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Research Article

PHYTOCHEMICAL ANALYSIS AND *IN - VITRO* ANTIMICROBIAL SCREENING OF SOME SELECTED MEDICINAL PLANTS OF NEPAL

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ABSTRACT

Nepalese medicinal plants have been known to relieve various diseases in Ayurveda. The aim of our study was to investigate the *in-vitro* antimicrobial effect of methanolic extracts of some selected medicinal plants collected from mountain, hills and terai regions of Nepal. Phytochemical screening of the 46 selected medicinal plant extracts was conducted to detect metabolites like flavonoids, terpenoids, tannins, polyphenols, steroids, glycosides, reducing sugars, cardiac glycosides, anthraquinone, carotenoids and saponins. The methanol soluble plant extracts inhibited the growth of gram-positive (*Staphylococcus aureus*, *Bacillus subtilis)* and gram-negative bacteria(*Salmonella typhi*, *Escherichia coli)*. Out of 46 screened medicinal plants, twenty three extracts possessed active antibacterial components against the test microorganisms. *Cleistocalyx operculatus*extractwas found inhibitory against all the microbes studied in this work. Antibacterial susceptibility test using its extract showed zone of inhibition against *Stayphylococcus aureus* (18.23 ± 0.15 mm/disc), *Bacillus subtilis* (18.1 ± 0.2 mm/disc), *E. coli* (16 ± 0.2 mm/disc) and *Salmonella typhi* (13.16 ± 0.15 mm/disc) at 10mg/ml concentration.Similarly, the extract of*Bridelia retusa*showed remarkable antibacterial property against *Staphylococcus aureus* (13.5 ± 0.2 mm/disc),*Bacillus subtilis* (11.16 ± 0.1 mm/disc), *E. coli* (16.23 ± 0.05 mm/disc) and *Salmonella typhi* (12.33 ± 0.2mm/disc). Our study showed that extracts of *Curcuma angustifolia*, *and Justicia adhatoda*, *Bauhinia variegate* containedbioactiveingredient against these organisms.

INTRODUCTION

According to world health organization (WHO) 25% of modern day medicines are derived from plant sources that were used traditionally as drugs and research focused on the traditional herbal medicines developed 75% of herbal drugs [1]. In developing countries majority of the population still relies on herbal medicine due to their adopted cultural practice, compatibility and lesser side effects[2]. WHO recorded 21,000 plant species with medicinal value throughout the world [3]. Wide varieties of higher plants are used in traditional medicine as a drug [4, 5]. Overproduction of oxygen is associated with the pathogenesis of many diseases [6]. Phytochemicals contain bioactive components with antioxidant and free radical scavenging properties which can be used asanti-inflammatory, anticancer, anti-aging compounds for treating cardiovascular, diabetes mellitus, obesity and neurodegenerative diseases [7].
Plant resources play a major role in the development of modern medicine since they contain vital components important for the drug discovery [8]. Worldwide,around 35,000-70,000 plant species are used in medicinal practices [9] and about 6500 plant species are used in Asia [10]. Ayurveda plays a major role in medicinal practice of Nepal. A policy of Ayurveda formulation was recognized in 1996 which includes the establishment and the development of the research for manufacturing drugs from local medicinal plants [11]. Nepal is fairly rich in plant resources which include 1600-1900 plant species commonly used in the medicinal practice from the ancient times [12].

There are only few systematic studiesconducted on medicinal plants of Nepal for assessing their antimicrobial activities. Infectious diseases account for approximately half of total deaths in tropical countries and they are considered a major threat to human health because of the unavailability of vaccines or limited therapeutics [13]. Infectious diseases caused by multi-resistant microbial strains are increasing and combating these diseases with natural products may be more efficient [14-17]. The most important bioactive constituents of plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides. Antibiotic or antimicrobial substances like saponins, glycosides, flavonoids, alkaloids are found in plants,

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yet these compounds have not been well established due to the insufficient knowledge and techniques [17-21]. The screening of the plant extracts represents our continuous attempt to find the species of plant that possess the antibacterial characteristics in the local area. Typhoid is a major health problem in Nepal. Antimicrobial resistance found in the different isolates of *Salmonella typhi* is threat to the cure of disease [22, 23]. Also *Escherichia coli* isolates from urinary tract infection isgaining antimicrobial resistance due to commonly used antibiotics in Nepal [24, 25]. There is a prevalence of Methicillin- resistant *Staphylococcus aureus* in the hospitals in Nepal [26].*Bacillus subtilis* is the most resistant bacteria against adverse conditions such as acidic, alkaline, oxidative, osmotic, heat or ethanol due to the formation of stress resistant endospores [27].

