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Antimicrobial And Antioxidant Activity Of Selected Ethnomedicinal Plant Species From Western Ghats Region Of Kodagu District.

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ABSTRACT
Background: *This study delved into the investigation of the potential antimicrobial and antioxidant activities of five indigenous medicinal plants originating from the Western Ghats region in Kodagu district. The plants examined, namely Cissus javana, Jasminum coarctatum, Litsea coriacea, Rubus fockei, and Breynia vitis-idaea, were assessed for their ability to combat various pathogens. Simultaneously, their antioxidant properties were evaluated, aiming to identify natural sources of free radical scavengers. This research sought to contribute to the understanding and validation of the bioefficacies associated with these traditional medicinal plants.*
Materials and Methods*: The investigations were carried out on the potential of five medicinal plants from the Western Ghats region of Kodagu district for their antimicrobial and antioxidant activities. The plants screened for these activities are Cissus javana, Jasminum coarctatum, Litsea coriacea, Rubus fockei, and Breynia vitis-idaea. The aqueous and methanolic extracts of the leaves were tested against both gram-positive and gram-negative bacteria. Agar well diffusion method was employed to determine antimicrobial activity. The minimum inhibitory concentrations (MIC) were determined through broth microdilution using a Resazurin assay. DPPH assay was used to analyze the antioxidant activity.*
Results: *The plant extracts exhibited significant antibacterial activity against S. aureus and E. coli, with E. coli being more susceptible. Litsea coriacea had the highest zone of inhibition, with 20mm and 16mm for E. coli in its methanol and aqueous extracts, respectively, and 18mm for S. aureus in its methanol extract. All plant extracts showed concentration-dependent activity, with IC50 values below 5µg/mL, indicating good potential as free radical scavengers. Methanol extracts had lower IC50 values than aqueous extracts, likely due to a higher concentration of polyphenolic compounds.*
Conclusion: *The results have suggested that Litsea coriacea could be a potential candidate for the development of natural antibacterial agents. Rubus fockei exhibited the most outstanding in vitro antiradical capacity among all the extracts tested. An attempt has been also made to scientifically validate the bio efficacies of these tribal medicines.*

Keywords: *Antibacterial; Antioxidant; Pharmacognosy; Ethnomedicine; Bioactivity; Western Ghats.*

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I. Introduction

Nature has been a source of therapeutic drugs since ancient times and an astounding number of contemporary medications have been isolated from natural sources, many of these isolations were based on the knowledge of traditional medicinal practices. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine[1]. Plants are made up of secondary metabolites which are formed as products of primary metabolism and produced for defense against predators. Examples of such metabolites are tannins, flavonoids, and alkaloids; they are known to be the brain behind the healing potentials of plants[2]. Utilization of synthetic antioxidants, that is, citric acid, propyl gallate, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) in foods, leads to many side effects. For instance, these synthetic antioxidants have carcinogenic effects in living systems and many reports indicated that they may enhance microsomal enzyme activity and also enlarge liver size[3]. Oxidative stress induced by free oxygen radicals is the main reason for various degenerative diseases such as gastric ulcers, cancer, atherosclerosis, and other conditions. Medicinal plants are the source of many antioxidants acting as active oxygen scavengers. Interest has been focused on antioxidants from natural sources to avoid the drawbacks of synthetic antioxidants. In some studies, the potent activity of antioxidants is attributed to phytochemical compounds present in considerable high amounts in plants[4]. The widespread use of commercial antimicrobial drugs for treating infectious diseases has led to the development of drug resistance in pathogenic microorganisms. This rise in multidrug-resistant bacteria is a

growing concern for the global population. It has resulted in higher infection rates and antibiotic resistance, posing a significant therapeutic challenge. To address this issue, alternative antimicrobial drugs need to be developed from various sources, including medicinal plants. These alternative sources may provide new and effective treatment options to combat drug-resistant pathogens[5]. The present investigation evaluated the anti-oxidant and antibacterial properties of five medicinal plants. The study aimed to assess the potential of these plants as alternative sources of antimicrobial agents against drug-resistant pathogens.

II. Materials And Methods

Study area: Kodagu, a small district of Karnataka State in southern India occupies about 4,100 Km^2 of land on the eastern slopes of the Western Ghats. The district lies between North latitude 11°56' to 12°56' and east longitude 75°22' to 76°11'. Kodagu has a temperate climate with an average temperature of 15°C. The average rainfall for the district is 2,692 mm per annum. Kodagu district embraces evergreen forest to scrub forest. It is a habitat for more than ten different types of ethnic indigenous tribal groups [6].

