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# ANTIOXIDANT AND WOUND HEALING PROPERTIES OF SOME SELECTED NEPALESE PLANTS

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#### **ABSTRACT**

Medicinal plants are significant sources of bioactive substances that need methodical investigation.

The chemical variety of natural goods offers many opportunities for the development of novel drugs.

The purpose of this research was to examine the biochemical characteristics of crude extracts from fifteen medicinal plants found in Nepal. Total flavonoid content (TFC), total phenolic content (TPC), and antioxidant activity were assessed using a colorimetric method, and the antibacterial activities were investigated by minimum measuring the inhibitory concentrations (MIC) using the broth dilution method and the zone of inhibition (ZoI) using the agar well diffusion method. Out of all the plant extracts under study, the methanolic extracts from Eupoterium adenophorum and Acacia catechu had the greatest TFC (10.23 ± 1.07 mg QE/gm) and TPC (55.21  $\pm$  11.09 mg GAE/gm). With an IC50 value of 1.3 µg/mL, Acacia catechu demonstrated excellent antioxidant activities. Myrica esculenta, Syzygium cumini, and Mangifera indica extracts were next demonstrate the to characteristics. Morus australis demonstrated antibacterial activity against the following pathogens: methicillin-resistant Staphylococcus aureus (MRSA) (ZoI: 19 mm, MIC: 0.19 mg/mL), Pseudomonas aeruginosa (ZoI; 20 mm, MIC: 0.05 mg/mL), Staphylococcus aureus ATCC 25923 (ZoI: 22 mm, MIC: 0.012 mg/mL), and Klebsiella pneumoniae (ZoI: 25 mm, MIC: 0.012 mg/mL). Broad-spectrum antibacterial activity was shown by an extract from Morus australis, Eclipta prostrata, and Hypericum cordifolium. It is advised that future research look into the medicinal plants' secondary metabolites to find further clinical effectiveness.

**Keywords:** minimum inhibitory concentration, secondary metabolites, medicinal herbs, and antibacterial activity.

# I. INTRODUCTION

The separation and identification of physiologically active chemicals and molecules from medicinal plants has resulted in innovative pharmaceutical treatments and advances. Secondary metabolites extracted from medicinal plants have played a significant role in upholding human health against various infectious diseases since ancient times. Plant extracts or their active phytoconstituents have been used as folk medicine by 80 % of the world's population in conventional therapies [1]. It is believed that over 50% of all modern clinical drugs are of natural product origin [2]. Multidrug resistance (MDR) is characterized as an acquired non-susceptibility to at least one antimicrobial agent from three or more categories [3]. Mobile genetic elements such as interferons, plasmids, and transposons are the most common carriers of antibiotic resistance among bacteria [4]. The rapid emergence of



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resistance to newly introduced antimicrobial agents, suggests that even a new antimicrobial agent would not be a complete solution to the problem [5]. MDR pathogens have raised a significant problem in public health by undermining the existing antibiotic-based treatment era, resulting in an increased mortality rate in patients [6]. MDR pathogens worsen the disease severity and put the value of antibiotics at risk, affecting the global economy [7]. It is anticipated that if the race of antimicrobial resistance (AMR) keep rising, it would take the lives of nearly ten million peoples annually by 2050 [8]. Thus, a new antibacterial agent is urgently needed to treat MDR induced infections caused by pathogens such as Enterobacteriaceae, Staphylococcus aureus, extended- spectrum βlactamase (ESBL) producing bacteria, among others [9].

Table 1: Description of medicinal plants used in this study.

