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LC/MS profile and biological evaluation of *Dalbergia sissoo* growing in Egypt desert

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Abstract: LC–HRESIMS Metabolomic profiling of *Dalbergia sissoo* (Roxb.) ex DC leaves ethanol extract resulted in the tentative characterization of 111 active constituents belong to different chemical classes. Among which four different classes of flavonoid were detected for the first time in the plant in addition to previously reported neoflavonoid and isoflvonoids, with qualitative and quantitative variation. They comprised twenty-one amino acids & other nitrogenous chemicals, five coumarins, eight phenolic acids, five anthocyanins, two alkaloids, and 46 flavonoids. Additionally, the stilbene glycoside E-3,4,5'-Trihydroxy-3'-glucopyranosylstilbene was found in the *D. sissoo* extract. Along with primary metabolites (Cytidine-3', 5'-cyclicmonophosphate), there are three growth regulators ((+/-)-cis, trans-abscisic acid, beta-indoleacetic acid, and Gibberelin A3). *D. sissoo* ethanolic leaf extracts successfully protected rabbits from infectious diarrhea when given orally. This is demonstrated by weight parameters, blood measures (Hb, Lymphocytes, Monocytes, and Eosinophil) and antibacterial effect against both gram +ve and gram -ve bacteria. The ethanolic leaves extract of *D. sissoo* significantly prolonged the onset of diarrhea and reduced the number of wet and total stools at doses of 300 mg/kg and 500 mg/kg as compared to the negative control so, it is highly recommended to use *D. sissoo* as a natural antidiarrheal drug.

Keywords: *Dalbergia sissoo*; LC–HRESIMS; Cytotoxic; Antidiarrheal; Antibacterial.

1. INTRODUCTION

Genus *Dalbergia* (Fabaceae) includes approximately 250 species of trees shrubs and vines in the tropics and 304 species worldwide. The most well-known species from these are the rosewoods [1]. Because of their exquisite colors and exceptional hardness and intensity features, these species are employed in high-end furniture, artwork, and musical instruments [2]. In various parts of the world, the plant *Dalbergia sissoo* (Roxb) ex DC (known as sisu, shisham, tahli, jag, and rosewood tree) is a significant medicinal plant. It is indigenous to southern Iran and the Indian subcontinent. It can be found in Mauritius, Sri Lanka, Burma, Pakistan, and India. It is critical to ecological balance and environmental conservation [3].

This tree's wood dries out quickly and is highly valued for use in the manufacture of upscale furniture. Commercial plywood is another application for it. The wood is great for making charcoal and fire [4]. Heavy metals and nickel ions are removed from industrial wastewater using sawdust [5]. Many ailments are treated with *D. sissoo* in traditional medicine. In addition to being given for fevers, concentrated bark extract was also used as a blood purifier and an anti-inflammatory for piles and sciatica. The oil was applied topically to infected ulcers and skin conditions. The wood was employed as a cooling, antileprotic, anthelmintic, aphrodisiac, expectorant, and anti-spasmodic properties were all used for aerial components. Leaf extracts have been utilized for jaundice, analgesia, antipyretics, antioxidants, and treatments for cancer and diabetes. Flowers were used to treat skin issues, purify the blood, and boost

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immunity [6, 7]. The most well-known applications of *D. sissoo* are as a source of timber, fuel, shelter, and shade. *D. sissoo* is also used for medicinal purposes. Researchers claim that it has a variety of biomodulatory properties, such as osteogenic activity, anti-inflammatory, anti-microbial, and antispermatogenic properties, bronchodilation, substantial antipyretic, analgesic, and estrogen-like effects were all demonstrated by extracts from aerial portions. Its wood has been employed in the Yunani medical system to cure a variety of ailments, including blood disorders, scabies, eye and nose disorders, burning feelings, boiling pee, stomach issues, syphilis, boils, eruptions, leprosy, and nausea. Dried leaves extract used as antibacterial, antiprotozoal, and anti-inflammatory properties. Leaf juice is also used to treat gonorrhea and has healing effects on eye conditions [6, 7].

Bark extracts have been demonstrated to have anthelmintic, antipyretic, aphrodisiac, expectorant, and antipyretic, and refrigerant in the ayurveda medicinal system. They are also used for managing anal problems, diarrhea, dyspepsia, leucoderma, and skin conditions[6, 8]. As a result, careful research must be done regarding the identification and purification of bioactive chemicals as well as any potential mechanisms of action that might be involved in treating such illness states.