Collection and identification: An ethnobotanical survey was conducted in the study area and multiple sources of information including the parts used, habits, ailments treated, and the formulations were recorded. Plants were selected for this study based on their medicinal use. The species were identified with the help of taxonomists and the aid of literature. Leaf samples of the selected species were collected from the Western Ghats region of Kodagu district for further bioprospection. The vernacular names, family, habits, parts used, and therapeutic formulations followed by ethnic groups are shown in Table 1.

Table 1: Selected medicinal plants for the evaluation of their bioactivity.

Sl. No.	Scientific Name	Vernacular Name	Family	Habit	Part Used	Therapeutic Formulation
1	*Cissus javana* DC.	Jone balli	Vitaceae	Climber	Leaves	Wound healing/ Carbuncles: Leaves are squeezed and the juice is applied onto the affected area and tied.
2	*Jasminum coarctatum* Roxb.	Mulleballi	Oleaceae	Climbing Shrub	Sap	Eye irritation: The sap obtained from the branches is collected and directly poured into the eyes which also gives a cooling effect.
3	*Litsea coriacea*	Erchikooti	Lauraceae	Tree	Leaves	Wound healing: The leaves are ground along with turmeric and tied onto the wound.
4	*Rubus fockei* K.N. Gandhi	Vaale pann	Rosaceae	Shrub	Root	Fever and cold: Root infusion is consumed for two days.
5	*Breynia vitis-idaea* (Burm.f.) C.E.C.Fisch.	Palli thoppu	Phyllanthaceae	Shrub	Leaves	Dermatitis: These leaves along with *Achyranthes aspera* L. leaves, *Azadirachta indica* leaves, and *Ocimum sanctum* leaves are made into a paste and boiled in oil, filtered, and applied on the affected area until it heals. Smallpox: These leaves along with *Coleus amboinicus* leaves and turmeric is ground and applied on the body and as well consumed.

Sample preparation: Plant materials were shade-dried, coarsely powdered, and both aqueous and methanol solvents were used for crude sample extraction using the Soxhlet apparatus. Then the methanol extracts were subjected to rotary evaporation and the aqueous extracts were subjected to lyophilization. The dried extracts obtained were stored in the refrigerator until further use.

Antimicrobial Activity

Test organisms: Microorganisms selected for the experiment were standard strains of *Staphylococcus aureus* (MTCC 96), and *Escherichia coli* (MTCC 443), which were procured from the Institute of Microbial Technology (IMTECH), Chandigarh.

Agar well diffusion method: The agar well diffusion method has been used to determine the antimicrobial activity. One ml of fresh bacterial strains of *Staphylococcus aureus* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria) was inoculated on the entire surface of the molten cooled agar plate of BHI broth

and Luria Bertani broth respectively at a viable count of 10^6 CFU/ml. Upon solidification, wells were made using a sterile cork borer (6 mm in diameter) into agar plates containing inoculums. Then, 100µl (3.7mg) of sample from mother stock was added to the respective wells of each plate. Gentamycin was used as a positive control since it is a broad-spectrum antibiotic. The plates were incubated at 37°C for 18 hours. The antimicrobial agent in the sample diffuses in the agar medium and inhibits the growth of the microbial strain tested. Antimicrobial activity was detected by measuring the zone of inhibition (including the diameter of the well) that appeared after the incubation period[7].

Minimum Inhibitory Concentration(MIC) Assay: Resazurin assay was used to determine the minimum inhibitory concentrations (MIC) of samples using the broth microdilution method. Serial dilutions of the stock solutions of the crude extracts in broth medium were prepared in a microtiter plate and the microbial suspensions were added in the microwells i. e. *E. coli* (10^6 CFU/ml) in Luria Bertani broth and *S. aureus* (10^6 CFU/ml) in BHI broth. 50µl (3.75mg/ml concentration) of different extracts were poured in the respective wells. 50µl (5mg, 100mg/ml) of Ampicillin was used as a positive control. To each well resazurin indicator solution (0.027g in 4ml sterile water) at 10µLwas added. The plate was covered with aluminum foil to prevent dehydration. The plate was incubated overnight at 37°C. The change in color from purple to pink was assessed visually and was recorded as positive for bacterial growth. The lowest concentration at which color change is observed is the MIC value for the test material and bacterial strain[8,9,10].

