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ORIGINAL RESEARCH ARTICLE

Phytochemical Screening and Antimicrobial Activities of Some Selected Medicinal Plants of Nepal

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ABSTRACT

The *in-vitro* antimicrobial activities of methanolic extract of sixteen plants and their comparison with standard antibiotics were investigated using disc diffusion method. The clinically isolated strains of bacteria like: *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Enterococcus species, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi and Salmonella paratyphi* were used for assay. The preliminary screening of phytochemical constituents of different plant extracts showed the presence of cardiac glycosides, flavonoids, tannins, polyphenols, saponins, carbohydrates and steroids. The methanolic extracts of *Adhatoda vasica* Nees, *Myrica esculenta* Buch Ham, *Urtica dioica* L, *Jasminum humile* L, *Rhododendron arborium* L, *Osyris wightiana* Wall, *Nyctanthes arbor-tristis* L and *Bombax ceiba* L were highly active against *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi* and *Salmonella paratyphi.* Similarly, the methanolic extracts *of Nardostachys grandiflora* DC and *Rhododendron anthopogon* D. Don were also very effective against *Klebsiella pneumonia, Enterococcus species, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi* and *Salmonella paratyphi.* Likewise, methanolic extract of *Woodfordia fruticosa* L showed considerable activity against *Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi and Salmonella paratyphi.* The *Candida albicans* had also maximum activity against methanolic extracts of *Myrica esculenta* Buch Ham, *Nardostachys grandiflora* DC, *Rhododendron arborium* L, *Osyris wightiana* Wall and *Rhododendron anthopogon* D. Don. But *Trichoderma viridae* showed almost no any response against all the methanolic plant extracts.

Keywords: medicinal plants, phytochemical screening, antibacterial activity, antifungal activity

INTRODUCTION

In the recent years, the use of herbal medicines has been extensively expanded all over the world. More than 80% of world population directly or indirectly depends on the traditional herbal medicines for their primary health care. In the context of Nepal, a large varieties of medicinal plants or any materials that derived from plants have been employed for the proper prevention and treatment of different kinds of diseases in all the traditional cultures[1]. Since researchers are looking on traditional knowledge about probable consequences of exercising these plants as herbal medicines, but there is no more enough progress in the use of herbal medicine as expected. The traditional knowledge regarding of medicinal plants and their use by indigenous cultures have been passed from one generation to another [2]. So, transfer of traditional knowledge about the herbal medicines would be supportive for the proper conservation of cultural tradition, biodiversity of medicinal herbs as well as a potential new drug development in the present and future prospect of country [3-5]. The herbal medicines have drawn a great attention to all the common people as well as pharmaceutical companies because of their cost effective and eco-friendly attributes[6].

The medicinal values of these plants are known due to the presence of some active chemical constituents [7]. These bio-active compounds are responsible for anti-microbial effect of plant extract *in-vitro*, for e.g. flavonoids, alkaloids, glycosides, saponins, tannins, terpenoids, carbohydrates and sterols etc. [8, 9]. Thus, the utilization of plant extract and phyto-chemical,

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both with known anti-microbial properties may lead to play a significant role for the discovery of new drugs in therapeutical treatments. Nowadays, anti-microbials derived from the plants have been receiving the increasing attention because synthetic anti-biotics have shown ineffectiveness against several human pathogenic organisms due to increasing drug resistance [10]. Therefore, a number of studies have been conducted for screening of plants in different countries to find out novel potent compounds for anti-microbial therapy [11]. Several such studies have been carried out on the basis of ethnobotanical knowledge which has greater number of positive results than that on the randomly selected plants. Ethno-botanical knowledge of traditional health care has greatly attracting the interest of pharmaceutical companies into research and developmental programs in the pursuit of novel drugs [12].

Nepal is a Himalayan country with great repository of natural products. So, there is a huge scope for the characteristic detailed study of such ethno botanical plants having significant medicinal values in Nepal [13-16]. Although a number of plants with antimicrobial potential have been identified, greater number still remains unidentified. So, there is a dire need of proper documentation and evaluation of therapeutic properties of several other medicinal plants found in Nepal with a special reference to their ability to fight against various diseases. Here, methanolic extracts of sixteen medicinal plants that collected from different parts of Nepal on the basis of ethno-pharmacological information have been subjected for their phytochemical screening and anti-microbial activities.