| Medicinal plants         | Voucher<br>specimen | Local<br>name | Parts<br>used |
|--------------------------|---------------------|---------------|---------------|
| Eclipta prostrata        | NCDB203             | Bhringaraj    | Whole plants  |
| Shorea robusta           | NCDB212             | Saal          | Leaves        |
| Smallanthus sonchifolius | NCDB214             | Yacon         | Leaves        |
| Hypericum cordifolium    | NCDB201             | Arelu         | Leaves        |
| Mangifera indica         | NCDB211             | Mango         | Leaves        |
| Morus australis          | NCDB210             | Kimbu         | Barks         |
| Psidium guajava          | NCDB206             | Guava         | Leaves        |
| Chrysanthemum indicium   | NCDB205             | Godawari      | Leaves        |
| Myrica esculenta         | NCDB208             | Kafal         | Leaves        |
| Urtica ardens            | NCDB213             | Sisnoo        | Buds          |
| Pterocarpus marsupium    | NCDB204             | Bijayasal     | Barks         |
| Eupoterium adenophoium   | NCDB202             | Banmara       | Leaves        |
| Zingiber officinale      | NCDB200             | Aaduwa        | Leaves        |
| Acacia catechu           | NCDB209             | Khair         | Barks         |
| Syzygium cumini          | NCDB207             | Jamun         | Leaves        |

Medicinal plants produce secondary metabolites that can tackle MDR pathogens. Furthermore, medicinal plants have immunomodulatory and antioxidant activity, which result in antibacterial properties. They have a wide range of immunomodulatory effects stimulating both non-specific and specific immunity [10].

Antimicrobial and antioxidant activity is found in phytochemicals such as vitamins (A, C, E, and K), tannins, carotenoids, polyphenols, flavonoids, alkaloids, saponins, pigments, [11]. enzymes, terpenoids, and minerals Nonetheless, analgesic, antibacterial, deodorizing, febrifuge, fungicidal, antiseptic, astringent, galactagogue, diuretic. antidepressant, insecticidal, antipyretic, and sedative properties have been recorded for volatile oils from plants (Blanco et al., 2009; Bekoe et al., 2018; Iscan et al. 2002).

However, microorganisms have continuously evolved with a wide range of metabolic mechanisms to overcome drug effects [6]. Plantderived drugs are a superior choice over synthetic drugs because of fewer side effects and Jacob adverse effects (Bindu Narendhirakannan R.T., 2019; Verma et al., 2018). Nepal is rich in biodiversity and geographical condition with diverse flora, and numerous species are believed to possess curative properties. However, most of these claims lack scientific validation. The plants selected for this study are being used routinely by the indigenous people as remedies against various human diseases since ancient times. Therefore, the selected plants may contain certain important bioactive compounds that could have some medicinal and antimicrobial properties and some therapeutic value based on phytochemical constituents and their secondary metabolites. Hence, the antibacterial activity of plant extracts reported here would be beneficial to identify some potent secondary metabolites as future drug candidates for the therapeutic measures of MDR-strains-induced infections in Nepal and beyond.



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# II. MATERIALS AND METHODS

#### **Bacterial isolates**

Eight MDR bacterial strains: Acinetobacter spp. (628), Citrobacter freundii (377), methicillinresistant Staphylococcus aureus (MRSA) (338), Klebsiella pneumoniae (386), Pseudomonas aeruginosa (484), Escherichia coli (2A), Morganella morganii (4331), and Xanthomonas spp. (767) were collected from the National Public Health Laboratory (NPHL), Kathmandu, and transferred aseptically to the laboratory of the Department of Biotechnology, National College for further study. All isolates were obtained from clinical specimens. Besides, ATCC strains such as E. coli 25922, S. aureus 25923, Salmonella Typhimurium 14028, and K. pneumoniae 700603 were also collected from the NPHL stored at -20°C for further studies.

# Collection of plant materials

Different parts (leaves, bark, fruit, roots, and were collected based stem) on the and ethnomedicinal traditional medicinal practices from different geographical regions of Nepal as depicted in Table 1 (Collection period: January to June 2017). The plant samples were identified by National Herbarium and Plant Laboratories, Godawari, Lalitpur, Nepal, and herbarium collections were deposited in the Department of Botany, National College, Khusibu, Kathmandu.

#### **Preparation of plant extracts**

The plant parts (mentioned in Table 1) were dried in the shade at room temperature, pulverized into the powders with the help of a grinding mill, and then soaked in methanol for 24 hours. Then, they were filtered, and the process was repeated three times with fresh methanol. To obtain plant extracts, the filtrates were concentrated in a rotary evaporator at 50 °C.