The leaves of the *D. sissoo* plant include trisaccharides, oligosaccharides, phenols, and neoflavenes, among other chemical components. Tectorigenin and biochanin found in the shisham flower. Flavonoids, dalbergichromene, cinnamylphenols, and 4-phenylchromene are present in stem-bark. Root-bark contains biochanin A, flavone, 7-hydroxy- 6-methoxyflavone, rotenoid, and dehydroamorphigenin, as well as chalcone (2, 3-dimethoxy-4′, -dimethylallyloxy-2′-hydroxychalcone), isoflavone (7-hydroxy-5-hydroxy-4′-methoxyisoflavone), and others [6].

The aim of this study; characterization of the metabolomic profile of the secondary metabolites of the *Dalbergia sissoo* ethanolic leaves extract, and evaluation effect of the extract as a successful protective natural product against rabbits' infectious diarrhea when given orally.

2. METHODS

2.1. Plant Collection

In March and April 2016, leaves of *Dalbergia sissoo* (Roxb.) ex DC were taken from plants grown at Matrouh Research Station in Egypt. Prof. Dr. Abduo Marie Hamed, a professor of plant ecology at the faculty of science, Al-Azhar University in Nasr City, and Dr. Therese Labib Youssef, a former manager and taxonomist at the Botanical Orman Garden in Giza, Egypt, generously authenticated the plant. The powdered leaves were stored in a tightly closed jar. Voucher specimens (code: Ds-04-2016) are kept in the Department of Pharmacognosy, Faculty of Pharmacy (Girls), Al-Azhar University.

2.2. Preparation of Plants Extracts

One kilogram of finely ground *Dalbergia sissoo* leaves were extracted with 8 L of 70% aqueous ethanol and at 70°C for 5 hours. The hot extract was then filtered using grade one filter paper and the filtrate was then concentrated using a rotary evaporator under reduced pressure. The semi-liquid product was then kept at -20°C until further examination, while being further dried in a water bath at 60°C.

2.3. Calculation of Total Phenolic Content (TPC)

According to Khan *et al*., TPC was quantified in an aqueous ethanol extract using the Folin-Ciocalteu reagent and measured calorimetrically at 765 nm [9].

2.4. Calculation of Total Flavonoid Content Determination (TFC)

According to Khan *et al.*, a modified colorimetric approach was used to quantify TFC in aqueous ethanol [9].

2.5. UPLC/ESI-qT0E-HRMS/MS Analysis

2.5.1. Mobile phase preparation of LC/MS analysis

Deionized water, MeOH, and acetonitrile were combined to create a reconstitution mobile phase working solution (MPWS) (50: 25: 25). The stock solutions were created by first adding 1 ml of the MPWS separately to 50 mg of the ethanol extract of the leaves. Each of the two solutions underwent two minutes of vortexing, ten minutes of ultrasonication, and ten minutes of 10000 rpm centrifugation. Following that, 1000 ml of reconstitution solvent was used to dilute 20: 1, 11 stock (50 mg/1000 ml) until the injection concentration was 1 g/l blank [10].

2.5.2. HPLC analysis conditions

The proteome and metabolomics team at the children's cancer hospital performed the LC/MS study (CCHU 57357). For MS/MS selective fragmentation analysis and the gathering of structural data, a quadruple time-of-flight (QTOF, with HR-TOF scan) mass spectrometer (Triple TOF 5600+, Sciex) is utilized in conjunction with an HPLC standard interface (Exion LC, Sciex). Positive and negative modes were also employed.

The process of chromatographic separation was employed using an In-line filter discs pre-column from Phenomenex and an Xbridge C18 column from Waters (2.1 x 50 mm, 3.5 m) at 400C. 5 mM HCOONH4, buffer (pH=3) containing 1% MeOH, 5 mM HCOONH4, buffer (pH=8) containing 1% MeOH, and 100% MeCN, respectively, were the mobile phases A, B, and C. The mobile phase's A and B were employed in the ionization's positive and negative modes, respectively, while C was used in both modes. The injection volume was 10 l, and the flow rate was 0.3 ml/min.

A sequence of linear gradients at intervals of 0, 1, 21, 25, 25, 01, and 28 had a ratio of 90:90:10:10:90:90 for A or B and 10:10:90:90:10:10 for C, which altered the separating process. The total ion chromatogram (TIC) was used for data processing, and the Master View was used to extract peaks based on characteristics that should have a Signal-to-Noise ratio of greater than 5 (non-targeted analysis) and intensities of the sample-to-blank ratio of larger than 5. Utilizing a built-in database and an online database, Sciex software was used to annotate features and remove isotopic peaks in order to identify peaks based on their fragments (MoNA-Mass Bank of North America).