MATERIALS AND METHODS

Collection of Plant materials

The different parts of plants were collected in September to January, 2013-2014 from the various places of Nepal at different altitudes. Voucher specimens identified by Rajendra Gyawali and Tirtha Maiya Shrestha (Department of Pharmacy, Kathmandu University, Dhulikhel, Nepal) and were deposited in Department of Pharmacy, Kathmandu University. The collected plants materials were then dried in the shade and stored at room temperature before the conduction of experiment.

Preparation of plant extracts

The cold extraction of plant samples was carried out by using the methanol as solvent. The air dried plant sample was blended in the home blender and 25 grams of each of finely ground plant powder sample was initially soaked in the 100 ml methanol in a conical flask and mouth of the flask was closed with aluminium foil to reduce the volatilization of the solvent. The flask was then allowed to keep in the rotary shaker for 7 days with constant shaking [17]. After 7 days, the solvent along with components were collected and was filtered using Whatman No.1 filter paper. The residue on the filter paper was again washed further with 100 ml methanol solvent. Traces of the methanol from the extract (filtrate) were removed by keeping it on the water bath at low temperature. The extracts thus obtained were the weighed and percentage of the yield was evaluated. Finally, the plant extracts were kept aseptically until use.

Phytochemical Screening

Phytochemical screening for the major chemical constituents of methanolic extracts of different plant samples was carried out according to the standard procedures [18].The plant material was screened for the presence of cardiac glycoside, carbohydrates, flavonoids, tannins, polyphenol, saponin, steroids, and terpenoids.

Test micro- organisms for antimicrobial assay

The bacterial strains used for the antibacterial assay were *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Enterococcus species, Salmonella typhi, Salmonella paratyphi, Proteus mirabilis and Staphylococcus aureus*. For the antifungal assay, *Candida albicans* and *Trichoderma viridae* were used. These test organisms were provided by Department of Microbiology, Dhulikhel Hospital, Kathmandu University Teaching Hospital, Nepal and were stored at -4^0 C.

Preparation of test solutions

Each test compound was dissolved in dimethyl sulfoxide to give stock solution of 5%, 10% and 15% in concentration. Then, these solutions were used for testing. Standard drugs or reference drugs used as antibiotics were Ciprofloxacin (30mcg), Chloramphenicol (30mcg/disc) and Nitrofurantoin (300mcg). Negative control was prepared using respective solvent (DMSO).

Preparation of inoculums

All the micro-organism cultures were revived in the appropriate media after susceptibility test in MacConkey Agar, Muller Hinton Agar and Blood Agar. Each the micro-organism, at a concentration of 1.5×10^6 cells/ml (adjusted to the 0.5 McFarland turbidity standards) was inoculated till

it reached the turbidity equal to that of the standard 0.5 McFarland solutions at 600 nm which is equivalent to 10^6– 10^8 CFU/ml. Then, these cultures were used for antimicrobial assay

Antibacterial activity

Sensitivity of antibiotics against test strains was assessed by agar disc diffusion method [19]. So, overall sensitivity was predicted with degree of clear zone surrounding the disc after 24 h in mm [20]. The definite amount of these bacterial media was dissolved in distilled water and pH was adjusted to 7.2. The sterilized media (15 to 20 ml) were poured into sterile petriplates. The plates were allowed to set at room temperature for 5 min and inoculum suspension was inoculated over the surface of medium uniformly using sterile cotton swab. Finally, the inoculum was allowed to dry for 5 min. Sterile, 6 mm diameter Grade No. 1 Whatman filter paper discs were impregnated with 5%, 10% and 15% (w/v) in the concentration of methanolic crude extract in DMSO. The loaded discs were placed on the surface of medium spread with 1.5 × 10^6 cells/ml (adjusted to the 0.5 McFarland turbidity standards) bacteria cultures. After holding the plates for 1 hour at room temperature, allowed to diffuse the test samples into the agar medium and they were incubated at 37±2°C for 24 hrs. Negative control was prepared using respective solvent. Ciprofloxacin (30mcg/disc), Chloramphenicol (30mcg/disc) and Nitrofurantoin (300mcg/disc) were used as positive control. After 24 h or incubation, the inhibition zones formed around the disc were measured with transparent ruler in millimeter (mm). Overall, cultured microorganisms with equal to or greater than 7 mm were considered susceptible to samples tested. All tests were performed in triplicates and antimicrobial activity was expressed as the mean diameter of zone of growth inhibition (mm) produced by the plant extract around the discs. Control discs contained DMSO only.

