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ANTI - BACTERIAL GUIDED FRACTIONATION OF *DALBERGIA SISSOO*

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Keywords:

Dalbergia sissoo, Ampicillin, Norfloxacin, gram-negative, Gram-positive

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ABSTRACT: Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of human kind. The plant *Dalbergia sissoo* contains various chemical constituent with specific value in ethno-medicinal practices and has significance in the field of health care system. The main objective of this study involves the evaluation of anti-bacterial activity of *D. sissoo* leaves and barks extracts of chloroform, ethyl acetate, acetone and methanol. The assay was done by bioassay guided fractionation by disc diffusion method on both *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) bacteria using Ampicillin for *S. aureus* and Norfloxacin for *E. coli* as positive control and DMSO (Dimethyl Sulphoxide) as a negative control. The anti-bacterial activity of methanol extracts of leaves was found to be more as compared to other extracts. *S. aureus* was found to be more susceptible to plant extracts than *E. coli*. Thus, the results obtained confirm the therapeutic potency of *D. sissoo* used in traditional medicine.

INTRODUCTION: According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs [1]. Generally, about 80% of total world population use plants based traditional medicine for their primary healthcare need [2]. Out of 2, 25,000 species of plants, only less than 10 percentage of study have been carried out for the medicinal uses [3]. A substantial number of various new antibiotics which are introduced in the market are obtained from natural or semi-synthetic resources [4]. The demand of plant based medication is increasing day by day in every country throughout the world due to the growing recognition that they are natural products, are non narcotic, with no side effects, are easily available at affordable prices and also sometimes a only one source of healthcare available for the poor [2].

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Plants have been used as a valuable source of natural products for maintaining human health for a long period of time [1]. Plant derived medicine might provide a cheap, biodegradable and effective control way in rural areas of various developing countries [5]. The medicinal plant(s) derived medicines have been a source of wide variety of biologically active compounds for hundreds of years and used extensively as crude material or as a pure compounds for treating various types of human / animals disease because they contains various components of therapeutic value [6].

Different parts of plants have been used throughout the world by human beings and as well as a animals as well as drugs and remedies for various kinds of diseases since time immemorial. In various parts of the world the administration of the decoction of plants is still in practice [4]. There are large number of practitioners of traditional medicinal system using medicinal plants which is generally over 1.5 millions in prevention, promotion and cure of the diseases [7]. Although pharmaceutical industries have produced a large number of new antibiotics in the last years, the resistance in the micro-organisms against these drugs has also been increased.

In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [1]. The microbial resistance is growing as a most important problem and the use of anti-microbial drugs is still uncertain. Therefore, appropriate actions must be taken to reduce this problem [1].

Dalbergia sissoo, a wind-dispersed tropical tree [8] belongs to the fabaceae family [9, 10] which is the third largest family of angiosperm plant with about 730 genera and more than 19400 species around, including the plants commonly known as legumes. The family fabaceae includes horticultural varieties and many species harvested which are as crops and for oils, fiber, fuel, timber, medicines, and chemicals. Different types of phytochemicals such as alkaloids, non-protein amino acids, amines, flavonoids, isoflavonoids, coumarins, phenyl-propanoids, anthraquinones, di-, sesqui- and triterpenes, cyanogenic glycosides, protease inhibitors and lectins have been reported in this family. The therapeutic effect of medicinal plant is generally due to the presence of phytochemicals such as alkaloids, terpenoides, glycosides, phenolic compounds, polysaccharides [11] and also accumulation of heavy metals [12].

It is an erect deciduous tree of about 25 meters in height with trunk of grey yellow in color and a diameter of 2 - 3 meters [9]. It contains leathery, pinnately compound leaves with alternate leaflets. They are broad, ovate, acuminate, glabrescent, petiolate with pointed tip. Flowers are whitish to pink, fragrant nearly sessile and the crown is oval in shape. The pods are oblong, flat, thin, strap like 4 - 8 cm long and 1 cm in wide with 1 - 4 seeds. It contains a kidney shaped seeds which are 4 - 5 mm, thin and flat with light brown in color. The taproot is long and numerous surface roots which produce suckers. The flowering period is generally March - May [13]. It is generally found in the attitude of 1400 m in open places and also found in western Asia, Afghanistan, India, Pakistan Bhutan, Bangladesh and subtropical Africa [14].