Antioxidant Activity

DPPH Assay: For the estimation of free radical scavenging activity, 0.5 mM of DPPH (2,2-diphenyl-1-picrylhydrazyl) solution is prepared in ethanol, and 1.0 ml of this solution is added to 2.0 ml of different concentrations of methanolic and aqueous extracts, vortexed and allowed to stand at 27 °C in a dark place for 30 minutes. Blank is prepared without adding the extract. Ascorbic acid at various concentrations is used as a standard. The absorbance of the prepared samples was measured using a UV spectrophotometer at a wavelength of 520 nm[11]. The capability of extracts to scavenge the DPPH radical is calculated using the following equation:

DPPH Scavenged (%) = [(Abs control- Abs test) / Abs control]x 100

Where Abs control is the absorbance of the control reaction and Abs test is the absorbance in the presence of the sample. The antioxidant activity of the methanolic leaf extract is expressed as IC50 and compared with the standard. The IC50 value is defined as the concentration in (µg/ml) of extracts that scavenges the DPPH radicals by 50%.

Statistical analysis: Linear regression model was used and the results were entered into the Microsoft Excel® software to allow for analysis and interpretation. To better understand the data, various graphs, and tables were extracted from it. These visual representations allowed for easier identification of patterns, trends, and correlations in the data.

III. Results

The results showed that plant extracts demonstrated significant antibacterial activity against *S. aureus* and *E.coli.* It can be noted from Table 2 that *E. coli.* was more susceptible to the plant extracts which is a gram-negative bacterium. *Litsea coriacea* had the highest zone of inhibition area of 20mm with methanol extract and 16mm with aqueous extract for *E. coli,* while the inhibition was 18mm for *S. aureus* in its methanol extract.

Table 2: Antibacterial activity in terms of zone of inhibition(mm) of plant extracts.

Sl. No.	Medicinal Plants	*Escherichia coli* (Gram -ve bacteria)		*Staphylococcus aureus* (Gram +ve bacteria)	
		Methanol extract	Aqueous extract	Methanol extract	Aqueous extract
M1, A1	*Cissus javana*	10 mm	12 mm	13 mm	-
M2, A2	*Jasminum coarctatum*	12 mm	-	-	-
M3, A3	*Litsea coriacea*	20 mm	16 mm	18 mm	-
M4, A4	*Rubus fockei*	18 mm	15 mm	18 mm	-
M5, A5	*Breynia vitis-idaea*	14 mm	-	-	-
	Gentamycin (Control)	21 mm	25 mm	22 mm	24 mm

Where,M- methanol sample, and A- aqueous sample

The methanol extracts of all samples exhibited antibacterial activity against *E. coli* (Fig. 1A) at a concentration of 7.5mg, with the zone of inhibitions measured as shown in Table 2. The Gentamycin disc (30mg, Himedia) used as control showed the zone of inhibition of 21mm in Fig. 1A. In the Resazurin assay (Fig. 1B), 50µl sample tested containing 3.75mg/ml extract with Log_2 dilution with methanol extracts had the following results; M1: 2^5 = 0.117mg, M2: 2^6 = 0.058mg, M3: 2^7 = 0.029mg, M4:2^6 = 0.058mg, M5:2^5 = 0.117mg represented MIC for *E. coli* inhibition.