#### **Determination of TPC and TFC**

Using Folin-Ciocalteu reagent and a 96-well plate-based colorimetric process, The TPC was calculated (Ainsworth & Gillespie, 2007; Bhandari et al., 2021). Initially, 20 µL of plant extract was mixed with 100 µL of Folin-Ciocalteu's reagent (1:10 v/v) and 80 µL of sodium carbonate (7.5%, w/v) in each wellcontaining standard and sample before incubation. Then, the sample was incubated at temperature, and absorbance room measured at 765 nm[15]. By comparing TPC to standard gallic acid, milligrams of gallic acid equivalents per gram of extract (mg GAE/gm) were determined. Likewise, for TFC, 20 µL of plant extract was mixed with 60 µL of methanol, 5 μL of potassium acetate (1 M), 5 μL of 10% aluminum chloride, and 110 µL of distilled water, then incubated at room temperature for 30 minutes, and the absorbance was measured at 415 nm[17]. Likewise, TFC was expressed as milligrams of quercetin equivalents per gram of extract (mg QE/gm extract) by comparing to standard quercetin [17].

# **Determination of antioxidant activity**

The antioxidant property was determined by discoloration assay based on the scavenging of 2, 2- diphenyl-1-picrylhydrazyl (DPPH) free radical (0.1 mM) (Brand-Williams et al., 1995; Aryal et al., 2021) at 517 nm using a multi-plate reader (Epoch 2, BioTek, Instruments, Inc., USA), maintaining 1 mg/mL of quercetin as a control. Crude extracts were allowed to react with DPPH free radicals for 30 minutes at room temperature. The scavenging of DPPH radical was calculated by using the following expression: (where optical density (OD) is the absorbance).

% Scavenging = 
$$100 - \frac{\text{(OD of extract)}}{\text{(OD of control)}} \times 100$$



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# **Antibacterial activity**

Using sterile cotton swabs moistened with the bacterial suspension, an inoculum suspension containing 1.5 x108 CFU/mL of bacteria was spread on firm Muller-Hinton Agar (MHA) plates (Balouiri et al., 2016; Marasini et al., 2015; Valgas et al., 2007). Using a sterile cork borer, wells were punched in plates (6 mm diameter) and micropipettes were used to fill the wells with a functioning suspension (50µL) of plant extracts (50 mg/mL), as well as neomycin (20 µg /mL), amikacin (30 mcg), and nitrofurantoin (30 mcg) as positive controls and 50 % DMSO as negative controls [23]. The MHA plates were incubated for 24 hours at 37°C and finally, the ZoI was determined after overnight incubation.

#### **Determination of MIC**

The broth dilution method was followed to determine MIC values of plant extracts as recommended by the Clinical and Laboratory Standards Institute [24]. Extracts of E. adenophorum, M. australis, E. prostrata, A. catechu, Z. officinale, P. marsupium, S. robusta, M. indica, S. sonchifolius, M. esculenta, U. ardens, H. cordifolium, S. cumini, P. guajava, and C. indicium showed significant antibacterial activity with larger ZoI, so they were selected for the determination of MIC value. The plant extracts were two-fold diluted to get a series of concentrations ranging from 25 mg/mL to 0.012 mg/mL in freshly prepared sterile nutrient broth. Then 20 µL of bacterial culture adjusted to 0.5 McFarland Standard was inoculated in each dilution tube and incubated at 37°C for 24 hours. The set-up included bacterial growth controls containing test tubes with media inoculated with 20 µL of bacterial inoculum only and negative controls with media and plant extract without bacterial inoculum. The MIC value was measured by choosing the lowest concentration of plant extract that inhibited the organism's growth in the test tubes, as determined by unaided observation. The bacterial growth in the tubes containing the plant extracts was compared to the control sample without the plant extracts to establish the growth endpoints. Each assay was carried out in triplicate to confirm the results.

# III. RESULTS

The researches on medicinal plants have been carried throughout the world to explore the bioactive compounds which could be used to make a preventive or treatment approach against various health complications. The ethnopharmacological applications of plants under study were depicted in Table 2.