2.6. Anti-diarrheal Effect

2.6.1. Drugs and chemicals

The ethanol was purchased from SIGMA® (Sigma-Aldrich®, St. Louis, USA), the trimethoprim and sulfamethoxazole from Alexandria, and the kaolin and pectin, both made by Eipico Pharmaceuticals, were acquired from a neighborhood pharmacy.

2.6.2. Animals and housing conditions

Albino New Zealand rabbits of either sex (♂/♀) (1.0-1.8 kg), approximately 6 months age with infectious diarrhea caused by *Escherichia coli* were chosen and exposed to pharmacological and haematological measures at the Teaching Veterinary Clinical Complex in Gharbia Governorate. The creatures were maintained in the Desert Research Center's animal housing in Cairo under controlled climatic conditions (23-25ºC). The animals were given a regular diet and unlimited access to tap water. Prior to the studies, the animals were fasted for 24 hours, but they had free access to water.

After receiving a strike to the back of the head, rabbits were sacrificed. The experiments performed complied with the rulings of the experimental protocol and animal care were conducted in accordance with the ethical procedures and policies approved by the animal care and use committee at the Faculty of Pharmacy, Al-Azhar University (No: 349-2022).

2.6.3. In vivo experimental anti-diarrheal design

Animals were placed into five groups, each with nine animals, and kept in individual cages. Animals from Group 1 were used as a negative control, and they received an oral dosage of ordinary saline (10 mL/kg). As a positive control, 2nd group; animals were administered a half-tablet (one tablet=800mg) oral dose of sulfamethoxazole + trimethoprim forte tablets twice daily. Animals in group 3 were used as positive controls and received a 5ml oral dosage of kaolin + pectin suspension twice daily. As test groups, the fourth and fifth animals received oral dosages of plant extract at a dose of 300 and 500 mg/kg, respectively.

Each medication was taken orally twice daily for five days while drinking fluids. After 4 hours, the animal cages were examined for the appearance of diarrheal spots and the frequency (number) of wet stools. Less diarrheal spot occurrence in the group 2nd, 3rd, 4th, and 5th animals was a sign of a potential anti-diarrheal effect. Prior to therapy and every day for five days following treatment, the faeces consistency score was kept track of. The control group was assumed to have 100% of all diarrheal faeces. The formula for percent inhibition (PI) was as follows [11]:

$$PI = \frac{\text{mean number of wet stools (negative control group} - \text{treated group)}}{\text{mean number of wet stools of the negative control group}} \times 100$$

2.6.4. Blood collection procedures from rabbits

After 24 hours had passed since the last treatment, blood samples were taken under aseptic conditions from rabbits in all groups. Blood was drawn from the rabbits' marginal ear vein after all of the rabbits' ears were carefully shaved with a blade to provide clear sight of the saphenous vein. For the examination of coagulation, haematological, and biochemical characteristics, blood samples were collected.

2.6.5. Hematological studies

An automated coulter counter was used to evaluate the blood sample that was taken from the rabbits in the lavender-topped tube containing tri-potassium EDTA (BD vacutainer) (DXH 500). Hematological parameters such as blood (Haemoglobin**,** Lymphocytes, Monocytes, and Esinophil)(immunological parameters) are measured.

2.7. Cytotoxic Activity

For cytotoxic activity against human lung carcinoma (A-549), colon carcinoma (HCT-116), human breast cancer (MCF-7), intestinal carcinoma (CACO), cervical carcinoma (Hela), larynx carcinoma (HEp-2), and hepatocellular carcinoma (HepG-2); aqueous ethanol extract of *D. sissoo* leaves was examined. The American Type Culture Collection provided the cells (ATCC, Rockville, MD). On RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 g/ml gentamycin, the cells were cultured. The cells were sub-cultured twice to three times a week at 37 ^{o}C in a humidified environment with 5% CO_2 [12].

2.8. Antibacterial Screening

Four important pathogenic and spoilage bacteria for food [*Staphylococcus aureus*, a gram-positive bacterium (ATCC 12600) and *Bacillus cereus* (ATCC 6051), in addition, *Escherichia coli*, (ATCC 11775) and *Pseudomonas aeuroginosa* (ATCC 10145) as gram-negative bacterium] were tested for antibacterial activity using 70% ethanol from *D. sissoo* leaves applying method which mentioned by Dutta, Raya, 2016 [13].

3. RESULTS

3.1. Calculation of Total Phenolic Content, total flavonoid and UPLC/ESI-qT0E-HRMS/MS Analysis

96.45 mg/g is the total phenolic content which expressed as Gallic acid equivalent (GAE) / g on dry basis

56.44 mg/g is total flavonoids content in *D. sissoo* leaves' which was expressed as mg of quercetin equivalent (QE) per gram of dry weight. (Standard Deviation = 21.1).