D. sissoo possesses various types of chemicals which can be extracted using various solvents. Polar compounds are extracted by polar solvents while non-polar compounds are extracted by non-polar solvents. It consists of various constituents such as alkaloids, saponins, coumarins, tannins, flavonoids, quinones, proteins, terpenoids, flavonoids, glycosides and sterols that have been investigated in several studies [15].

D. sissoo has been traditionally used for different medicinal purposes to cure various types of diseases [16] and is reported as an folk medicine and is used mainly as aphrodisiac, expectorant, emesis, ulcers, leucoderma, dysentery, stomach troubles, skin diseases [17], anti-diarrhoeal [18], stimulation of new bone formation [19], anti-resorptive [20], anti-inflammatory [21], anti-fungal [11], anti-bacterial, anti-helminthic, bronchodilator, abortifacient, anti-pyretic, aphrodisiac, expectorant, analgesic [22], astringent, bleeding, piles, varicose veins [4], osteogenic [23], reversible suppression of spermatogenesis [24], in heart problems [25, 26], menstrual disorders [27] as gastroprotective [28], during jaundice [29] and also to treat sore throats, syphilis, gonorrhea and also showed anti-oxidant activity [30] which is almost two times higher than Selenium and Vitamin E. The tribal peoples of Odisha use the extract of *D. sissoo* for the treatment of scalding of urine [31].

E. coli are usually present as commensals and also have several virulent factors and colonize in a biofilm fashion that results a variety of intestinal and extra intestinal diseases. Therefore, it is necessary or the need to develop newer, safer, cheaper and effective cheaper anti-microbial agents to tackle this problem. The plant source can be the alternative solution for this problem, which can be found naturally [32]. This research is aimed to collect, extract, evaluate and to compare *in vitro* anti-bacterial activity of *D. sissoo* in different solvent systems against *S. aureus* and *E. coli*.

MATERIALS AND METHODS:

Chemicals and Equipments:

Solvents: Chloroform (Thermo Fisher Scientific, India Pvt. Ltd., Mumbai), ethyl acetate (Thermo Fisher Scientific, India Pvt. Ltd., Mumbai), acetone (Thermo Fisher Scientific, India Pvt. Ltd., Mumbai) and methanol (RCFL Ltd., Haryana) were used as solvents. Water was prepared in the laboratory with Distilled water plant.

Chemicals: Chemicals used in the experiments were Mueller Hinton Agar (HiMedia Laboratories

Pvt. Ltd., Mumbai), Nutrient Broth (HiMedia Laboratories Pvt. Ltd., Mumbai) and DMSO (Thermo Fisher scientific, India Pvt. Ltd; Mumbai).

Test Organisms: The test organism used for the research was *E. coli* and *S. aureus* collected from Lumbini Zonal Hospital.

Antibiotics: Ampicillin and Norfloxacin antibiotic discs were used which was purchased from Himedia, India.

Equipments: Equipments used in the experiments were beakers (50 ml, 100 ml, 500 ml), volumetric flasks (500 ml, 1000 ml), micropipette, pipettes (1 ml, 10 ml, 50 ml), round bottom flask (1000 ml), cotton, plastic bottle, aluminium foils, detergents, conical flasks, test tubes, measuring cylinders (50 ml, 500 ml), spatulas (stainless steel), plant cutter, paper sheets, glass rods, washing brush, funnels, filter paper (Whatman no.1), scale, marker, gloves, mask, stand punching machine, inoculating loop, forceps, sterile filter paper.