In the present study we screened 46 ethnobotanically selected medicinal plants for their anti-bacterial activities against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Bacillus subtilis*. Out of 46 medicinal plants, twenty three plants were effective towards these organisms and *Cleistocalyx operculatus*, *Bridelia retusa, Justicia adhatoda,Bauhinia variegata, Curcuma angustifolia* were found to be rich in antimicrobial compounds.

MATERIALS AND METHODS

Plant materials

The plant samples were collected from three different ecological regions of Nepal (mountain, hill and terai) based on their ethnobotanical uses. The plants were collected from National Herbarium and Plant Resources, Ministry of Forests and Soil Conservation, Godawari, Nepal. The plant parts used in the study were leaves, barks, roots and aerial which were authenticated by the research officer of the above centre,

Preparation of the plant extract

All samples were air dried at room temperature and grinded in milling machine. The powdered material was then weighed (50 g), soaked in methanol for 72h and filtered using Whatman No 1 filter paper. The filtrate was concentrated in vacuum at 40°-50°Cusing a rotary evaporator at 190-220 rpm for 24 h to obtain the crude extract.

Phytochemical Screening

The methanolic extracts obtained from different parts of plant species were analysed for the presence of polyphenols, alkaloids, flavonoids, tannin, carotenoids, saponins, reducing sugars, cardiac glycosides, steroids, terpenoids, glycoside and anthraquinone according to standard methods [20, 21].

Alkaloids

0.2g of the extract of each plant sample was weighed in a test tube to which picric acid solution was added. An orange- red precipitate indicated the presence of alkaloids.

Flavonoids

5 ml of methanolic extract was warmed and metal magnesium was added. To the reaction mixture 5-6 drops of concentrated hydrochloric acid was added and observed for development of red colour for the presence of flavonoids.

Steroids

2ml of acetic anhydride was added to 0.5ml methanolic extract followed by adding 2 mL of sulphuric acid. The color changed from violet to green or blue indicating the presence of steroids.

Terpenoids: About 0.2g of the plant extract was mixed with 2 ml of chloroform followed by 3 mL of concentratedSulphuric acid to each mixture. The formation of reddish brown coloration at the interface indicated the presence of terpenoids.

Reducing sugars: Each samplewas shaken with distilled water followed by filtration. To the filtrate, few drops of Fehling's solution A and B were added and boiled for few minutes. The appearance of an orange red precipitate confirmed the presence of reducing sugars.

Glycosides

5 ml of extract was taken in a test tube to which 25 ml of dilute Sulphuric acid was added and boiled for 15 minutes. Then the tube was cooled,neutralized with 10%NaOH followed by the addition of 5 ml of Fehling'ssolution. Formation of red precipitate indicated the presence of glycosides.

Polyphenols

The methanolic extract was mixed with water and to this solution, 1% (w/v) ferric chloride solution (3drops) was added. A greenish colour was developed indicating the presence polyphenols.

Tannins

About 0.5g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue black coloration which confirmed the presence of tannins.

Cardiac glycoside

To 0.5g of the extract diluted to 5ml in water, 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was under layered with 1 mL of concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristics of cardenolides.

Anthraquinone

0.5g of the extract was boiled with 10mL of Sulphuric acid and filtered while hot. Then the filtrate was shaken with 5mL of chloroform. The chloroform layer was pipetted into another test tube and 1ml of dilute NH_3 was added. Finally the resulting solution was observed for colour change to confirm the presence of anthraquinone.

Saponins

To 0.5g of extract was added 5 mL of distilled water in a test tube. The solution was then shaken vigorously and observed for a stable persistent froth which indicated the presence of saponins.