UPLC/ESI-qTOF-HRMS/MS and metabolomic profiling of the secondary metabolites of *D. sissoo* leaves ethanol extract showed numerous physiologically active compounds belonging to various chemical groups. Four other types of flavonoids were found in the plant for the first time in addition to previously described neoflavonoid and isoflvonoids, which were most prevalent and showing both qualitative and quantitative variation.

They comprised twenty-one amino acids, other nitrogenous chemicals, five coumarins, eight phenolic acids, five anthocyanins, two alkaloids, and 46 flavonoids. Additionally, E- 3, 4, 5'- Trihydroxy -3'- glucopyranosylstilbene glycoside was found in the *D. sissoo* extract. Along with primary metabolites (Cytidine-3', 5'-cyclicmonophosphate), there are three growth regulators ((+/-)-cis, trans-abscisic acid, beta-indoleacetic acid, and Gibberelin A3). Screening study led to the tentative identification or preliminary characterization of 111 compounds (Fig.1, 2 &Table 1).

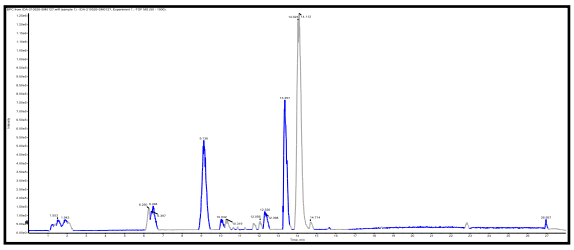

Figure 1. UPLC-QTOF-ESI-MS/MS Base Peak chromatogram (BPC) of *D. sissoo* leaves ethanol extract (Negative mode).

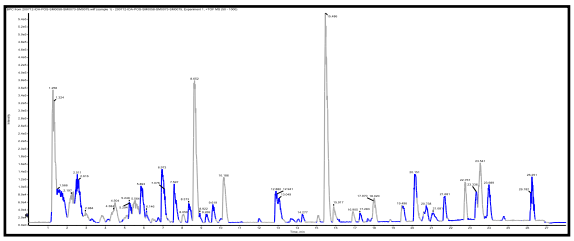

Figure 2. UPLC-QTOF-ESI-MS/MS Base Peak chromatogram (BPC) of *D. sissoo* leaves ethanol extract (Positive mode).

3.2. % Inhibition of Wet Stools and Haematological Parameters

At doses of 300 mg/kg and 500 mg/kg, the ethanolic leaves extract of *D. sissoo* considerably delayed the beginning of diarrhea and decreased the quantity of wet and total faeces in as compared to the adverse control. In addition, at doses of 300 mg/kg and 500 mg/kg, there is a significant difference between doses as, the % decreases of wet stools was 59.09% followed by 75.23% respectively (Fig.3). In comparison to the negative control, the % decreases in wet stools was 59.09% to nearly equal Kaolin/pectin (60.9%) at dose 300 mg/kg of Dalbergia extract and to nearly equal with Sulfamethoxazole + trimethoprim (80%) at dose 500 mg/kg of Dalbergia extract (75.23%) which is considered significantly different.

The findings of this investigation show that *D. sissoo* significantly increases haemoglobin (Hb) values ($P< 0.05$). It causes a considerable reduction in values of lymphocytes, monocytes & eosinophils. All blood parameter values significantly dropped except (Hb) (Table 2).

Table 1. Distribution of the tentatively identified metabolites in ethanolic extract of the *D. sissoo* leaves