Instruments:
Instruments Used in the Experiment were:

- Digital balance (ATX224, SHIMADZU Corporation, Philippines).
- Rotary evaporator (R-210/215, BUCHI Labortechok AG, Switzerland).
- Vacuum pump (V-700/215, BUCHI Labortechok AG, Switzerland).
- Refrigerator (LG) purchased from India.
- Grinder
- Distilled Water (DW) plant.
- Autoclave (S.M. Scientific Instruments Pvt. Ltd., Delhi).
- Hot air oven (S.M. Scientific Instruments Pvt. Ltd., Delhi).
- Incubator (S.M. Scientific Instruments Pvt. Ltd., Delhi).
- Sonicator (Indosati Scientific Lab Equipments).

Collection of Plant Parts: The leaves and barks were collected from Ramapur of Rupandehi district in July, 2016 from Western Development Region of Nepal. A voucher is deposited at the herbarium in the Museum of Material Medica, Crimson College of Technology and was identified with the help of botanist Mr. Homnath Pathak, PNC (Prithvi Narayan Campus) and pharmacognosist Mr. Ravin Bhandari, M. Pharma.

Drying: Collected plant materials were cleaned with tap water and were then rinsed with distilled water. The remaining water was wiped with the help of clean cloth. They were then air dried in shade under the newspaper at room temperature in a well ventilated room. The drying was carried out for 15 days with proper checking at regular interval.

Comminution of Dried Plants: After the plant parts were dried, they were grinded to a coarse powder using a portable cutting machine and then grinded using grinder. The grinded powder was kept in air tight plastic bottle, sealed in order to prevent contamination and stored at a room temperature in a dark place until use.

Extraction Procedure:

- 100 gm of powder (leaves and barks) was taken and kept for first maceration for 24 hours in 700 ml of chloroform.
- Then it was filtered by Whatman filter paper No.1 and the chloroform extract and residue was separated in different beakers.
- The residue left was then again dissolved in ethyl acetate and kept for 24 hours and after then filtered and separated.
- The same procedure was done in acetone and methanol.
- The whole extraction procedure was done in the room temperature and using a Whatman filter paper No.1.

Evaporation of Extracts: The filtrates obtained from extraction process were then evaporated to dryness using rotary vacuum evaporator. All the extract of both leaves and barks were evaporated at a temperature of 40 °C and 20 rpm. The concentrates were kept in a beaker and the percentage yields of the extract were calculated. Then, the concentrate kept in a beaker were covered with aluminium foil and stored in the refrigerator at temperature of 4 °C until use.

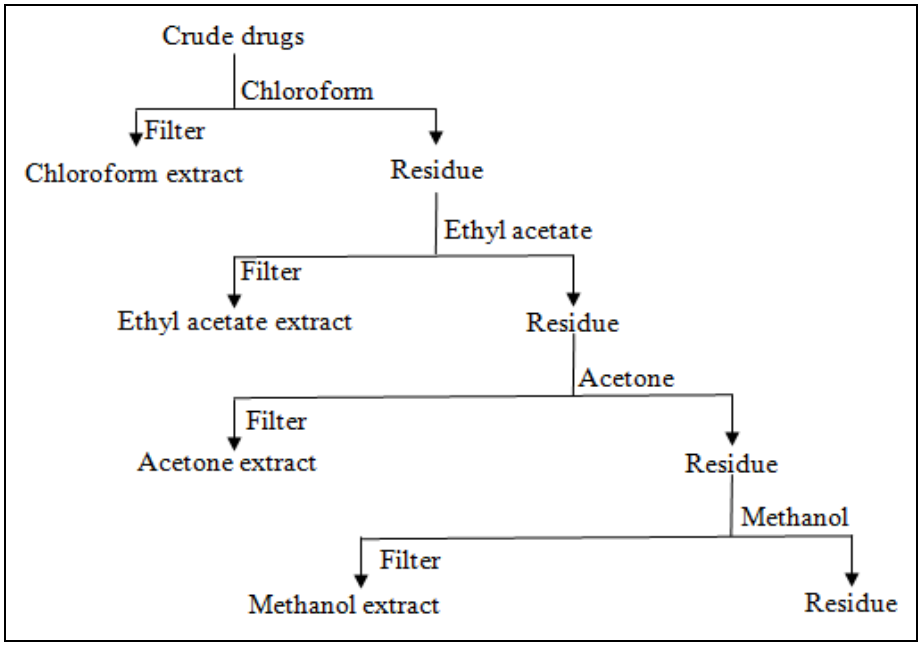

FIG. 1: ANTI-BACTERIAL GUIDED FRACTIONATION

Anti-bacterial activity Test: *D. sissoo* leaves and barks extracts were evaluated for anti-bacterial activity by disk diffusion method respectively.