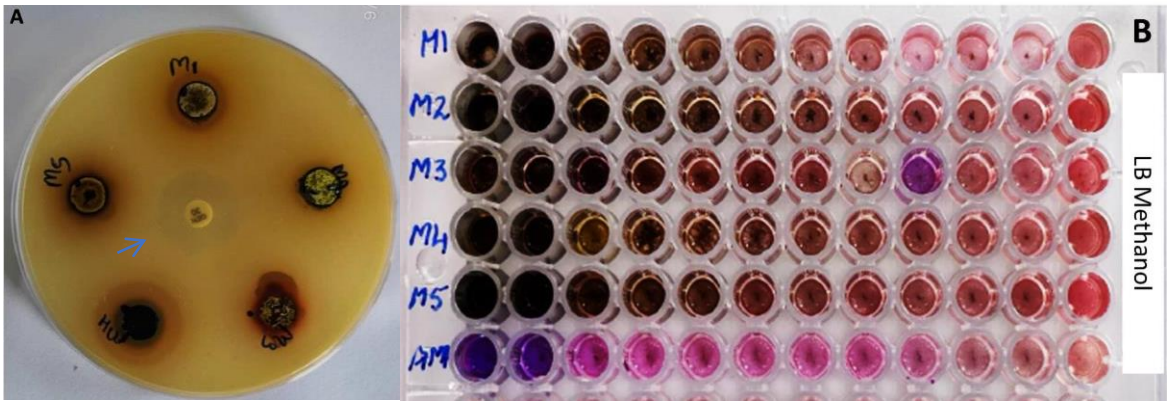


Fig. 1: (A) Antimicrobial activity of methanol samples on *E. coli*. The arrow indicates the zone of inhibition. (B) Resazurin plate assay after 12h [Blue color indicates inhibition and pink indicates growth; the test organism is *E. coli*) Lane 1,2,3,4,5: Test compound (Log_2 dilution + broth + resazurin + *E. coli*) Lane 6: Positive control (Ampicillin in serial dilution + broth + indicator + *E. coli*).

The aqueous extracts of *Cissus javana*, *Litsea coriacea*, and *Rubus fockei* exhibited antibacterial activity on *E. coli* (Fig. 2A) at a concentration of 7.5mg, with the zone of inhibitions as shown in Table 2. The Gentamycin disc (30mg, Himedia) used as control showed the zone of inhibition of 25mm in Fig. 2A. In the Resazurin assay (Fig. 2B), 50µl sample tested containing 3.75mg/ml extract with Log_2 dilution with Aqueous extracts had the following results; A1: 2^3 = 0.468mg, A2: 2^2 = 0.937mg, A3: 2^1 = 1.85mg, A4: 2^3 = 0. 0.468mg, A5: 2^3 = 0.468mg represented MIC for *E. coli* inhibition. Amp (5mg) had no MIC for *E. coli* inhibition.

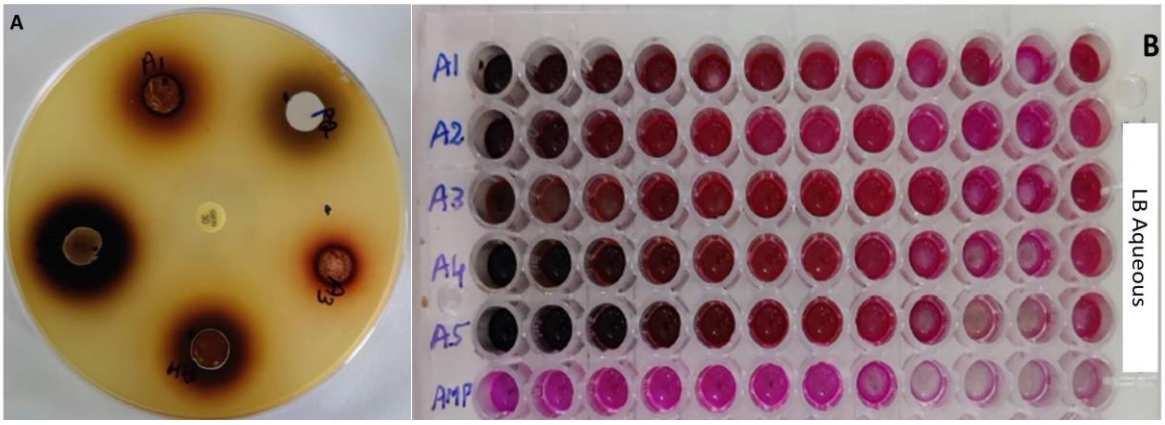


Fig. 2: (A) Antimicrobial activity of aqueous sample on *E. coli*. (B) Resazurin plate assay after 12h (Blue colour indicates inhibition and pink indicates growth; the test organism is *E. coli*) Lane 1,2,3,4,5: Test compound (Log_2 dilution + broth + resazurin + *E. coli*) Lane6: Positive control (Ampicillin in serial dilution + broth + indicator + *S. aureus*).

The methanol extracts of *Cissus javana*, *Litsea coriacea*, and *Rubus fockei* exhibited antibacterial activity on *S. aureus* (Fig. 3A) at concentrations of 7.5mg, with the zone of inhibitions as shown in Table 2. The Gentamycin disc (30mg, Himedia, Mumbai) used as control showed the zone of inhibition of 22mm in Fig. 3A. In the Resazurin assay (Fig. 3B), 50µl sample tested containing Log_2 dilution with methanol extracts (3.75mg) and Amp (5mg) had no MIC for *S. aureus* inhibition.

