al.* 2013). Arévalo-Híjar *et al.* (2018) reported that no cytotoxic activity was observed in different plant parts of *M. oleifera*.

Conclusions

Our ethnobotanical survey reveals that the whole plant parts (roots, stem bark, leaf, flower and fruit/seed) of *M. oleifera* are traditionally used by local people for treatment of various ailments and six antibacterial ailments (inflammation, tuberculosis, hysteria, diabetes, piles and tumors). The therapeutic value has been consented with the findings of biochemical analyses. The phytochemical screening test showed the presence of phenols,

glycosides, phytosterols, flavonoids, saponins, tannins, terpenoids and proteins from root, bark, leaf and seed, all are potential for antibacterial treatment. Bark showed the high antibacterial activity against *B. subtilis* followed by leaf, whereas seed shows its best against *S. aureus* and root against *E. coli*. It shows that all plant parts are worth consisting therapeutic compounds. Plant use as nutraceutical is also important. As higher analogy in comparative assessment and other potential as nutraceuticals, the further bioprospecting research of *M. oleifera* deemed necessary.

We have compiled and summarized traditional uses, phytochemical and antibacterial test of different parts of *M. oleifera*. Although considerable interests have been devoted in elucidating the phytochemical and pharmacological aspects of the plant, many noteworthy lacunae still exist and are yet to be resolved. Recent phytochemical screening and antibacterial test have increasingly validated the traditional use of *M. oleifera* especially as anti-tubercular, anti-inflammations, anti- diabetic and tumors. Therefore, in depth studies must be undertaken in order to test and to better understand its age-old use in the Nepalese system of medicine.

Declarations

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