Preparation of Solution of Extract: Each 150 mg of the extracts used were dissolved in 1.5 ml of Dimethyl Sulphoxide (DMSO) to make a solution of concentration 100 mg/ml of each chloroform, ethyl acetate, acetone and methanol extract. Then it was mixed properly by vigorous shaking with the help of sonicator and then covered with aluminium foil. It was then kept in refrigerator at 4 °C until used [33, 34].

Preparation of Inoculum: Bacterial suspension of *Staphylococcus aureus* and *Escherichia coli* was prepared by addition of nutrient agar. Nutrient agar was prepared by pouring 6.5 gm of Nutrient Broth in 500 ml of distilled water with proper shaking after mixing them. It was autoclaved for 15 min at 121 °C at 15 lbs pressure. About 10 ml of the autoclaved nutrient medium was poured in test tube and fixed the slanting position. It was the allowed to solidify for 15 min. After then a loop was taken and then a colony of each bacterium were streaked in the different slant and incubated at 37 °C in incubator for 48 hr. In the prepared slants 10 ml of freshly prepared sterile saline solution was added and the colonies formed on the medium were scraped with an inoculating loop. Turbid solution of each bacterium was obtained and kept for further use.

Preparation of Filter Paper Discs: Whatman filter paper no. 1 was used to prepare the paper discs approximately 6 mm in diameter by a paper punching machine. These paper discs were sterilized by using autoclave.

Preparation of Culture Media: 38 gm of Mueller Hinton agar was weighted and placed in the conical flask and was dissolved with 1000 ml of distilled water and mixed vigorously. The solution obtained after mixing was heated to boiling in order to ensure the proper mixing. The media in the conical flask was then covered with cotton and wrapped with aluminium foil. It was then sterilized in autoclave for 15 min at 121 °C at 15 lbs pressure. The autoclaved / sterilized media was then let to cool to 40 - 50 °C and was poured in petri-plates having the diameter of 6 cm and let them solidified and was kept under acceptable environment on refrigerator until used.

Inoculation of Inoculums in the Petri-Plates: Optimally within few minutes after adjusting the turbidity of the inoculums suspension (tube containing suspension of microorganism), a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This helped to remove excess inoculums from the swab. The dried surface of the media plate was inoculated by streaking the swab over the entire sterile media surface.

This procedure was repeated by streaking of two more times, rotating the plate to ensure properly distribution of inoculums. This process was performed for each two microorganism *E. coli* and *S. aureus*. All the plates were labelled with name of micro-organisms, concentration of the plant on disc, standard antibiotic dose at backside. Then such labelled disc was placed in incubator.

Application of Plant Extracts to the Prepared Filter Paper Discs: A 10 µl micropipette was adjusted so that a single press on the micropipette was delivered a concentration of 1 mg/disc of plant extract from the concentration of 100 mg/ml to filter paper discs. In same way it was adjusted to the 10 µl of micropipette so that a single press (1mg/disc) on the micropipette was deliver a DMSO on filter paper discs.

Anti-bacterial Assay: The extracts were tested for their effect against the growth of pathogenic bacteria by disc diffusion method. The paper disc was then kept in the media where 1 mg/disc extract (sample) and DMSO (negative control) was poured by the help of 10 µl micropipette and was allowed to diffuse for 1 hr. The antibiotics (standard) norfloxacin and ampicillin were also then placed on such culture media. Total 48 petridisc was used, for each single extract 3 petridisc was used. The plate was then incubated at 37 °C for 24 hr for bacterial growth. After the specified time, the zone of inhibition of extract and antibiotics were measured. Triplicates were maintained for all this experiments.