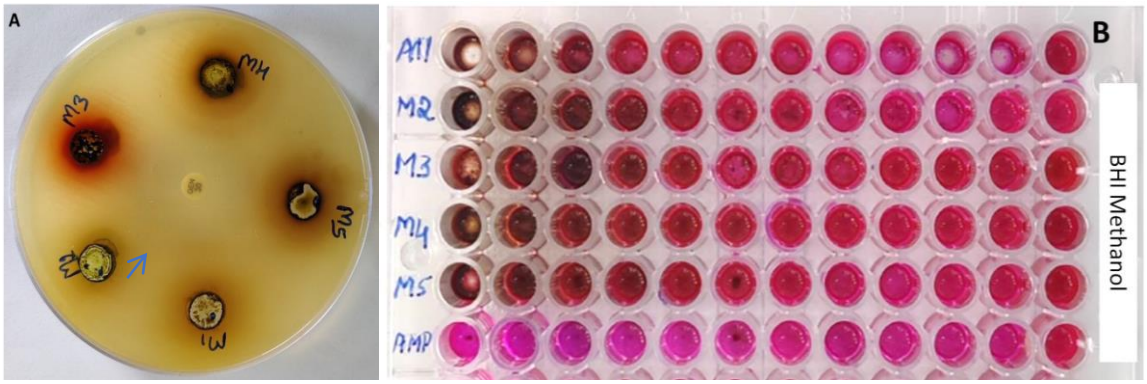


Fig. 3: (A) Antimicrobial activity of methanol sample on *S. aureus*.(B) Resazurin plate assay after 12h (Blue color indicates inhibition and pink indicates growth; the test organism is *S. aureus*) Lane 1,2,3,4,5: Test compound (Log_2 dilution + broth + resazurin + *S. aureus*), Lane 6: Positive control (Ampicillin in serial dilution + broth + indicator + *S. aureus*).

The aqueous extracts exhibited no antibacterial activity on *S. aureus* (Fig. 4A) at concentrations of 7.5mg. The Gentamycin disc (30mg, Himedia) used as control showed the zone of inhibition of 24mm in Fig. 4A. In the Resazurin assay (Fig. 4B), 50µl sample tested containing Log_2 dilution with aqueous extracts (3.75mg) and Amp (5mg) had no MIC for *S. aureus* inhibition.

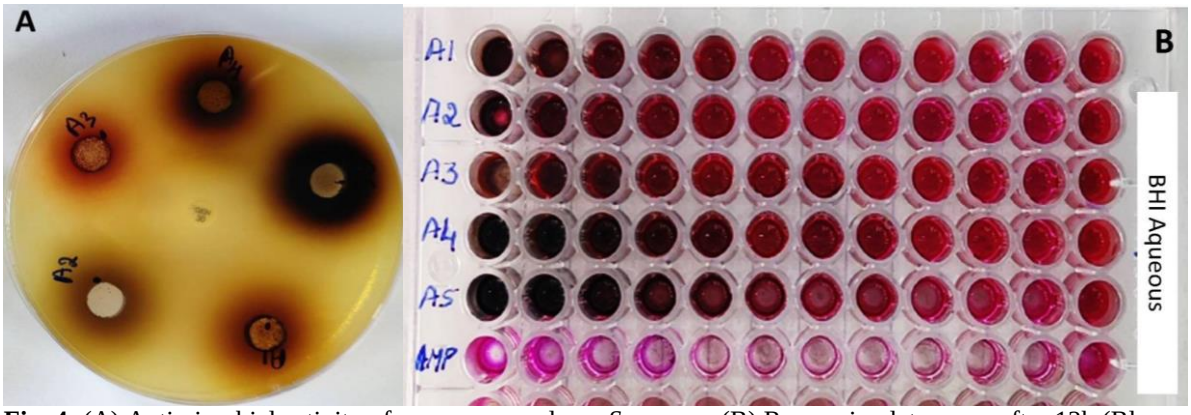
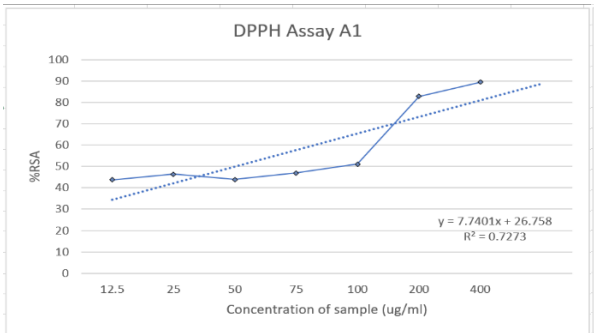


Fig. 4: (A) Antimicrobial activity of aqueous sample on *S. aureus*. (B) Resazurin plate assay after 12h (Blue color indicates inhibition and pink indicates growth; the test organism is *S. aureus*) Lane 1,2,3,4,5: Test compound (Log_2 dilution + broth + resazurin + *S. aureus*), Lane 6: Positive control (Ampicillin in serial dilution + broth + indicator + *S. aureus*).