Carotenoids

About 1g of sample was extracted with 10 mL of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85% Sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

Microorganisms

The organisms used were *Bacillus subtilis*, *Stayphylococcusaureus*, *Salmonella typhi* and *E. coli.* They were obtained from Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal. The microorganisms were maintained on nutrient agar slant at 4°C.

Antimicrobial Screening of methanolic extract

The antibacterial property of crude extracts of medicinal plants was screened against four test microorganisms mentioned above by agar well diffusion method.

Sterile Muller Hinton agar (MHA) plates of approximately 4mm thickness were prepared. 8 h broth culture of each bacterium was seeded into the plates. Using a sterile cork borer no 5, a well of 6 mm in diameter was cut out in the plates. Then 50 µl of the extract was introduced into respective well. One of the wells was filled with pure methanol asa control. The plates were then left for half an hour with the lid closed to let the extract diffuse into media. The plates were incubated at 37 ^{0}C for 18-24 h. On the next day, the diameter of the zone of inhibition was measured. The experiment was done in triplicate and mean diameter of zone of inhibition was calculated as mentioned before [28, 29].

RESULTS AND DISCUSSION

Various species of the plants found in different geographical regions of Nepal were collected for the purpose of our study.

Table 1 List of medicinal plants used in this study with their therapeutic uses. Plants were collected from the different regions of Nepal. The traditional name, parts of the plant that are used with their therapeutic value is mentioned in the table.