Measuring Zone Sizes: Following incubation, the zone sizes were measured to the nearest millimeter using a ruler. The diameter of the disc was included in the measurement. When measuring zone diameter, it was round up to the next millimeter. The plate was held a few inches above a black, non-reflecting surface illuminated with reflected light. The plate was viewed using a direct, vertical line of sight to avoid any parallax that might result in misreading. The zone measured was recorded on the recording sheet. If the placement of the disc or the size of the zone did not allow to read the diameter of the zone, then it was measured from the centre of the disc to a point on the circumference of the zone where a distinct edge was present (the radius) and the radius of the disc was reduced. The measurement was then multiplied by 2 to determine the diameter.

RESULTS AND DISCUSSION:

Determination of Yield Value: The crude drug was extracted in four different solvent system *i.e.* chloroforms, ethyl acetate, acetone and methanol to compare anti-bacterial activity with four different solvent. Among them methanol extract have higher yield value than other solvent systems.

Following table that shows the yield value of extract. The yield values of each extract were calculated as:

$$\text{Yield value} = \frac{\text{Extracts obtained}}{\text{Total amount of crude drug}} \times 100$$

TABLE 1: EXTRACTION YIELD VALUE

S. no.	Parts of Plant	Solvent	Wt. of extracts (gm)	Extract Yield value (%)
1	Barks	Chloroform	0.2726	0.2726 %
2	Barks	Ethyl acetate	0.1615	0.161 5%
3	Barks	Acetone	0.3706	0.3706 %
4	Barks	Methanol	1.701	1.701 %
5	Leaves	Chloroform	1.3532	1.3532 %
6	Leaves	Ethyl acetate	0.7613	0.7613 %
7	Leaves	Acetone	0.6239	0.6239 %
8	Leaves	Methanol	3.219	3.219 %

Anti-bacterial Activity Test: In the present study, the *in vitro* anti-bacterial activity of leaves and barks extracts of *D. sissoo* were done using chloroform, ethyl acetate, acetone and methanol solvents by the presence or absence of inhibition zones. Results of the anti-bacterial activity obtained using disc diffusion assay are summarized in **Table 2** and **Table 3**. Out of eight extracts different extracts showed different zone of inhibition. The data indicated that the extracts displayed a variable degree of anti-bacterial activity on different tested strains and the micro-organisms tested exhibited variable sensitivity against the different plant extracts.

The positive control for gram-positive strain and gram-negative strain was Ampicillin and Norfloxacin, respectively. DMSO was used as negative control for the study.

According to **Table 2**, methanol, acetone and ethyl acetate extract exhibit no zone of inhibition against *E. coli*. While, the zone of inhibition of 11 ± 2 mm, 7.67 ± 0.57 mm and 7.2 ± 0.57 mm were obtained against *S. aureus* respectively. The maximum zone of inhibition was seen against *S. aureus* in comparison to *E. coli*, which was in case of methanol extract. Positive control drug Norfloxacin shoed the zone of inhibition of 25 mm against *E. coli* and Ampicillin showed the zone of inhibition of 19.67 ± 0.577 mm against *S. aureus*.

According to **Table 3**, acetone, methanol and chloroform extract exhibit no zone of inhibition against *E. coli*. While, the zone of inhibition of 10 mm, 7.67 ± 1.53 mm and 5 ± 1 mm were obtained against *S. aureus* respectively. The maximum zone of inhibition was seen against *S. aureus* in comparison to *E. coli*, which was in case the of acetone extract. Positive control drug Norfloxacin showed the zone of inhibition of 25 mm against *E. coli* and Ampicillin showed the zone of inhibition of 20 mm against *S. aureus*.