All extracts presented IC_{50} values below 5µg/mL (Table 3), showing extremely good potential as free radical scavengers. Methanol extracts showed lower IC_{50} values compared to aqueous extracts and hence showing that those extracts are better radical scavengers which might be due to the chemical composition, especially rich in polyphenolic compounds. *Rubus fockei* exhibited an outstanding *in vitro* antiradical capacity whose IC_{50} was the least among all other extracts with an IC_{50} value of 2.35µg/ml for aqueous extract and 2.04 µg/ml for methanol extract. Ascorbic acid which was used as positive control showed an IC_{50} value of 32.73 µg/ml which was much higher than the samples tested. It is important to notice that the work has been carried out with crude extracts instead of pure substances. Phenolic compounds from medicinal plants possess strong antioxidant activity and may help to protect the cells against oxidative damage caused by free radicals. Consequently, if the studies were to be conducted with isolated compounds in in-vivo conditions the specimens might prove to be excellent radical scavengers.

Table 3: IC_{50} values of the DPPH radical scavenging activities of selected medicinal plants.

Medicinal Plants	**IC_{50} value (µg/ml)**	
	Aqueous extract	**Methanol extract**
Cissus javana	3.00	2.13
Jasminum coarctatum	3.63	2.46
Litsea coriacea	3.54	2.20
Rubus fockei	2.35	2.04
Breynia vitis-idaea	3.83	4.30


DPPH Assay A1
%RSA
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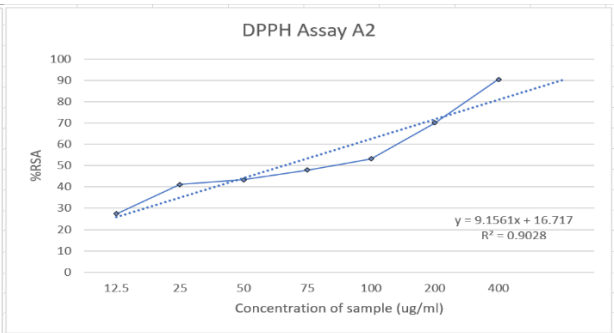
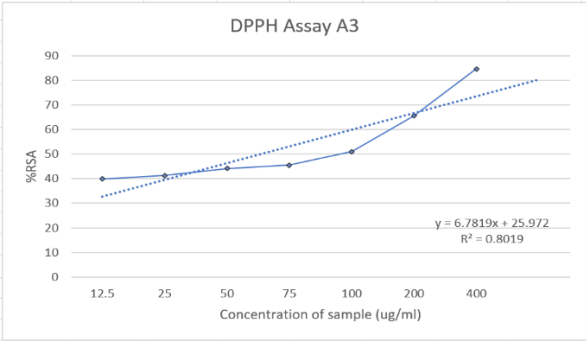
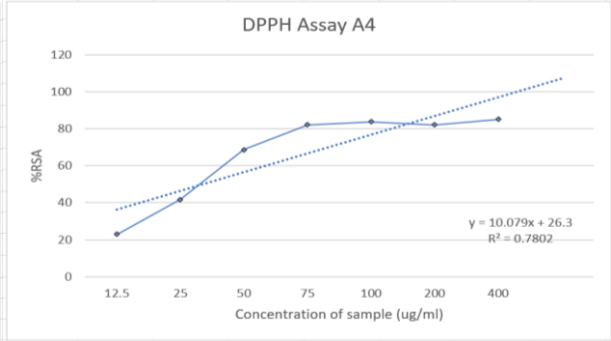
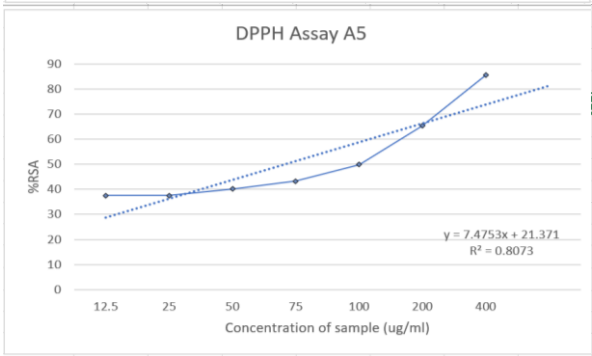
R² = 0.7273
12.5 25 50 75 100 200 400
Concentration of sample (ug/ml)