Antifungal Activity

The antifungal activity was tested by disc diffusion method [21]. The blood agar plates were inoculated with each fungal culture. The filter paper discs (6 mm diameter) impregnated with varying concentrations of plant extracts. DMSO was used to dissolve the extract and was completely evaporated before application on test organism-seeded plates. Blank disc impregnated with solvent followed by drying off was used as negative control and Amphotericin B (10μg per disc) was used as positive control. The activity was determined after 1 week of incubation at 28± 2°C. The diameters of the inhibition zones were measured in mm.

RESULTS

Phytochemical Screening

The preliminary screening of phytochemical constituent of different plant extracts revealed the presence of cardiac glycosides, flavonoids, tannins, polyphenols, saponins, Carbohydrates and steroids but the terpenoids and alkaloids were absent. The studies have shown that the plant extract: *Adhatoda vasica* Nees (leaf), *Artemisia vulgaris* L, *Nardostachys grandiflora* DC, *Urtica dioica* L., *Jasminum humile* L, *Rhododendron arborium* L., *Osyris wightiana* Wall, *Nyctanthes arbor-tristis* L, *Colquhounia coccinea* Wall, *Adhatoda vasica* Nees (flower), *Rhododendron anthopogon* D.Don *and Woodfordia fruticosa* L. contain the major pharmaceutical constituents such as Tanins, Flavonoids and Steroids. These are the bio-active components which are known to be as bactericidal, pesticidal or fungicidal in the nature [22, 23]. It has also been reported that the above mentioned compounds are fundamentally secondary metabolites and have the capacity to produce a definite physiological actions on the body [24]. The tannins in the plant are known to be astringents which are useful in the wound healing and are anti-parasitic, they are used for treating intestinal disorders such as diarrhea and dysentery [25]. They are also known to show curative activity against several pathogens [26]. The presence of these metabolites suggests a great potential of these plant sample as a major source of useful phytomedicines.

Antimicrobial activity of the combination of plant extracts and antibiotic discs

The results for antimicrobial activity of different plant extracts under study against bacteria are shown in (**Table 2-4)**. The diameter of zone of inhibition was generally found to be increased with increase in concentration of plant extract. Whereas, the diameter of zone of inhibition produced by some of plant extract was unusual with increase in concentration.

Result shows the in-vitro combined activities of the methanolic plant extracts and conventional antibiotics against different test organisms, the combinations produced varying zone of inhibition. The methanolic plant extracts like *Urtica dioica* L., *Bombax ceiba* L., *Adhatoda vasica* Nees (leaf), *Adhatoda vasica* Nees(flower) in the respective order and Ciprofloxcin(30mcg/disc) as standard

are highly effective to inhibit the activity of *E. Coli* . The methanolic extracts of *Urtica dioica* L., *Nardostachys grandiflora* DC, *Myrica esculenta* Buch Ham, *Rhododendron anthopogon* D.Don, *Bombax ceiba* L , *Adhatoda vasica* Nees and Chloramphenicol (30mcg/disc) as standard show the greater the diameter of zone of inhibition against *Klebsiella pneumonia.* Similarly, methanolic extract of *Berberis aristata* DC, *Jasminum humile* L, *Adhatoda vasica* Nees and Chloramphenicol (30mcg/disc) as standard showed the efficient activity as compared to other plant extract against *Staphylococcus aureus* (**Table 2**). The plant extract of *Nardostachys grandiflora* DC, *Rhododendron anthopogon* D.Don, *Colquhounia coccinea* Wall, *Urtica dioica* L., *Woodfordia fruticosa* L. and Nitrofurantoin (300mcg/disc) as standard showed higher activity than other plant extract against *Enterococcus species* (**Table 3**).