TABLE 2: ANTI-BACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *DALBERGIA SISSOO* LEAVES

S. no.	Chemicals	Diameter of Zone of Inhibition (mm) (mean ± SD)	
		E. coli	***S. aureus***
1.	Chloroform	5.33 ± 0.57	0
2.	Ethyl acetate	0	7.2 ± 0.57
3.	Acetone	0	7.67 ± 0.57
4.	Methanol	0	11 ± 2
5.	Negative control (DMSO)	0	0
6.	Ampicillin	0	19.67 ± 0.577
7.	Norfloxacin	25	0

TABLE 3: ANTI-BACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *DALBERGIA SISSOO* BARKS

S. no.	Chemicals	Diameter of Zone of Inhibition (mm) (mean ± Standard)	
		E. coli	***S. aureus***
1.	Chloroform	0	5 ± 1
2.	Ethyl acetate	9.67 ± 1.53	7.33 ± 1.15
3.	Acetone	0	10
4.	Methanol	0	7.67 ± 1.53
5.	Negative control (DMSO)	0	0
6.	Ampicillin	0	20
7.	Norfloxacin	25	0

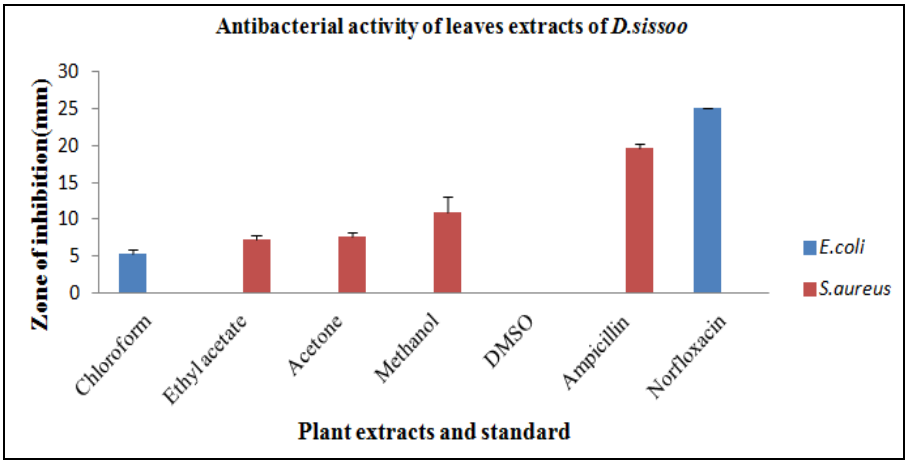

FIG. 1: ANTI-BACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *DALBERGIA SISSOO* LEAVES

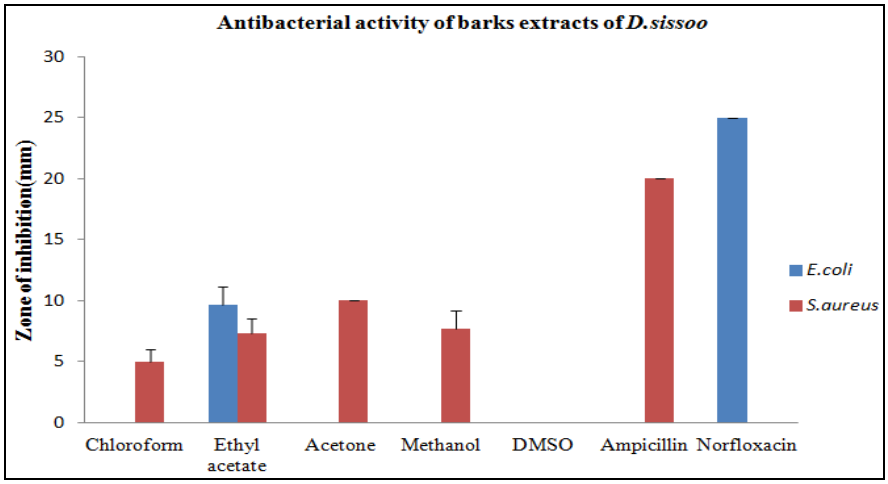

FIG. 2: ANTI-BACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *DALBERGIA SISSOO* BARKS