# Yields, TPC and TFC of plant extracts

The percentage yield of plant extracts varied from 5.94% to 28.47% (Table 3). Extracts of H. cordifolium had the highest percentage yield (28.47%), followed by A. catechu (23.0%), P. guajava (21.82%), and M. esculenta (19.02%). Noticeably all plant extracts were found to be in semi-solid inconsistency.

Table 2: Medicinal plants selected understudy with their ethnopharmacological applications



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| Family                     | Ethnopharmacological applications  |
|----------------------------|--|
|                            | Used as an anti-inflammatory, antivonom [25], anti-aging, hepatoprotective, anti-viral antimicrobial agents. Bithiophenes and 5-(but-3-yes-1,2-diel)-5-(hydroxy-mathyl   |
| Asteraceae                 | 2,25thiuphene solated from this plant used as artibacterial and antibyperglycomi<br>[26], [27].  |
|                            | Used in the treatment of ulcer, cough, itching, leprosy, anthelmintic [28]. Antibacteria   |
| 1-12-1400 HV014 0          | wound bealing and anti-inflammatory activity due to the presence of polyphenols  |
| Dipterocarpaceae           | flavoroids, and triterpenoids, etc. Ursolic acid extracted from this plant is responsible  |
|                            | for showing antibacterial activity [29].   |
|                            | Used as a functional food, antioxidant, antimicrobial, probiotic, growth promoter [30]   |
| Asteraceur                 | Leaves extract contains the compounds faxtuanin and enhydrin show antibacteria<br>activity [31].   |
| Hypericaevae               | Treatment of back pain and broken boxes, an antidepressant [32]. Dermatological resurclogical, and traumatological problems, antibacterial activity [33].  |
|                            | Used for gastric disorders, mouth sores, tooth pain, and dermatological disorders. [34   |
|                            | Treatment for diabetes, infertility, ethanolic extract of M. indice showed significan  |
| Ansombacese                | antibactorial activity. Methanolic extract displayed cytotoxicity against the pancreatic<br>cancer cell line. Magniforin (5) from plant extract showed antimic robial effect [35], [36].   |
|                            | Treatment for Sever, protect the liver, improve eyesight, strengthen joints, lower blood   |
| Moracous                   | pressure [37]. Leaves contain 1-deoxynajirimycin known to have potential u   |
|                            | glucosidase inhibition activity. The piperidine alkaloid and glycoproteins from the<br>extract of M. australis have been used for antidiabetic agents [38].  |
|                            | Used for ulcers, wounds, toothache, anti-allergic effects, anti-cancer effects, and anti-  |
| Myrtacuae                  | hyperglycemia [39]. Used effectively in diabetes, diarrhea, dysentery, pain relact, cough  |
|                            | gastrocententis, hypertension, caries. The hypoglycemic components in Psidion gusjoo   |
|                            | might be due to oleanolic acid, arjunolic acid, ursolic acid, and glucuronic acid [40].  |
|                            | Used for hypertension, pneumonia, colitis, stomatitis, fever, neurological problems  |
| Asteraceus                 | headache [41], antipyretic purpose, treatment of cephalgia, vertigo, and ey-<br>inflammations [42].  |
|                            | Used for cough, aremia, asthma, chronic dysentery, fever, sores, tumors, nasal catarrh   |
| Microscope                 | piles, throat complaints, ulcers, and urinary dischargeo[43]. Used against differer<br>disease conditions such as; articliabetic, artiallergic, artimicrobial, anti-ulcer, arti  |
| 2001/2007                  | hypertensive, antioxidant, and higher phynolic and flavonoid compounds including   |
|                            | myricetin, myricanel, and myricanone have anti-inflammatory properties. [44]   |
|                            | Used for diabetes, diarrhea, excessive menstrual bleading, urinary disorders, respiratory  |
| Photogram                  | problems, ulcors, asthma, rheumatism, high blood pressure [45]. Treatment for sprains  |