No.	Compounds	No.	Compounds
1) Flavonoids		**2) Anthocyanin**	
1.	3'-Methoxy-4',5,7-trihydroxyflavonol	**1.**	Cyanidin-3-*O*-(2"-*O*- β -xylopyranosyl- β -gluco-pyranoside)
2.	3,5,7-trihydroxy-4'-methoxyflavone	**2.**	Delphinidin-3-*O*-(6"-*O*-α-rhamnopyranyl -β-gluco-pyranoside).
3.	Acacetin	**3.***	Cyanidin-3-glucoside
4.	Apigenin	**4.***	Peonidine-3-*O*-glucoside chloride
5.	Apigenin 8-*C*-glucoside	**5.***	Petunidin-3-*O*-beta-glucopyranoside
6.	Baicalein-7-*O*-glucuronide	**6.***	Cyanidin-3-*O*-rutinoside
7.	Daidzein	**3) Amino acids and other nitrogenous**	
8.	Eriodictyol-7-*O*-glucoside	**1.**	2'-Deoxyadenosine 5'-monophosphate
9.	Eriodictyol-7-*O*-neohesperidoside	**2.**	2'-Deoxyuridine
10.	Hesperetin	**3.**	3-Methylxanthine
11.	Hesperetin-7-*O*-neohesperidoside	**4.**	Adenine
12.	Isoquercitrin	**5.**	Carnosine
13.	Isorhamnetin-3-*O*-glucoside	**6.**	gamma,gamma-dimethyallyl pyrophosphate ammonium salt
14.	Kaempferol-3-*O*-(6-p-coumaroyl) -glucoside	**7.**	Guanosine-5'-diphosphate sodium salt
15.	Kaempferol-3-*O*-α-L-arabinoside	**8.**	Inosine
16.	Kaempferol-3-*O*-alpha-L-rhamnoside	**9.**	Inosine-5'-monophosphate
17.	Kaempferol-3-*O*-glucoside	**10.**	L-(-)-Phenylalanine
18.	Kaempferol-7-neohesperidoside	**11.**	L-5-Oxoproline
19.	Luteolin	**12.**	L- β -Homoisoleucine
20.	Luteolin-3', 7-di-*O*-glucoside	**13.**	Para nitro phenol
21.	Neohesperidin dihydrochalcone	**14.**	Pyridoxal 5-phosphate
22.	Phlorizin	**15.**	Riboflavin-5-monophosphate sodium salt
23.	Quercetin-3-D-xyloside	**16.**	Thymidine
24.	Quercetin-3-*O*-arabinoglucoside	**17.**	Uridine 5'-diphosphoglucuronic acid
25.	Quercetin-3,4'-*O*-di- β -glucopyranoside	**18.**	Uridine 5'-diphosphoglucuronic acid
26.	Quercitrin	**19.**	Xanthosine-5'-monophosphate
27.	Rhoifolin	**20.**	Glycine-Betaine
28.	Quercetin-3-*O*-arabinoglucoside	**21.**	L-(-)-Phenylalanine
29.	Quercetin-3,4'-*O*-di- β -glucopyranoside	**4) Stilbene glycosides**	
30.	Rhoifolin	**1.**	E-3,4,5'-Trihydroxy-3'-glucopyranosylsti
31.	Syringetin-3-*O*-galactoside	**5) Phenolic acids:**	
32.*	Genistein	**1.**	3-(4-Hydroxyphenyl)prop-2-enoic Acid
33.*	3,5,7-trihydroxy-4'-methoxyflavone	**2.**	5-Methoxysalicylic acid
34.*	(+-)-Taxifolin	**3.**	Caffeic acid
35.*	3,3'4',5-tetrahydroxy-7-methoxyflavone	**4.**	Chlorogenic acid
36.*	Daidzein-8-C-glucoside	**5.**	D-3-Phenyllactic acid

Table 1 Cont. Distribution of the tentatively identified metabolites in ethanolic extract of the *D. sissoo* leaves.

No	Compounds	No.	Compounds
37.	Sissotrin	**6.***	*trans*-Cinnamate
38.	Hyperoside	**7.***	Sinapyl aldehyde
39.	Gossypin	**8.***	1-*O*-β-D-glucopyranosyl sinapate
40.	Rhoifolin	**6) Coumarins.**	
41.	Datiscin	**1.**	Daphnetin
42.	Isosakuranetin-7-*O*-neohesperidoside	**2.**	Esculin
43.	Kaempferol-3-*O*-rutinoside	**3.***	7-Hydroxy-4-methylcoumarin
44.	Diosmin	**4.***	6,7-Dihydroxycoumarin
45.	Isorhamnetin-3-*O*-rutinoside	**5.***	3,4-Dimethoxycinnamic acid
		8) Growth regulators	
7) Sugars and sugar		**1.**	(+/-)-*cis*,trans-abscisic acid
1.	alpha-D-Galactose-1-phosphate	**2.**	Beta-indoleacetic acid
2.	Cytidine-3',5'-cyclicmonophosphate	**3.**	Gibberelin A3
3.	D-(+)-Galacturonic acid	**9) Other acids and miscellaneous compounds**	
4.	D-(+)-Raffinose	**1.**	D-(-)-Quinic acid
5.	D-(+)-Trehalose	**2.**	D-(+)-Malic acid
6.	L-(+)-Tartrate	**3.**	gamma-Linolenic acid
7.	Maltitol	**4.**	Ketoisoleucine
8.	Mucate	**5.**	Maleic acid
9.	Sucrose	**6.**	1-Myristoyl-2-hydroxy-sn-glycero-3-phosphate
10.	D-(-)-Erythrose	**10) Alkaloids**	
		1. Trigonelline **2.** Harmaline	