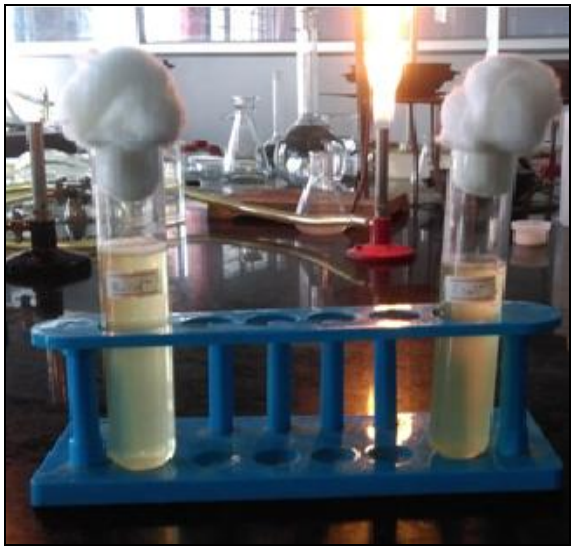

FIG. 3: STANDARD SOLUTION OF *E. COLI*

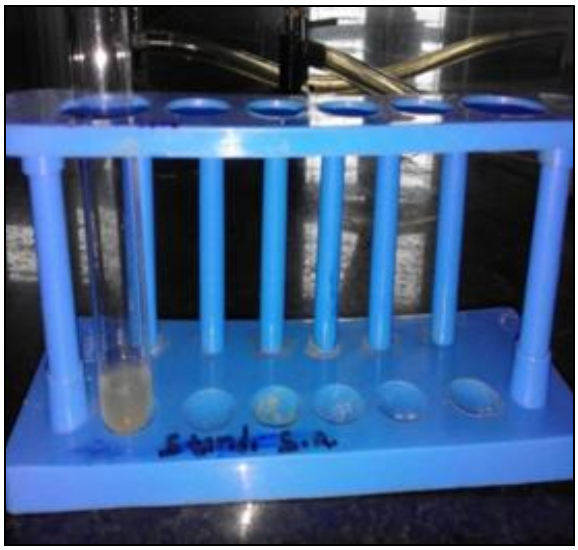

FIG. 4: STANDARD SOLUTION OF *S. AUREUS*

FIG. 5: MEASURING ZONE OF INHIBITION

The present study was conducted to obtain preliminary information on the anti-bacterial activity of leaves and barks extracts of *D. sissoo* in four different solvents (methanol, chloroform, ethyl acetate and acetone) from Western region of Nepal by the help of disc diffusion method. It was seen that the extraction yield in methanol was higher than that of other solvent systems. The highest

extraction yield for methanol barks extract was 1.701% and methanol leaves extract was 3.219% of *D. sissoo* plant. The factors such as; the age of the plant and polarity of the different solvents used may also affects the yield of the plant extracts [32].

In the present investigation, the anti-bacterial efficacy of leaves and barks extracts of *D. sissoo* plant were evaluated. The anti-bacterial efficacy of plant extract was done for four different solvent (methanol, chloroform, ethyl acetate and acetone).

In the leaves extracts at the concentration of 1 mg/disc the of the maximum zone of inhibition against *S. aureus* was obtained in methanol extract with the zone of inhibition of 11 ± 2 mm while there is no inhibition against *E. coli* in methanol extracts. Also, the maximum zone of inhibition of extract against *E. coli* was obtained in chloroform extract with the zone of inhibition of 5.33 ± 0.57 mm while there is no inhibition against *S. aureus* in chloroform extract.

Similarly, for the barks extracts at the concentration of 1 mg/disc the maximum zone of inhibition against *S. aureus* was obtained in acetone extract with the zone of inhibition of 10 mm while there is no inhibition against *E. coli* in acetone extract. Also, the maximum zone of inhibition of extract against *E. coli* was obtained in ethyl acetate extract with the zone of inhibition of 9.67 ± 1.53 mm while the maximum zone of inhibition against *S. aureus* in ethyl acetate extract was 7.33 ± 1.15 mm.

In the leaves extract of methanol, acetone and ethyl acetate extract exhibit no zone of inhibition against *E. coli*. While, the zone of inhibition of 11 ± 2 mm, 7.67 ± 0.57 mm and 7.2 ± 0.57 mm were obtained against *S. aureus* respectively. The maximum zone of inhibition was seen against *S. aureus* in comparison to *E. coli* which was in case of methanol extract.