DPPH Assay A2
%RSA
y = 9.1561x + 16.717
R² = 0.9028
12.5 25 50 75 100 200 400
Concentration of sample (ug/ml)


DPPH Assay A3
%RSA
y = 6.7819x + 25.972
R² = 0.8019
12.5 25 50 75 100 200 400
Concentration of sample (ug/ml)


DPPH Assay A4
%RSA
y = 10.079x + 26.3
R² = 0.7802
12.5 25 50 75 100 200 400
Concentration of sample (ug/ml)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%RSA
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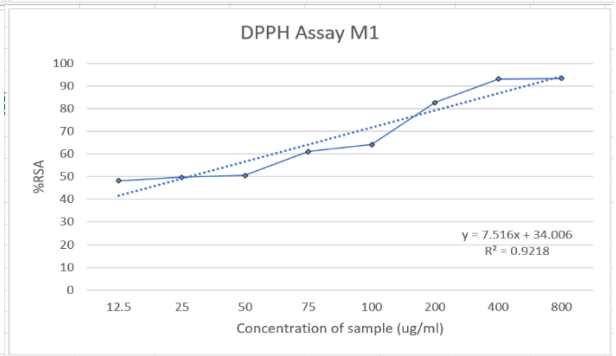
R² = 0.8073
12.5 25 50 75 100 200 400
Concentration of sample (ug/ml)


DPPH Assay M1
%RSA
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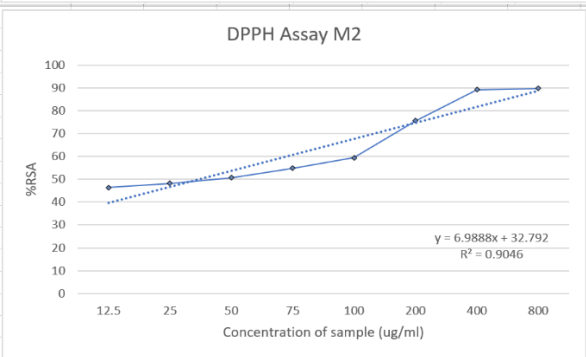
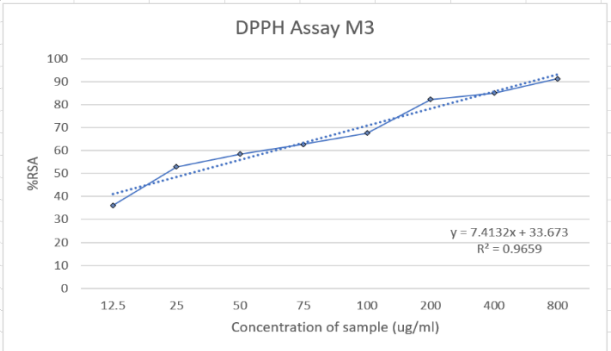
R² = 0.9218
12.5 25 50 75 100 200 400 800
Concentration of sample (ug/ml)


DPPH Assay M2
%RSA
y = 6.9888x + 32.792
R² = 0.9046
12.5 25 50 75 100 200 400 800
Concentration of sample (ug/ml)


DPPH Assay M3
%RSA
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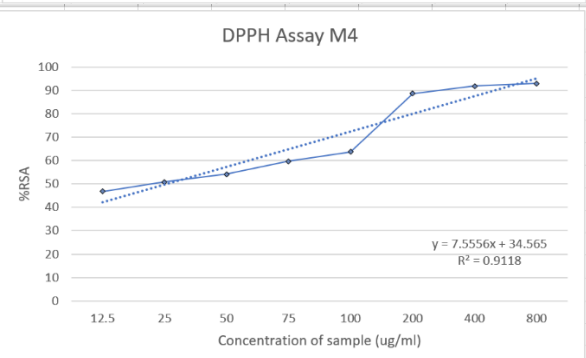
R² = 0.9659
12.5 25 50 75 100 200 400 800
Concentration of sample (ug/ml)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%RSA
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R² = 0.9118
12.5 25 50 75 100 200 400 800
Concentration of sample (ug/ml)