The methanolic extract of *Urtica dioica* L., *Rhododendron arborium* L., *Osyris wightiana Wall, Nyctanthes arbor-tristis* L, *Colquhounia coccinea* Wall, *Rhododendron anthopogon* D.Don, *Nardostachys grandiflora* DC and Ciprofloxcin(30mcg/disc) as standard are highly effective to inhibit the activity of *Pseudomonas aeruginosa* rather than other plant extract (**Table 3**). The plant extracts of *Adhatoda vasica* Nees, *Myrica esculenta* Buch Ham,

Nardostachys grandiflora DC, *Rhododendron arborium* L., *Osyris wightiana* Wall, *Rhododendron anthopogon* D.Don, and Chloramphenicol (30mcg/disc) as standard show the greater the diameter of zone of inhibition against *Proteus mirabillis* (**Table 3**). While plant extracts of *Artemisia vulgaris* L, *Berberis aristata* DC, *Myrica esculenta* Buch Ham, *Nardostachys grandiflora* DC, *Osyris wightiana* Wall, *Bombax ceiba* L., *Rhododendron anthopogon* D.Don, showed good inhibition activity against *Salmonella typhi* and *Salmonella paratyphi* in which Chloramphenicol (30mcg/disc) was used as standard (**Table 4**).

Plethora of terpenoidal research showed, have reported the membrane disruption and inhibitory effect of terpenoids against fungi and bacteria [27]. Studies have shown that saponins have heamolytic property, induced cytotoxicity effect [28], expectorant action [29], antitumor and anti-mutagenic activities and can lower the risk of human cancers, by preventing cancer cells from growing [30]. Saponins have the property of precipitating and coagulating red blood. These plants are used to stop bleeding and in treating wounds [31]. They exhibit foaming properties and cell membrane- permeabilizing properties. Their soapy character is due to their surfactant properties [32]. Thus the secondary metabolites identified in the plant materials used in the study could be responsible for antimicrobial activity exhibited by the different extracts of the plants. Their varied occurrences in various plant extracts however indicate that probably, their therapeutic effect(s) are not the direct effect of a single group or compound, but rather that the compounds possibly act in combination to bring about an effect.

All most of all the antibiotics used for the positive control showed the good inhibitory effect on the microbes except *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia* and *Salmonella paratyphi* which were highly resistant to Penicillin-G. Among all the antibiotics, highest zone of inhibition were observed with Ciprofloxcin(30mcg/disc) against *E. coli* and *Salmonella paratyphi.*

Antifungal activity of methanolic plant extracts

Trichoderma viridae has showed no activity against all most of all the methanolic plant extracts, but very less activity in the methanolic extract of *A. vulgaris* L , *M. esculenta* Buch Ham, *N. grandiflora* DC, *W. fruticosa* L. DMSO referred as blank and it has showed considerable activity. Amphotericin B (10μg per disc) as standard has showed average activity. Similarly, *Candida albicans* also has maximum activity against methanolic extracts of *M. esculenta* Buch Ham, *N. grandiflora* DC, *R. arborium* L., *O. wightiana* Wall and *R. anthopogon* D.Don

whereas remaining plant extract showed very poor activity . Here, DMSO referred as blank with minimum activity and standard, Amphotericin B (10µg per disc) has activity within the range. The effect of different methanolic plant extract on the *Candida albicans* and *Trichoderma viridae* has also found remarkable (**Table 5**)

Table 1: Plants collected based on ethnobotanical uses from the different parts of Nepal.