No.*: Compounds were detected in positive mode

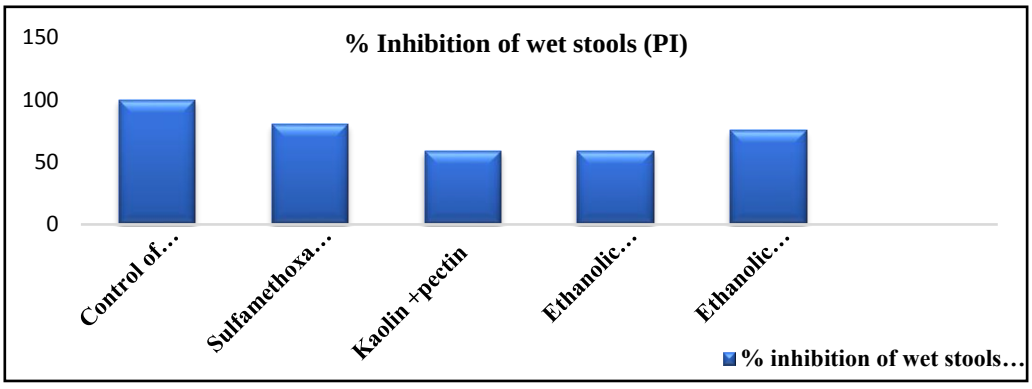

Figure 3. % Inhibition of wet stools (PI) of ethanolic leaves extract of *D. sissoo* in comparison with Sulfamethoxazole + trimethoprim and Kaolin +pectin.

The findings of this investigation show that *D. sissoo* significantly increases haemoglobin (Hb) values ($P< 0.05$). It causes a considerable reduction in values of lymphocytes, monocytes & eosinophils. All blood parameter values significantly decreased except (Hb) (Table 2).

3.3. IC_{50} Values of the *D. sissoo* Extract and Vinblastine Sulphate

Different cell lines were used to test cytotoxicity of *D. sissoo* leaves aqueous alcoholic extract. Human lung carcinoma (A-549), human colon carcinoma (HCT-116), human breast cancer (MCF-7), human intestinal carcinoma (CACO), human cervical carcinoma (Hela), human larynx carcinoma (HEp-2), and human hepatocellular carcinoma (HepG-2) were detected with IC50 values of 80.05 and 60.02 g/ml Fig.4.

Table 2. Haematological parameters of four groups of animals before and after treatments.

Types of blood cells / Groups	Hb			Lymphocytes			Monocytes			Esinophil		
	Before	After	Sig.	Before	After	Sig.	Before	After	Sig.	Before	After	Sig.
Gp1: Control of infected animals	8.4	8.2	ns	49	55	*	3.1	3.5	*	2.8	3.8	*
Gp2: Sulfamethoxazole + trimethoprim	8.1	8.5	*	48	45	*	3.4	3.2	*	3.8	3.1	*
Gp3: Kaolin +pectin	8.2	8.4	ns	44	44	ns	3.5	3.4	ns	3.7	3.4	*
Gp4: Ethanolic extract (500mg/kg)	8.1	8.4	*	45	42	*	3.2	3.0	*	3.2	2.6	*

ns, non-significant; *, Significantly different from the normal control group at P≤0.05.