Table 1 List of plants used in this study with their therapeutic uses

S.N.	Scientific Name	Family	English Name	Common Name	Locality	Parts used	Therapeutic uses	References
1	*Oxalis corniculata*	Oxalidaceae	Wood sorrel	Chari amilo	Chitwan	Whole plant	Antioxidant, antibacterial	[42]
2	*Drymaria diandra*	Caryophyllaceae	Chickweed	Abhijhalo	Chitwan	Whole plant	Anti HIV, sinusitis	[43]
3	*Melia azadarach*	Meliaceae	China berry	Bakaino	Chitwan	Leaf	Insecticide	[44]
4	*Cyperus rotundus*	Cyperaceae	Nut grass	Mothe jhar	Chitwan	Whole plant	Antidiabetic, dysyntery	[45]
5	*Cissampelos pareira*	Menisermaceae	Abuta	Batulpate	Chitwan	Aerial parts	antitumor, cytotoxic	[46]
6	*Coccinia grandis*	Cucurbitaceae	Ivy gourd	Kunruk	Chitwan	Aerial parts	Jaundice, leprosy	[47]
7	*Euphorbia hirta*	Euphorbiaceae	Snake weed	Dudhe jhar	Chitwan	Whole plant	Antihelmintic, antiinflammatory	[48]
8	*Cynodon dactylon*	Poacceae	Balama grass	Dubo	Chitwan	Whole plant	Antibacterial	[49]
9	*Ageratum houstonianum*	Asteraceae	Garden Ageratum	Gandhe jhar	Chitwan	Whole plant	Toxic	[50]
10	*Curcuma angustifolia*	Zingiberaceae	Turmeric	Beshar	Daman	Rhizomes	Antioxidant, antibacterial	[51]
11	*Shorea robusta*	Dipterocarpaceae	Saltree	Sal	Chitwan	Bark	Analgesics, antibacterial	[52]
12	*Acacia catechu*	Fabaceae	Cutch tree	Khayar	Chitwan	Bark	Antidiuretics, coolant	[53]
13	*Lyonia avalifolia*	Ericaceae	Pieris elliptica	Aanger	Syangja	Leaf	Skin diseases, Antiparasite	[54]
14	*Pterocarpus santalinus*	Fabaceae	Red sandalwood	Rakta chandan	Chitwan	Leaf	Skin care, cooling agent	[55]
15	*Desmostachya bipinnata*	Poacceae	Halfa grass	Kush	Chitwan	Aerial parts	Diarrhea, indigestion, asthma	[56]
16	*Cinnamomum tenupile*	Lauraceae	Sugandhakokila	Sugandha Kokila	Chitwan	Leaf	Imparting odour	[57]
17	*Justicia adhatoda*	Acanthaceae	Malabar nut	Asuro	Syangja	Leaf	Cardiac depressant	[58]
18	*Aegle marmelos*	Rutaceae	Golden apple	Bel	Chitwan	Leaf	Gastrointestinal, antivirus	[59]
19	*Mahonia napaulensis*	Berberidaceae	Mahonia napaulesis	Jamane mandro	Kathmandu	Leaf	Dyeing	[60]
20	*Phyllanthus emblica*	Phyllanthaceae	Emblic	Amala	Chitwan	Leaf	Diabetes	[61]
21	*Berberis aristata*	Berberidaceae	Tree turmeric	Chutro	Kathmandu	Leaf	Antifungal, antibacterial	[62]
22	*Tinospora sinensis*	Menisermaceae	Tinospora	Gurjo ko lahara	Syangja	Aerial parts	Anticancer, antidiabetic	[63]
23	*Cuscuta reflexa*	Convolvulaceae	Giant dodder	Aakash belli	Syangja	Aerial parts	Antibacterial, carminative	[64]
24	*Leucas cephalotes*	Ranunculaceae	Bara Halkusha	Bish mara	Syangja	Leaf	Poisonous	[65]
25	*Drynaria propinqua*	Polypodiaceae	Broken bone repairing	Commeri	Syangja	Bark	Strengthen bones, headache	[66]
26	*Centella asiatica*	Mackinlayaceae	Centella	Ghottapre	Kaski	Aerial parts	Brain stimulating, healing	[67]
27	*Asparagus filicinus*	Asparagaceae	Asparagus	Kurilo	Syangja	Aerial parts	Antioxidant, antibacterial	[68]
28	*Achyranthes bidentata*	Amaranthaceae	Oxknee	Datiwan	Syangja	Aerial parts	Toothache, inflammatory	[69]
29	*Bridelia retusa*	Phyllanthaceae	Kantakoi, Kanta	Gayo	Syangja	Bark	Antirheumatic, antifungal	[70]
30	*Litsea cubeba*	Lauraceae	Exotic verbena	Sidharlo	Syangja	Aerial parts	Antidepressant	[71]
31	*Cleistocalyx operculatus*	Oxalidaceae	Water Banyan	Kyamuno	Syangja	Bark	Muscular swelling	[72]
32	*Bauhinia variegata*	Fabaceae	Mountain ebony	Koiralo	Syangja	Bark	Asthma and ulcer	[73]
33	*Pogostemon amarantoidea*	Labiatae	Night jasmine	Rudilo	Syangja	Aerial parts	Cough, cold	[74]
34	*Betula alnoides*	Betulaceae	Indian birch	Sour	Manang	Bark	Cure diabetes	[75]
35	*Scoparia dulcis*	Scorphulariaceae	Broomweed	Chini jhar	Chitwan	Whole plant	Hypertension, bronchitis diabetes	[76]
36	*Bergenia ciliata*	Saxifragaceae	Hairy bergenia	Pakhanvedh	Manang	Root	Dissolving stone in the body	[77]
37	*Periploca calophylla*	Asclepiadaceae	Callophyllum wight	Shikari lahara	Manang	Aerial parts	Cerebral fever	[78]
38	*Astilbe rivularis*	Saxifragaceae	Thulo ausadhi	Thulo ookhati	Manang	Root	Dysentry, diarrhoea	[79]
39	*Piper mullesna*	Piperaceae	Kathali champa	Pipla	Syangja	Aerial parts	Asthma and bronchitis, antitumor	[80]
40	*Bombax ceiba*	Bombacaceae	Malabar semal	Simal	Chitwan	Bark	Oxylosis activity	[81]
41	*Calotropis gigantean*	Apocynaceae	Crown flower	Aak	Chitwan	Leaf	Paralysis, swelling	[82]
42	*Annona reticulata*	Annonaceae	Custard apple	Sarifa	Chitwan	Leaf	Toxic to intestinal worms	[83]
43	*Callicarpa sp.*	Labiatae	Verbenaceae	Dhaichamle	Chitwan	Aerial parts	Rheumatism, stomach trouble	[84]
44	*Mimosa pudica*	Fabaceae	Humble plant	Lajjawati	Chitwan	Aerial parts	Toxic alkaloids, neurology	[85]
45	*Ziziphus mauritiana*	Rhamnaceae	Wild berry	Bayar	Chitwan	Leaf	Gastrointestinal, antivirus	[86]
46	*Cascabela thevetia*	Apocynaceae	Lucky nut tree	Karbir	Chitwan	Aerial parts	Heart stimulant, poisonous	[87]