Scientific Name (Code)	Local Name(collection site)	Family	Parts Used	Traditional Use
Adhatoda vasica Nees	Asuro (Dhulikhel)	Acanthaceae	Leaves	Used for cough, chronic bronchitis.
Artemisia vulgaris L	Titepati (Syanja)	Asteraceae	Leaves	Used in asthma, purgative, skin- diseases like scabies & on ulcers.
Berberis aristata DC	Chutro (Daunne)	Berberidaceae	Bark	To cure for Jaundice, diarrhea, fever, eye infection.
Myrica esculenta Buch Ham	Kaphal (Kavre)	Myricaceae	Bark	To control excessive bleeding during means, cough and cold.
Nardostachys grandiflora DC	Jatamansi (Gosaikunda)	Valerianaceae	Root	Used as essential group of ailments such as hysteria, cholera, palpitations, epilepsy and similar convulsive disorders.
Urtica dioica L	Sisno (Dhilikhel)	Urticaceae	Leaves, shoots	To cure for anemia, to purify blood and for tonic.
Jasminum humile L	Jaai (Dhulikhel)	Oleaceae	Flower	Used in the treatment of Ringworm, infestation, stomach disorders.
Rhododendron arborium L	Laliguras (Panauti)	Ericaceae	Bark	Used as possible anti-inflammatory and hepatoprotective activities.
Osyris wightiana Wall	Nundhiki (Panauti)	Santalaceae	Leaves	Used as a wild herbal tea, acts as a labor inducing agent, to plaster around the fractured bone.
Nyctanthes arbor-tristis L	Parijat (Dhilikhel) "Night Jasmine"	Oleaceae	Leaves	Used in several ailments including asthma, cough, rheumatism, high blood pressure.
Colquhounia coccinea Wall	Sano Tusare (Langtang)	Labiatae	Leaves, Stem	Used as culinary herbs such as basil, mint, sage, savory, lavender and perilla.
Adhatoda vasica Nees	Asuro(Dhulikhel)	Acanthaceae	Flower	Used for Cough, chronic bronchitis.
Rhododendron anthopogon D.Don	Sunpati (Gosaikunda)	Ericaceae	Leaves and stem	Used as good decongestant and is antispasmodic for the respiratory system, also known to help reduce inflammation , tonic for the adrenal glands.
Bombax ceiba L	Simal (Bardiya)	Malvaceae	Flower, Bark	Used to remove internal inflammation in the body.
Nardostachys grandiflora DC	Jatamansi	Valerianaceae	Rhizome	Used as essential group of ailments such as hysteria, cholera, palpitations, epilepsy and similar convulsive disorders.
Woodfordia fruticosa L	Dhairo (Daunne)	Acanthaceae	Flower	In dysentery and to care of bleeding in stool.

Table 2: Antibacterial activity of methanolic extracts of different plants against human pathogenic bacteria. Concentration of extract: 5%, 10% and 15% (w/v in DMSO). Ciprofloxacin (30mcg) as Standard [30.33±0.57], Chloramphenicol (30mcg) as Standard [21±1.0]and Chloramphenicol (30mcg) as Standard [23.33±1.15].

Plant extract	*Escherichia coli* (Ciprofloxacin as std.)			*Klebsiella pneumonia* (Chloramphenicol as std.)			*Staphylococcus aureus* Chloramphenicol as std.)		
	Concentration			Concentration			Concentration		
	5%	10%	15%	5%	10%	15%	5%	10%	15%
A. vasica Nees	12.3±0.8	11.7±0.6	12.7±1.0	11.16±1.04	11.66±0.76	15.16±0.76	-	-	-
A. vulgaris L	8.7±0.6	7.3±0.3	-	11.83±1.04	13.16±1.25	15.16±0.76	-	-	-
B. aristata DC	6.8±0.28	7.2±0.8	7.7±0.8	14.16±0.76	14.50±0.50	12.16±0.76	10.83±0.28	9.33±1.04	13.16±0.76
M. esculenta Buch Ham	10.2±0.8	7.5±0.5	8.2±0.8	14.33±1.25	14.50±0.50	14.00±0.50	-	-	-
N. grandiflora DC	10.3±1.5	8.5±1. 3	-	13.00±0.50	15.50±0.50	15.16±1.04	-	-	-
U. dioca L	12.7±1.5	12.8±0.3	14.3±1.2	16.16±0.57	16.33±1.25	15.66±0.76	-	-	-
J. humile L	7.2±0.3	9.7±0.6	9.8±0.3	11.16±0.76	15.16±0.76	13.50±1.32	8.33±0.76	9.33±1.52	7.33±0.28
R. arborium L.	10.2±1.3	11.2±0.8	9.2±0.8	-	-	-	-	-	-
O. wightiana Wall	9.3±0.6	10.2±0.8	9.8±1.0	13.33±1.04	11.83±0.76	12.83±0.76	-	-	-
N. arbor-tristis L	8.8±0.8	10.2±0.8	10.7±0.6	11.16±1.04	12.66±01.04	11.33±0.76	11.16±1.04	-	-
C. coccinea Wall	8.2±0.8	9.5±0.5	10.5±0.5	13.50±0.86	13.83±0.76	14.83±0.76	-	-	-
A. vasica Nees	11.2±0.8	9.7±0.6	12.3±1.5	7.83±0.28	9.16±1.04	9.83±0.76	11.16±0.76	-	11.33±0.76