In the barks extract of acetone, methanol and chloroform extract exhibit no zone of inhibition against *E. coli*. While, the zone of inhibition of 10 mm, 7.67 ± 1.53 mm and 5 ± 1 mm were obtained against *S. aureus* respectively. The maximum zone of inhibition was seen against *S. aureus* in comparison to *E. coli* which was in case the of acetone extract.

In the study conducted by Parmar HKIS *et al*., 2014 the anti-bacterial activity of methanol extracts of leaves against *E. coli* at the concentration of 5 µg/ml, 100 µg/ml showed the zone of inhibition of 2 mm for 5 µg/ml and 7 mm for 100 µg/ml respectively [16].

In the study conducted by Hussain M *et al*., 2014 the anti-bacterial activity of methanol extracts of whole plant plant (pods, leaves, stem bark, root bark) against *E. coli* and *S. aureus* showed the zone of inhibition of 19 mm against *E. coli* and 18 mm against *S. aureus* respectively and methanol extracts of leaves only against *E. coli* and *S. aureus* showed the zone of inhibition of 17 mm against *E. coli* and 16.80 mm against *S. aureus* respectively [22].

While in our study the anti-bacterial activity of leaves and barks extracts of *D. sissoo* on methanol solvent against *E. coli* are 0 mm (no zone of inhibition) and for leaves and barks extracts of *D. sissoo* on methanol solvent against *S. aureus* are 11 ± 2 mm for leaves extract and 7.67 ± 1.53 mm for barks extract respectively.

Our test results showed that gram-positive bacteria *i.e. S. aureus* was more susceptible to selected plant extracts than gram-negative bacteria *i.e. E. coli*.

Gram-positive bacteria were more susceptible to the plant extract than gram-negative bacteria which contradict the previous reports that plant extracts are more active against gram-positive bacteria than gram-negative bacteria. These differences may be attributed due to the differences in their cell wall structure. Gram-negative organisms are considered to be more resistant due to their outer membrane acting as a barrier to many environmental substances, including antibiotics to the fact that the cell wall in gram-positive bacteria is of single layer, whereas the cell wall of gram-negative is multilayered structure [35, 36].

The plant *D. sissoo* contains the different phytochemicals [11] which may be responsible for the broad-spectrum anti-bacterial activity of the plant extract. It is therefore important to point out that the crude extracts of this plant need to be further purified through anti-bacterial activity guided fractionation to isolate and identify the compounds responsible for the anti-bacterial activity.

In addition, this result form a good basis for selection of the plant for further phytochemical and pharmacological investigation and suggests that the plant extract contain certain constituents with anti-bacterial properties that can be used as anti-bacterial agents in new drugs for the therapy of infectious diseases caused by pathogens. However, such plants must not be dangerous, be effective and that preparations are not adulterated or made harmful by parasites and micro-organisms. The most active extracts should be further subjected for the isolation of therapeutic anti-bacterial and carry out pharmacological evaluation [37].

CONCLUSION: The present study suggests / highlights that the barks and leaves extract of *D. sissoo* in different solvent system can also be strongly recommended for consideration of anti-bacterial property. This study provides an important basis for the use of extracts from the investigated plant for the treatment of infections associated with the studied micro-organisms.

The anti-bacterial activity of *D. sissoo* against *S. aureus* was more as compared to *E. coli* in both the leaves and barks extracts.

Thus, the results obtained confirm the therapeutic potency of *D. sissoo* used in traditional medicine and the use of this plant by indigenous people against a number of infectious diseases since generation. This forms a good basis for the selection of the plant for further different investigations. So, present work gives a direction for future investigators to carry out research on the extracts of the plant so that they could get some medicinally important drugs / chemicals. Also the experiment provides some scientific justification for the utilization of plant extracts to treat infectious diseases.

Further studies are required to identify the exact chemical constituents present in the barks and leaves extracts of this plant responsible for anti-bacterial activity. Finally, there is a need to explore this area further to understand the potentiality of the active plants towards the development of new era medicines.

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CONFLICT OF INTEREST: Nil.

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