Their scientific name, local name, geographical location, parts used, their therapeutic value are presented in table1.Phytochemical screening of 46 medicinal plants showed the abundance of different bioactive components in different proportions denoted by +/- grading that shows their level

present in plants extracts individually (Table 2). The extracts were rich in secondary metabolites such as polyphenols, alkaloids, flavonoids, steroids, terpenoids, tannin, cardiac glycosides, reducing sugars and saponins.Microbial resistance is a global problem increasing rapidly, thus the use of new compounds belonging to polyphenols along with the clinically used antibacterial agents may be valuable [30]. Alkaloid comprises a diverse class of compounds possessing scaffolds with antibacterial property such as metronidazole and the quinolones [31]. Flavanoids are ubiquitously present in photosynthesizing cells and commonly found in flower, fruits, leaves, bark, tea and honey.

Saponin has been shown to possess significant hypo cholesterol, hypertensive and cardiac depressant properties [35].

The results on screening of *In-vitro* antibacterial activity of methanolic extracts of 46 medicinal plants are listed in table 3. Out of these, twenty three medicinal plants (italicized in red and black bold letters) were found active against all the bacterial strains used in our study and five plants (indicated in red bold letters) were the potent ones (table 4). For validating of antibacterial activity of screened medicinal plant extracts, standard drugs were tested against all the bacterial strains (table 5).

Table 2 Results of the phytochemical analysis of studied medicinal plants. Methanolic extracts from different plants were tested for the presence of polyphenols, steroids, flavonoids, Alkaloids,glycosides, reducing sugars, tannin, Cardiac glycoside, Anthra-quinone, Carotenoids and saponins using different biochemical assays as mentioned before.