R. anthopogon D.Don	9.7± 0.6	9.2±0.3	8.7±0.6	13.50±0.50	14.16±0.76	15.16±0.76	-	-	7.00±0.50
B. ceiba L	13.5±0.9	12.2±0.3	11.3±1.2	12.50±0.5	15.16±1.25	16. 00±0.5	12.16±1.25	-	-
N. grandiflora DC	7.7±0.8	7.7±1.2	9.2±0.8	10.83±1.04	11.16±0.76	14.00±0.50	7.50±0.50	7.83±0.28	-
W. fruticosa L	8.16±0.28	8.5±1. 3	8.7±0.6	11.16±0.76	11.66±0.76	10.7±0.6	8.2±0.8	9.33±1.04	-

Table 3: Antibacterial activity of methanolic extracts of different plants against human pathogenic bacteria. Concentration of extract: 5%, 10% and 15% (w/v in DMSO). Nitrofurantoin (300mcg) as Standard [13.33±1.53], Ciprofloxacin (30 mcg/disc) as Standard [15±1.0]and Chloramphenicol (30mcg) as Standard [20.66 ±1.15]

Plant extract	*Enterococcus Species* (Nitrofurantoin as std.)			*Pseudomonas aeruginosa* (Ciprofloxacin as std.)			*Proteus Mirabillis* (Chloramphenicol as std.)		
	Concentration			Concentration			Concentration		
	5%	10%	15%	5%	10%	15%	5%	10%	15%
A. vasica Nees	-	-	-	-	-	-	12.66±0.57	13.33±0.57	13.00±1.00
A. vulgaris L	7.0±0.50	-	-	7.33±0.57	7.16±0.28	-	7.16±0.28	9.00±1.00	7.66±0.57
B. aristata DC	-	-	-	7.16±0.28	7.33±0.57	7.50±0.5	-	-	-
M. esculenta Buch Ham	7.83±0.76	7.33±0.57	-	6.83±0.28	8.83±0.76	-	10.83±1.25	8.66±1.15	8.000±1.00
N. grandiflora DC	8.50±0.50	8.66±0.28	10.16±0.28	-	-	-	8.66±0.57	9.66±0.57	12.16±0.28
U. dioca L	7.83±0.76	8.66±0.57	9.50±0.50	7.50±0.50	8.50±1.33	9.50±0.50	-	-	-
J. humile L	7.16±0.28	-	7.5±0.50	-	-	-	6.83±0.28	7.16±0.28	6.83±0.28
R. arborium L.	-	-	8.16±0.28	9.00±0.50	9.16±1.32	-	9.33±0.57	9.66±0.57	11.33±0.57
O. wightiana Wall	7.16±0.28	7.33±0.28	8.00±0.50	7.83±0.28	9.16±0.28	8.50±0.50	11.66±0.57	13.00±1.00	13.83±0.28
N. arbor-tristis L	-	7.66±0.28	-	7.83±0.76	7.83±0.76	11.00±1.00	7.16±0.28	8.00±1.00	8.33±0.57
C. coccinea Wall	8.33±0.76	7.83±0.76	8.83±0.76	8.16±0.76	7.16±0.28	10.16±1.25	-	-	-
A. vasica Nees	7.16±0.28	7.33±0.28	7.33±0.28	-	-	-	6.66±0.28	-	-
R. anthopogon D.Don	9.16± 0.28	9.50±0.50	8.00±1.0	7.00± 0.50	9.50±0.50	9.83±1.04	9.33± 0.57	10.33±0.57	9.33±0.57
B. ceiba L	7.16±0.28	-	7.16±0.28	8.16±0.76	9.33±0.76	-	-	-	-
N. grandiflora DC	-	-	-	10.00±1.00	13.50±1.32	13.16±1.25	7.33±0.57	6.66±0.28	8.00±1.00
W. fruticosa L	8.5±1. 3	9.00±0.50	7.5±0.5	9.50±0.50	8.66±0.57	11.33±0.76	8.66±1.15	9.66±0.57	10.00±1.00

Table 4: Antibacterial activity of methanolic extracts of different plants against human pathogenic bacteria. Concentration of extract: 5%, 10% and 15% (w/v in DMSO). Chloramphenicol (30mcg/disc) as Standard [24.66±0.57] and Ciprofloxacin (30 mcg/disc) as Standard [27.66±0.57].