| Urficacione:               | kidney stones, hemorrheids, flu, fever, hepatoprotective, nephroprotective effect, etc [46].   |
| Colorosso                  | Stomachache, cholera, dysentery, urinary complaints, tongue disease, toothache, ara  |
| - more said                | cough are all treated. [47]. Treatment of diabetes, jaundice, and an ofcer [48].   |
|                            | Used for treatment of emetic, diaphoretic, stimulant, tonic, fever, cuts and wounds  |
|                            | analgesic [49]. Used as an anti-inflammatory, blood coagulant, antimicrobial, antisoptic   |
| Asternome                  | and analysis; antipyretic Isomers of mono-cathographic acid present in I   |
|                            | almostorium exhibit potent anti-inflammatory, anti-bacterium, and anti-obesity   |
|                            | properties [50].   |
|                            | Treatment of diabetes bush blood resource concer atomachache manues authoris   |
|                            | Treatment of diabetes, high blood pressure, cancer, stomachache, nauses, asthma-<br>roservators disorders [51]. Treatment for diabetes blood pressure, atomach ache world-   |
| Zingiberaceae              | respiratory disorders [51]. Treatment for diabetes, blood pressure, stomach ache, weigh  |
| Zingiberaceae              |  |
| Zingibetaceae              | rispiratory disorders [51]. Treatment for diabetes, blood pressure, stomach ache, weigh<br>loss, diarrhea, and sausea. Geraniol present in Z. officieale shows potential anti-   |
| SSSSTEMMAN)                | respiratory disorders [51]. Treatment for diabetes, blood pressure, stomach ache, weigh<br>loss, diarrhoa, and rousen. Geraniol present in Z. officivale shows potential anti-<br>inflammatory and antioxidant offects [52].   |
| Zingi beraceae<br>Fabaceae | respiratory disorders [51]. Treatment for diabets, blood prossus, stimuta tachs, weigh-<br>bless, diarrha, and nausae. Caraini prosent in Z. efficiel shows potential ari<br>inflammatory and antioxidates effects [52].<br>It can be used to treat colds, cought, soch, bods, and sain evoptions. Monding masses<br>antipyratics, and acute and chronic wound basiling [53]. The lost constituents of Artistical near catesfrine and transfers, which have antinqual, nativing, antibreating anti-  |
| SSSSTEMMAN)                | rospiratory disorders [51]. Toutiment for diabeto, blood prosume, stimuth ache, weigh-<br>loss, diarrhas, and naunce. Ceranity provent in Z. efficient observe potential arti-<br>iraliammakery and antionidated officis [52].  It can be used in treat colds, cought, ukern, beda, and skin eruptions, blooding masses<br>antipyratics, and acute and chromic userual basiling. [53]. The losy constituents of A<br>statistic are catachin and trailodin, which have antifungal, antiviral, antibusterial, anti-<br>rialiammakery, and anticidated prospersions. [53].  |
| SSSSTEMMAN)                | respiratory disorders [51]. Toutiment for diabets, blood prossus, stimuta tachs, weigh-<br>box, diarrha, and nausae. Carmid prosent in Z. efficient shows potential ari<br>inflammatory and antioxidates effects [52].  It can be used to rest code, cought, sorbs, back, and sain everptions. Moniting masses<br>antisynotics, and acute and chronic wound bouling. [53]. The lay constituents of Ar-<br>storials are catestion and tastiction, which have antitionally, antivioral, antibuteviol, and<br>inflammatory, and antioxidates properties. [53].  Usual for diabetes mellions, constipations, storteck-docks, 110V, inflammation locuorrhoxul |
| SSSSTEMMAN)                | rospiratory disorders [51]. Toutiment for diabeto, blood prosume, stimuth ache, weigh-<br>loss, diarrhas, and naunce. Ceranity provent in Z. efficient observe potential arti-<br>iraliammakery and antionidated officis [52].  It can be used in treat colds, cought, ukern, beda, and skin eruptions, blooding masses<br>antipyratics, and acute and chromic userual basiling. [53]. The losy constituents of A<br>statistic are catachin and trailodin, which have antifungal, antiviral, antibusterial, anti-<br>rialiammakery, and anticidated prospersions. [53].  |
|                            | Asteracoae  Dipterocarpacoae  Asteracoae   |

Table 3: Physical characteristics and percentage yield of the crude extracts.