Table 2: Results of the phytochemical analysis of studied medicinal plants

S.N.	Name of the plants	Polyphenols	Steroids	Flavonoids	Alkaloids	Glycosides	Reducing sugars	Tannin	Cardiac glycoside	Anthra-quinone	Carotenoids	Saponins
1	*Oxalis corniculata*	++	++	-	++	-	-	+	-	+	+	-
2	*Drymaria diandra*	+	+	+	-	-	-	+	-	-	+	-
3	*Melia azedarach*	+	++	++	++	-	-	+	-	-	-	-
4	*Cyperus rotundus*	-	+	-	-	-	-	+	-	-	+	-
5	*Cissampelos pareira*	+	-	--	++	-	-	+	-	-	+	-
6	*Coccinia grandis*	++	++	--	--	-	-	-	-	-	+	-
7	*Euphorbia hirta*	+	++	+	++	-	-	-	-	+	+	-
8	*Cynodon dactylon*	--	++	+	--	-	-	+	-	+	-	-
9	*Ageratum houstonianum*	+++	--	--	+	-	-	+	-	-	+	-
10	*Curcuma angustifolia*	--	--	--	++	+	+	+	++	+	-	-
11	*Shorea robusta*	++	--	+	--	-	-	+	+	+	-	-
12	*Acacia catechu*	++	--	+	--	+	+	-	+	+	-	+
13	*Lyonia ovalifolia*	++	-	++	--	-	-	+	+	+	-	+
14	*Pterocarpus santalinus*	++	++	++	+	-	-	-	-	-	+	+
15	*Desmostachya bipinnata*	--	++	-	++	-	-	+	-	+	+	-
16	*Cinnamomum tenuipile*	--	++	++	+	-	-	-	-	+	-	+
17	*Justicia adhatoda*	+	++	--	++	-	-	+	+	-	+	-
18	*Aegle marmelos*	++	++	++	++	-	-	+	+	-	+	+
19	*Mahonia napaulensis*	++	+	+	++	-	+	-	+	+	-	-
20	*Phyllanthus emblica*	+++	++	++	--	-	-	-	+	+	-	-
21	*Berberis aristata*	++	++	+	++	-	-	-	-	+	-	-
22	*Tinospora sinensis*	--	--	+	+	-	+	+	-	+	-	-
23	*Cuscuta reflexa*	+	--	+	++	-	-	+	+	-	-	-
24	*Leucas cephalotes*	--	++	+	++	-	-	-	+	-	+	-
25	*Drynaria propinqua*	--	--		++	-	-	-	-	-	-	+
26	*Centella asiatica*	--	++	+	+	-	-	-	-	+	-	-
27	*Asparagus filicinus*	--	-	+	++	-	+	+	-	+	+	-
28	*Achyranthes bidentata*	--	--	-	++	-	+	-	-	-	-	+
29	*Bridelia retusa*	++	--	+	--	+	+	+	+	-	-	-
30	*Litsea cubeba*	++	--	--	--	-	+	+	+	+	-	-
31	*Cleistocalyx operculatus*	+++	--	+	--	+	+	-	+	+	-	-
32	*Bauhinia Variegata*	+	--	+	--	-	+	+	+	+	+	+
33	*Pogostemon amarantoides*	++	++	+	-	-	-	-	-	+	-	-
34	*Betula alnoides*	++	--	--	-	+	+	+	+	+	+	+
35	*Scoparia dulcis*	++	+	+	++	+	+	-	+	+	-	+
36	*Bergenia ciliata*	++	--	++	--	+	+	-	+	-	-	+
37	*Periploca calophylla*	+	--	+	--	-	-	+	-	-	-	-
38	*Astilbe rivularis*	--	--	-	+	+	-	-	+	+	-	-
39	*Piper mullesna*	--	--	++	++	-	-	-	-	-	-	-
40	*Bombax ceiba*	+	--	++	-	+	+	+	+	+	-	+
41	*Calotropis gigantea*	--	++	++	++	-	-	-	-	+	-	-
42	*Annona reticulata*	+	+	+	++	+	+	+	+	+	+	-
43	Callicarpa sp.	++	++	+	++	-	+	-	+	-	+	-
44	*Mimosa pudica*	++	--	+	++	-	-	-	+	+	-	+
45	*Ziziphus mauritiana*	++	--	++	+	-	+	+	+	+	-	-
46	*Cascabela thevetia*	--	--	--	-	-	--	+	+	+	+	-

Flavanoids possess antibacterial, antifungal and antiviral properties [32]. Similarly, glycosides play an important role in defense mechanism against microorganisms, insects and herbivores [33]. This explains the antibacterial activity of the stem, bark, aerial parts and leaves extracts of plants [34].

Previous author have described that an inhibition zone of 14 mm or greater which include the diameter of disc was conceived as high antimicrobial activity [36].Screening for the zone of inhibition (ZOI) by the plant extracts indicated that

most of them have ZOI > 14mm for at least one organism which confirmed their medicinal value in future prospects.

Medicinal plants are the back bone of traditional medicine and plant extracts show antibacterial activities due to the presence of different chemical constituents, in the extract, which are classified as active antimicrobial compounds [37]. Plants have the capacity to synthesize a diverse array of chemicals and understanding of how these phytochemicals function in plants may improve our knowledge of the mechanism by which they benefit humans. In humans phytochemicals have complementary and overlapping actions including anti-oxidative, detoxification, immunomodulatory, anti-inflammatory, and anti-bacterial and anti-viral properties [38].