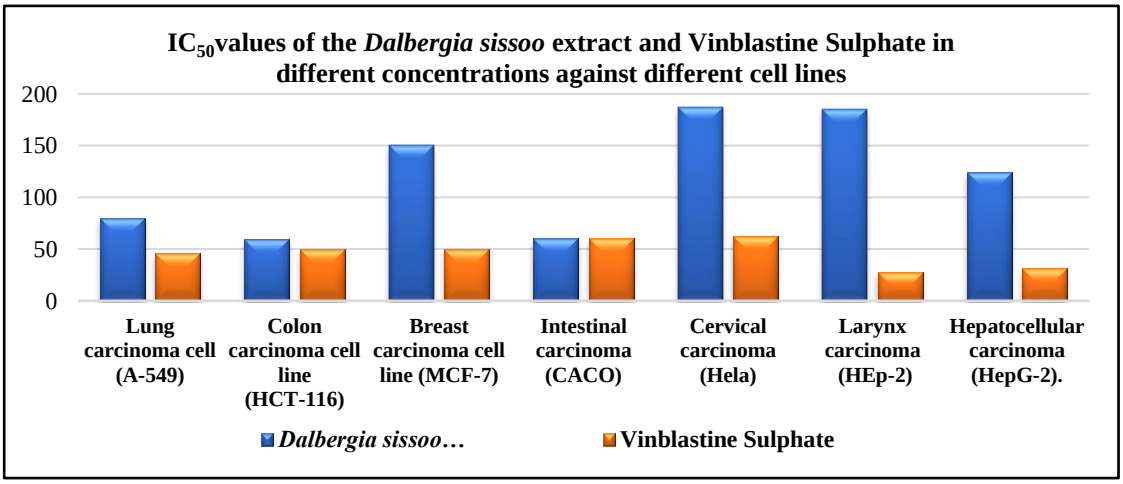

Figure 4. IC_{50} values of the *D. sissoo* extract and Vinblastine Sulphate in different concentrations against different cell lines.

3.4. Antibacterial Activity

Gram-negative bacteria have some resistance to 70% ethanol leaf extract (*Pseudomonas aeuroginosa*, 16.66 mm). Additionally, it demonstrated a high significant antibacterial effect against gram +ve (*Bacillus cereus*, 24.66 mm, *Staphylococcus aureus*, 21.66, and gram -ve bacteria *Escherichia coli*, 25.00) and gram -ve bacteria *Bacillus cereus*, 25.00 when compared to the reference antibacterial drugs norfloxacin and cefixime (Fig.5).

4. DISCUSSION

Using Gallic acid equivalent (GAE) / g. to express the content of total phenols, which was determined to be 96.45 mg/g. This value is consistent with that reported by Kaur A. *et al.* [14] who evaluated the contents of total phenolics of various extracts of *D. sissoo* at 50.8 mg/g. However, Total phenolic content was 58.06 mg/g of the extract like gallic acid equivalents (GAE) [15]. The total flavonoids value is 56.44 mg/g, is found to be in agreement with Yasmeen *et al.*, [16].

A range of biologically active components belonging to different chemical classes were characterized as a result of metabolomic profiling of the secondary metabolites of *D. sissoo* (Roxb.) leaves ethanol extract using UPLC/ESI-qTOF-HRMS/MS. The secondary metabolites occur in a wide range of concentrations along with enormous variations in their chemical and physical properties. In addition to previously described neoflavonoid and isoflvonoids, which were most prevalent and showed quantitative as well as qualitative variation, four other types of flavonoids were detected in the plant for the first time.

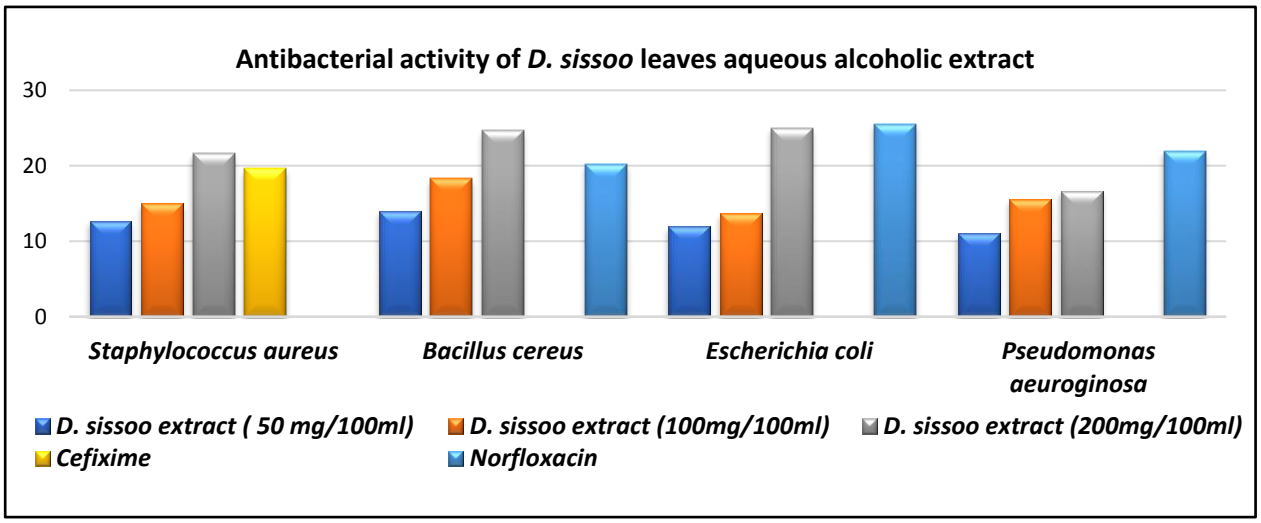

Figure 5. Antibacterial activity of *D. sissoo* leaves aqueous alcoholic extract**.**