| Medicinal plants         | Local Name   | Dry weight of plant (gm) | Percentage yield (% |
|--------------------------|--------------|--------------------------|---------------------|
| Highericans confifelians | Arelu        | 40                       | 28.46               |
| Auxir adahy              | Khayr        | 50                       | 23.9                |
| Position guasara         | Gueva        | 50                       | 21.82               |
| Murior escalente         | Kaful        | 50                       | 19.02               |
| Syzagiam carette         | Jamun        | 50                       | 17.0                |
| Manggion indica          | Mango        | 50                       | 14.9                |
| Chryslethereine indicine | Godawari     | 50                       | 13.44               |
| Zirgiber officinale      | Cinger       | 50                       | 12.5                |
| Smallanthus soughth/iss  | Ground apple | 50                       | 11.16               |
| Распоситрые выплырами    | Bijayasul    | 50                       | 11.07               |
| Espoterium admoplerum    | Banmara      | 50                       | 30.42               |
| Stores relevas           | Sel          | 30                       | 9.1                 |
| Exlipte presinate        | Hheringraj   | 70                       | 6.54                |
| Adverse miestrolio       | Kimbu        | 34.9                     | 6.03                |
| Littics imless           | Signato      | 50                       | 5.94                |

# **Antioxidant activity**

Free radical scavenging activity was used to assess the antioxidant activity of plant extracts, and the resulting degree of decolorization is stoichiometric in terms of the number of electrons captured from plant extracts. The results of antioxidant abilities of plant extracts were compared with standard quercetin (IC50 2.28  $\mu$ g/mL). Among them, methanolic extract of A. catechu, M. esculenta, S. cumini, and M. indica showed promising antioxidant properties with IC50 ranging 1.3-1.80  $\mu$ g/Ml.

# **Evaluation of antibacterial activity**

Plant extracts were examined for antibacterial activity against eight MDR bacteria and four ATCC bacterial species adopting the agar well diffusion technique. The extracts of M. australis, S. robusta, and M. indica showed the largest ZoI i.e. 21 mm at 50 mg/mL towards E. coli ATCC 25922 in agar plates. Meanwhile, only E. prostrata extract showed 7 mm of the ZoI against K. pneumoniae ATCC 700603. The M. australis extract showed 22 mm of the ZoI against S. aureus ATCC 25923, which was the highest among the ZoI shown by plant extract. Similarly, M. australis extract showed the highest ZoI against three MDR bacterial strains, K. pneumoniae, MRSA, and P. aeruginosa with 25 mm, 19 mm, and 20 mm, respectively.

# IV. DISCUSSION

In developing health care, the search for new medicines with better or enhanced therapeutic actions derived from medicinal plants with significance ethnobotanical has increasingly valuable [57,58]. Extraction is the most important step in obtaining the plant's bioactive compounds, and the yield is determined by the solvent and extraction method used [59]. In this study, methanol was used as a solvent with a percentage yield of H. cordifolium being the highest (28.46 %) followed by A. catechu (23 %) (Table 3). The methanolic extract of A. catechu showed the highest TPC, while the extract of E. adenophorum showed the highest TFC values of  $55.21 \pm 11.09 \text{ mg GAE/gm}$  and  $10.23 \pm 1.07 \text{ mg}$ QE/gm respectively (Table 4 and Table 5). A. catechu had the highest free radical scavenging activity in the DPPH assay, followed by M. esculenta, S. cumini, and S. robusta. Flavonoid and phenolic compounds from plants have been shown to have free radical scavenging activity and antioxidant properties, according to previous research [60]. The methanolic extract of A.



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catechu shows the IC50 of about  $84.9 \pm 1.9$  µg/mL while  $1.30 \pm 0.05$  µg/mL in our study [19]. The difference might be due to environmental variation, temperature, harvesting time, and temperature. These antioxidant mechanisms defend humans from infections and degenerative diseases by inhibiting and scavenging free radicals [61].