Table 3 Antimicrobial screening of plant extracts

Zone of inhibition (ZOI) mm (well diameter 6 mm)					
Name of the plant	**Control (mm)**	***Staphylococcus aureus***	***Salmonella typhi***	***Escherichia.coli***	***Bacillus subtilis***
Oxalis corniculata	6	-	-	-	-
Drymaria diandra	6	6.06 ± 0.15	6.26 ± 0.15	6.1 ± 0.1	6.23 ± 0.1
Melia azedarach	6	9.2 ± 0.17	8.13 ± 0.2	10.03 ± 0.05	8.03 ± 0.05
Cyperus rotundus	6	-	-	7.06 ± 0.1	9.1± 0.2
Cissampelos pareira	6	-	-	-	-
Coccinia grandis	6	-	-	-	-
Euphorbia hirta	6	9.3 ± 0.17	10.16 ± 0.15	9.06 ± 0.2	12.13 ± 0.1
Cynodon dactylon	6	9.16 ± 0.15	8.9 ± 0.1	7.1 ± 0.17	9.0 ± 0.0
Ageratum houstonianum	6	-	8.06 ± 0.2	9.23 ± 0.2	9.03 ± 0.05
Curcuma angustifolia	6	14.4 ± 0.1	15.06 ± 0.2	14.03 ± 0.05	15.26 ± 0.05
Shorea robusta	6	12.2 ± 0.17	13.23 ± 0.15	10.3 ± 0.17	11.06 ± 0.2
Acacia catechu	6	8.16 ± 0.2	15.13 ± 0.05	12.03 ± 0.05	10.06 ± 0.1
Lyonia ovalifolia	6	6.46 ± 0.2	8.23 ± 0.1	7.1 ± 0.2	8.53 ± 0.05
Pterocarpus santalinus	6	-	-	-	-
Demostachya bipinnata	6	-	7.2 ± 0.17	-	7.06 ± 0.05
Cinnamomum tenuipile	6	7.06 ± 0.2	-	-	-
Justicia adhatoda	6	13.26 ± 0.1	15.13 ± 0.2	12.2 ± 0.17	10.06 ± 0.05
Aegle marmelos	6	9.3 ± 0.1	6.16 ± 0.2	6.16 ± 0.05	16.03 ± 0.05
Mahonia napaulensis	6	-	-	-	-
Phyllanthus emblica	6	13.1 ± 0.2	10.23 ± 0.05	8.36 ± 0.25	11.2 ± 0.2
Berberis aristata	6	11.3 ± 0.17	12.03 ± 0.05	6.33 ± 0.05	9.06 ± 0.2
Tinospora sinensis	6	-	-	-	-
Cuscuta reflexa	6	-	7.06 ± 0.1	8.03 ± 0.15	7.36 ± 0.05
Leucas cephalotes	6	-	8.2 ± 0.17	9.03 ± 0.05	10 ± 0.1
Drynaria propinqua	6	-	-	-	-
Centella asiatica	6	-	-	-	-
Asparagus filicinus	6	7.3 ± 0.05	7.06 ± 0.05	10.03 ± 0.05	7.16 ± 0.23
Achyranthes bidentata	6	-	-	-	-
Bridelia retusa	6	13.5 ± 0.2	12.33 ± 0.2	16.23 ± 0.05	11.16 ± 0.1
Litsea cubeba	6	10.13 ± 0.11	10.0 ± 0.0	10.15 ± 0.2	13.03 ± 0.05
Cleistocalyx operculatus	6	18.23 ± 0.15	13.16 ± 0.15	16 ± 0.2	18.1 ± 0.2
Bauhinia variegata	6	14.1 ± 0.1	10.06 ± 0.2	11.13 ± 0.1	12.16 ± 0.2
Pogostemon amarantoides	6	6.03 ± 0.05	7.26 ± 0.2	6.13 ± 0.2	10.16 ± 0.01
Betula alnoides	6	11.3 ± 0.1	9.06 ± 0.05	10.13 ± 0.05	13.3 ± 0.3
Scoparia dulcis	6	-	-	-	-
Bergenia ciliata	6	12.06 ± 0.2	12.16 ± 0.1	12.23 ± 0.15	16.13 ± 0.1
Periploca calophylla	6	-	-	-	-
Astilbe rivularis	6	13.43 ± 0.2	11.13 ± 0.2	6.06 ± 0.5	6.16 ± 0.1
Piper mullesna	6	-	-	-	-
Bombax ceiba	6	10.26 ± 0.05	9.06 ± 0.05	8.16 ± 0.1	6.2 ± 0.17
Calotropis gigantea	6	12.23 ± 0.2	7.23 ± 0.15	-	-
Annona reticulata	6	6.36 ± 0.3	8.06 ± 0.2	17.03 ± 05	12 ± 0.17
Callicarpa sp.	6	9.53 ± 0.05	11.06 ± 0.1	10.13 ± 0.05	12.16 ± 0.05
Mimosa pudica	6	-	-	-	-
Ziziphus mauritiana	6	9.03 ± 0.05	12.16 ± 0.05	9.23 ± 0.1	15.2 ± 0.17
Cascabela thevetia	6	-	-	-	-