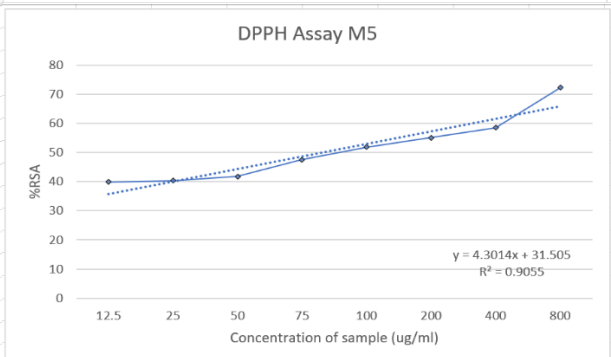
DPPH Assay M5
%RSA
y = 4.3014x + 31.505
R² = 0.9055
12.5 25 50 75 100 200 400 800
Concentration of sample (ug/ml)

Fig. 5. Graphs representing free radical scavenging activity of Aqueous and Methanolic crude extracts of five species *Cissus javana, Jasminum coarctatum, Litsea coriacea, Rubus fockei,* and *Breynia vitis-idaea* respectively.

IV. Discussion

The zones of inhibition induced by the test organisms indicated their susceptibility to the plant extracts and it was observed that it varied from one organism to another and from one plant extract to another. The plant extracts were active against both Gram-positive and Gram-negative bacteria, though they were more active against the latter. The results may be of great importance since *Escherichia coli* is a type of bacteria that is commonly found in the human and animal gastrointestinal tract. However, certain pathogenic strains of *E. coli* can cause various infections, including gastrointestinal infections, urinary tract infections, meningitis, peritonitis, and septicemia. Data obtained through surveillance indicate that resistance to antimicrobial agents is most prevalent in E. coli strains that have been in use for the longest period of time in both human and veterinary medicine [12].

Being rapid, simple, and independent of sample polarity, the DPPH method is very convenient for the quick screening of many samples for radical scavenging activity[13]. DPPH is a type of free radical that can maintain stability at room temperature and creates a purple solution when mixed with ethanol. When an antioxidant molecule is present, DPPH undergoes a reduction reaction that results in a colorless solution. This reduction process happens when certain compounds can donate hydrogen atoms, and it is based on the mechanism of the DPPH free radical. Many plants contain secondary metabolites like flavonoids, tannins, and phenolic acids that are rich in phenolic compounds. These compounds have antiradical properties due to their ability to donate hydrogen atoms. The IC50 was employed to ascertain the antioxidant efficacy of the samples. The lower the IC50 value of the sample, the greater its ability to scavenge the radical. All the plants exhibited a concentration-dependent activity(Fig. 5).

The effectiveness of medicinal plants in fighting against microbes is not solely dependent on the type of plant species. There are various other factors that play a role in determining the antimicrobial properties of these plants, including altitude, temperature, illumination, and moisture. These factors can impact the production and processing of secondary metabolites in medicinal plants. Furthermore, the location of medicinal plants can also influence the composition and concentration of active ingredients present in them[14]. The food and pharmaceutical industries are currently very keen on finding natural antioxidant and antimicrobial agents with no adverse effects and thus it has become a pressing need to find effective bio compounds from plant extracts.

Different extracts obtained from selected ethnomedicinal plants were studied to determine their antioxidant and antimicrobial properties. According to our results, in most cases, the highest radical scavenger capacity was detected in both methanol and aqueous extracts, which indicates that polyphenols may be responsible. These species may be used as a source of natural antioxidants to stabilize food against oxidative deterioration or as biologically active products to protect cells from damage and senescence.

V. Conclusion

The findings obtained from the current study provide a basic scientific explanation for the traditional usage of *Cissus javana*, *Litsea coriacea*, and *Rubus fockei* as a remedy against microbial pathogens. Nevertheless, it is imperative to conduct in-vivo experiments on medicinal plants to assess the toxicity of their active compounds, identify any potential adverse effects, study their pharmacokinetic properties, and determine the minimum inhibitory concentration (MIC) required in infected tissues and organs. Further improvement in the antimicrobial activity of these medicinal plants could be achieved by purifying their active components and determining appropriate dosages for effective administration.

Thus, plants studied can be utilized to prevent oxidative damage brought on by free radicals, and diseases brought on by pathogenic bacteria. The findings of this study may lead to the development of novel and effective treatment options for infectious diseases. Further research has to be carried out to isolate and characterize active compounds from the plants to ascertain their bio efficacies.

VI. Acknowledgement

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