The present study showed selected plant extracts possessed antibacterial activity; E. prostrata showed potential antibacterial activity against the ATCC strain of E. coli, S. aureus, and K. pneumoniae with ZoI ranging from 7 mm to 11 mm. Meanwhile, against MDR strains, the extract of E. prostrata showed ZoI against Acinetobacter spp. (628), K. pneumoniae (386), Morganella morganii (4331), and Xanthomonas spp. (767). Previous studies also support the antibacterial and antifungal activity of E. prostata (Chung et al., 2017; Khanna & Kannabiran, 2008). Cherdtrakulki at et al. (2015) reported that bioactive compounds isolated from the aerial parts of E. prostrata such as triterpenoids, 3acetylaleuritolic acid, stigmasterol, a mixture of triterpenoids, fatty esters, and aromatic components, had effective antimicrobial activity against Corynebacterium diphtheria NCTC 10356, Morexella catarrhalis, Streptococcus pyogenes and Saccharomyces cerevisiae ATCC 2601. Another study suggests the presence of alkaloids, cardiglycosides, phytosterol, beta-amyrin, polyacetylene, caffeic acid, stigmasterol, daucosterol on E. prostrata extracts and are found to be effective against K. pneumoniae, S. dysenteriae, E. coli, S. Typhi, B. subtilis, P. aeruginosa, and S. aureus [26]. Recently, ecliprostins A, B, and C isolated from this plant showed MIC of 25.0, 6.25 and 25.0 uM, respectively towards the growth of S. aureus [64]. M. australis extract showed a wide range of antibacterial activity against the MDR strains of Acinetobacter spp. (628), methicillin-

resistant S. aureus (MRSA) (338),K. pneumoniae (386), P. aeruginosa (484), and Xanthomonas spp. (767) with MIC value of 3.12 mg/mL, 0.19 mg/mL, 0.012 mg/mL, 0.05 mg/mL and 0.05 mg/mL respectively. A similar kind of result was observed by Wei et al. (2016) against a wide range of pathogens such as S. aureus, Fusarium roseum, S. faecalis, B. cereus, E. coli, K. pneumoniae, P. aeruginosa, Salmonella enterica serovar typhi, C. freundii, Candida albicans, Microsporum audouinii, B. subtilis, Micrococus flavus, and Salmonella abony due to presence of phytoconstituents such as that mulberrofuran, moracins, oxyresveratrol, morusin, and kuwanon C isolated from methanolic extract of Morus plant's root bark. Other plant extracts such as P. marsupium, M. esculenta, H. cordifolium also exhibited antibacterial activity against MDR strains with varying MIC values.

The plant extracts might have a wide variety of phytochemicals that have different mechanisms of action for their antimicrobial activity [66]. By inhibiting enzymes and highly oxidizing compounds, phenol or hydroxylated phenol inhibits bacterial development, likely through reaction with sulfhydryl groups or nonspecific interactions with proteins [67]. Antimicrobial effects are possibly due to flavonoid's ability to bind to extracellular and soluble proteins, as well as bacterial cell walls, inactivate enzymes, and disrupt microbial membranes [68]. Tannins function as antimicrobials by binding to adhesins, inhibiting enzymes, depriving bacteria of their food, forming a complex with the cell wall, disrupting membranes, and complexing metal ions [69]. Terpenoids and essential oils show antimicrobial activity by membrane disruption by the lipophilic compounds. Alkaloid acts as an antimicrobial agent by intercalating into the cell wall and DNA of parasites [10]. These results indicate that



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Nepalese medicinal plants contain different phytochemicals that need to be explored further to acquire a future drug candidate against MDR pathogens.

#### V. **CONCLUSION**

In addition to being used in the creation of medications to treat a wide range of ailments, medicinal plants have long been employed as traditional healers for a number of illnesses. A. catechu's bark extract had a high TPC (55.21 ± 11.09 mg GAE/gm), whereas the leaves extract of E. andenophorum had the greatest TFC (10.23 ± 1.07 mg QE/gm). The broad-spectrum antibacterial activity of Morus australis suggests that it might be the source of a future medication to treat illnesses linked to multidrug resistance. Similar to this, additional plant extracts with possible antibacterial action against clinical isolates of MDR bacteria included E. prostrata, M. esculenta, P. marsupium, and H. cordifolium. Future research is expected to look into the potential use of these plants in ethnomedicine and medication development to treat diseases brought on by pathogens resistant to drugs.

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