They comprised twenty-one amino acids, other nitrogenous chemicals, five coumarins, eight phenolic acids, five anthocyanins, two alkaloids, and 46 flavonoids. Additionally, the stilbene glycoside E-3, 4, 5'-Trihydroxy-3'-glucopyranosylstilbene was found in the *D. sissoo* extracts. Additionally, in addition to main metabolites (Cytidine-3', 5'-cyclicmonophosphate), three growth regulators ((+/-)-cis, trans-abscisic acid; beta-indoleacetic acid; and Gibberelin A3) were also present [17].

Aegle marmelous fruit and leaves of *Dalbergia sissoo* have been used successfully to treat diarrhoea and dysentery [18]. Consequently, at doses of 300 and 500 mg/kg, the ethanolic extract of leaves considerably delayed the onset of diarrhea and decreased the number of wet and total faeces, compared to the control, at doses of 300 and 500 mg/kg, the % decreases of wet stools was 59.09% and 75.23% respectively (Fig. 3).

In comparison to the negative control, the percentage decreases in wet stools were 59.09% to nearly equal with Kaolin/pectin (60.9%) at dose 300 mg/kg of *Dalbergia sissoo* extract and to nearly equal with Sulfamethoxazole + trimethoprim (80%) at dose 500 mg/kg of *Dalbergia sissoo* extract (75.23%).

Red blood cells, include the haemoglobin protein (Hb), that is responsible for providing oxygen to body organs and getting the lungs' carbon dioxide back. A low count of red blood cell is indicated if an abnormally low haemoglobin level is seen (anemia). Numerous factors, such as vitamin shortages, bleeding, infectious diarrhea, and chronic illnesses, can contribute to anemia [19]. A shift from lymphocyte- to neutrophil-predominant differential counts is more common in rabbits with infectious diseases than a greater WBC count. Therefore, acutely ill rabbits may have normal differential counts but a decline in their overall WBC count.

It is evident that when compared to the reference drug vinblastine sulphate, which had IC50 value of 60.2 g/ml. The plant's ethanolic extract under investigation displayed the most potent cytotoxic effect against the colon (HCT-116) and nearly equal anticancer activity against intestinal carcinoma (CACO) cell line. When there is several simple phenolic compounds & flavonoids in *D. sissoo* leaves, which enable them to guard against disorders of severe oxidative damage, may be responsible for the reported ethanolic leaf extract's effectiveness [20].

Additionally, the leaf extract of *D. sissoo* had IC50 value of 80 g/ml against Lung carcinoma cell line, which was roughly half that of the reference medication (vinblastine sulphate) (A-549). Moreover, the leaf extract of *D. sissoo* shown mild anticancer activity against human breast cancer (MCF-7) as well as cervical, larynx, hepatocellular carcinomas (HepG-2) and cancers of the cervix, larynx, and liver.

The existence of phenolic molecules and flavonoids could be the basis of the antibacterial effects of the ethanolic leaf extract of *D. sissoo* [21].

5. CONCLUSIONS

Anthocyanins, phenolic acids, flavonoids, and glycosides that are physiologically active were found in the leaves of *D. sissoo*. Rabbits successfully received oral dose of an ethanolic leaf extract from *Dalbergia sissoo* (Roxb) to protect them from infectious diarrhea. Its antibacterial properties against gram +ve and gram -ve bacteria are supported by weight parameters and blood parameters (Hb, Lymphocytes, Monocytes, and Esinophil) (immunological parameters). Additionally shown the strongest cytotoxic effect against the intestinal cancer (CACO) and colon (HCT-116) cell lines. This will open up new possibilities for treatments using natural plants instead of synthetic medications.

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Conflicts of Interest: The authors declare no conflict of interest concerning the publication of the current study.

Ethical Statement: The Al-Azhar University Faculty of Pharmacy's animal care and use committee accepted the ethical practices and policies used in the conduct of the studies, which were carried out in compliance with the protocol's rules (No: 349-2022).

Author Contribution: El-Azzazy E A performed the extraction, carry out the biological activities; Magda T formulated the research point and designed the experiments, participated in the supervision of the work. Mohamed R A participated in the supervision of the work. All authors have participated in writing and preparing the manuscript for publication through revision.

List of Abbreviations: Total Phenolic Content (TPC); Total Flavonoid Content (TFC);: Gallic Acid Equivalent (GAE);: Quercetin Equivalent (QE), ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-QTOFMS/MS).

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