Plant extract	*Salmonella typhi* (Chloramphenicol as std.)			*Salmonella paratyphi* (Ciprofloxacin as std.)		
	Concentration			Concentration		
	5%	10%	15%	5%	10%	15%
A. vasica Nees	-	-	-	-	-	-
A. vulgaris L	9.50±0.50	8.66±0.57	9.66±0.57	8.66±1.15	11.33±0.57	8.33±0.57
B. aristata DC	10.16±0.76	9.66±0.57	9.33±0.57	-	-	-
M. esculenta Buch Ham	9.33±0.57	9.66±1.15	12.33±0.57	-	-	-
N. grandiflora DC	13.00±1.00	13.33±0.57	13.50±0.5	11.00±1.00	10.33±0.57	12.00±1.00
U. dioca L	-	-	-	11.33±1.15	12.00±1.00	9.00±1.00
J. humile L	-	-	-	-	-	-
R. arborium L.	8.33±0.57	8.33±0.57	11.00±1.00	10.33±0.57	9.66±0.57	11.33±0.57
O. wightiana Wall	12.00±1.00	12.33±0.57	13.33±0.57	12.33±0.57	11.33±0.57	12.33±0.57
N. arbor-tristis L	9.66±0.57	9.33±0.57	10.00±1.00	12.66±0.57	9.33±0.57	11.00±1.00
C. coccinea Wall	-	-	-	7.11±0.28	6.66±0.28	-
A. vasica Nees	-	-	-	-	-	-
R. anthopogon D.Don	11.66± 0.57	13.33±0.57	13.66±0.76	-	-	-
B. ceiba L	13.33±0.57	11.66±0.57	13.16±0.28	-	-	-
N. grandiflora DC	-	-	-	-	-	-

W. fruticosa L	10.66±0.57	8.66±1.15	12.66±0.57	10.16±0.76	-	9.33±0.57

Values are diameter of zone of inhibition in mm (Mean ± SD). (–) No activity.

Table 5: Screening of antifungal assay of methanolic plant extracts. Amphotericin B (10µg per disc) as standard (13±1.0**)**

Plant name	**Diameter of Zone of Inhibition (mm)**					
	Candida albumins			***Trichoderma viridae***		
	5%	**10%**	**15%**	**5%**	**10%**	**15%**
A. vasica Nees	-	-	-	-	-	-
A. vulgaris L	-	-	-	Slightly	-	-
B. aristata DC	-	-	-	-	-	-
M. esculenta Buch Ham	10.33±1.52	7±0.5	-	Slightly	-	-
N. grandiflora DC	10.33±0.57	12.33±0.57	10±0.5	-	-	-
U. dioca L	-	-	-	-	-	-
J.humile L	-	-	-	-	-	-
R. arborium L.	8.66±0.57	-	9.33±0.57	-	-	-
O.wightiana Wall	14.33±0.57	13.33±1.15	15±1.0	-	-	-
N. arbor-tristis L	-	-	-	-	-	-
C. coccinea Wall	-	-	-	-	-	-
A. vasica Nees	-	-	-	-	-	-
R. anthopogon D.Don	14.33±0.57	11.66±0.57	14.33±0.57	-	-	-
B. ceiba L	-	-	-	-	-	-
N. grandiflora DC	8.33±0.57	7.16±0.28	6.66±0.28	**Slightly**	-	-
W. fruticosa L.	7.66±0.57	9.33±0.57	10.33±0.57	**8.66±0.57**	-	-

Values are diameter of zone of inhibition in mm (Mean ± SD). (–) No activity

CONCLUSION

The present study reveals that several medicinal plants of Nepal are rich in bioactive secondary metabolites. The methanolic extract of *A. vasica* L, *M. esculenta* Buch Ham, *U. dioica* L, *J. humile, R. arborium* L, *O. wightiana Wall, N. arbor-tristis* L *and B. ceiba* L was highly active against *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis , Salmonella typhi and Salmonella.* Similarly, the methanolic extract *of N. grandiflora* DC and *R. anthopogon* D.Don was also very effective against *Klebsiella pneumonia, Enterococcus Species, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi* and *Salmonella paratyphi.* The methanolic extracts of *M. esculenta, N. grandiflora, R. arborium* L, *O. wightiana* Wall and *R. anthopogon* D.Don showed remarkable inhibition of *Candida albicans* while *Trichoderma viridae* showed almost no any response against all plant extracts. With the evidence of antibacterial and antifungal activities of the methanolic extracts, it can be suggested that further work needs to be done to identity the chemical natures of the active principles as well as their modes of actions on the bacterial cells and their roles in diseases curing.

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