From our study it was revealed that out of 46 medicinal plants twenty-three were found to be the source of potent antibacterial agents. Importantly, the plant extracts of *Cleistocalyx operculatus* has remarkable ZOI (18.23 ± 0.15, 16 ± 0.2, 18.1 ± 0.2) against *S.aurues,E.coli and B.subtilis* respectively. Similarly extracts of *Bridelia retusa* is active against *E.coli* (ZOI: 16.23 ± 0.05).*Bauhinia variegate* showed ZOI 14.1 ± 0.1 against *S.aurues*.*Justicia adhatoda* is effective for *S.typhi* (ZOI: 15.13 ± 0.2). *Curcuma angustifolia* is potent against all the test organisms (ZOI: 14.4 ± 0.1, 15.06 ± 0.2, 14.03 ± 0.05, 15.26 ± 0.05 for *S.aurues,S.typhi, E.coli, B.subtilis* respectively). Other plant extracts showed moderate to good antibacterial activities ranges from10-15 mm/disc against the tested microorganisms. Our study showed that Nepalese medicinal plant extracts are the best sources of antibacterial agents in comparison to the plants of previously studied [39].

Table 3. Antimicrobial screening of plant extracts. Methanolic extracts of different plants (10mg/ml) were screened for the presence of anti bacterial compound against gram positive organisms like *Stayphylococcus aureus, Bacillus subtilis* and gram negative organisms like *Salmonella typhi,E.coli in* the terms of zone of inhibition by disc diffusion method. Out of 46 medicinal plants, 23 plants extract (indicated in bold letters)

contained antibacterial compounds against all the test organisms, while out of 23 plants, 5 plants (indicated in bold red letters) were the most potent for at least one test organisms. The experiment was done in triplicate and the data are indicated as maean ± standard deviation of zone of inhibition.

The plant extracts are more potent towards gram positive bacteria than the gram negative bacteria. The results are in agreement with previous studies which indicated that plant extracts were more active against gram positive bacteria than those of gram negative bacteria [36, 40]. Different species of plants influence their activity against microbes tested due to the difference in microbe's cell wall compound [41]. Significant bacterial growth inhibitory potential by these compounds offers a new area of research for the establishment of their structure activity relationship and mode of action of these compounds.

Table 4. Antimicrobial activity of drugs against gram positive and negative organism. For comparative analysis of strength of antibacterial activity of the plant extracts, standard antibacterial drugs were tested against the bacteria used in our study.

Table 4 Antimicrobial activity of drugs against gram positive and negative organism.

	Zone of inhibition (ZOI) mm				
Positive control	**concentration (µg)**	***Stayphylococcus aureus***	***Bacillus subtilis***	***Salmonella typhi***	***E.coli***
Ciprofloxacin	5	32		30	46
Erythromycin	15	22	20		30
Nalidixic acid	30				24
Chloramphenicol	30			25	

CONCLUSION

From the results of our study, we can conclude that the most of the plant extracts are valuable source of antimicrobial constituents and could be used for the isolation and identification of active ingredients. The result showed that *Cleistocalyx operculatus*, *Justicia adhatoda,Bauhinia variegate, Bridelia retusa, and Curcuma angustifolia* are promising plants for the treatment of bacterial infections. Moreover the methanolic extracts of the plants possess a diverse class of compounds which opens a new area of research for screening of these compounds for antifungal, antiviral, anti-inflammatory, anti allergic and anticancer activity.

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