Phytochemical Profiling and FT-IR Spectroscopic Analysis of Selected Anti-Leishmanial Plants

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Abstract

Bioactive chemicals found in medicinal plants have long been used to treat and prevent a wide range of human illnesses. As they are essential in the fight against infectious disorders like leishmaniasis. Eucalyptus globulus, Lawsonia inermis, and Syzygium jambolana were selected to determine the phytochemical components in their leaves that may have anti-leishmanial qualities. The leaves of selected plants were dried and grounded into fine powder and extracted using a solvent system of methanol, chloroform and water, (80ml:10 ml:10 ml). After filteration, the filterate was concentrated using a rotary evaporator. The extracts were subjected to phytochemical analysis, organoleptic evaluation and Fourier-transform infrared (FTIR) spectroscopy. The results showed the occurrence of flavonoids, saponins, terpenoids, phenolics and tannins but no proteins was found in leaves of aforementioned plants. FTIR analysis of Eucalyptus globulus leaves revealed the presence of alkenes and hydroxyl functional groups that indicating phenols, carboxylic acids or alcohols. Similarly Lawsonia inermis's FTIR analysis revealed several functional groups by identification of characteristic vibrational bands. Numerous functional groups, such as phenolic compounds, carboxylic acids and alkyl groups were detected in Lawsonia inermis FTIR analysis. FTIR spectrum showed 11 distinct peaks indicating the presence of important functional groups such as carboxylic acids, phenolics, alkyl groups, and aromatic rings in leaves of Syzygium jambolana. These peaks indicated a high concentration of bioactive substances with strong anti-leishmanial effects, such as alkaloids, flavonoids, and phenolic compounds. Due to high medicinal potential, these plant were considered as potent sources of bioactive constituents to be used against leishmaniasis.

Keywords: *MP (Medicinal Plants), ID (Infectious Disease), L (Leishmaniosis), PC (Phytochemical compounds), FTIR.*

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https://doi.org/10.58932/MULH0027

Introduction

Many disorders are treated with phytochemicals that are derived from medicinal plants (Sharma & Moin, 2020). Phytochemicals provide protection against many diseases because of their natural defense properties (Krishnaiah et al., 2007). Macrophages are home to intracellular protozoan parasites called leismania. Different strategies are used by both natural substances and manufactured medications to suppress or eradicate them.Various phytochemical substances are extracted from plant seeds, roots, and leaves and are being investigated for the production of new medications to treat leishmaniasis. Alkaloids, polyphenols, and essential oils are only a few of the many bioactive substances that plants produce; many of these hydrophobic molecules are thought to be powerful anti-leishmanial medicines because of their high biological activity (Machado et al., 2014). By breaking parasite membranes, generating reactive oxygen species, modulating host immune responses, and inhibiting protein and DNA synthesis and phytochemical substances such as bioflavonoids and phenolics derived from plants have anti-leishmanial properties (Fonseca-Silva et al., 2011).

Syzygium jambolana is a member of the family Myrtaceae and possesses significant levels of kaemferol, glucoside, anthocyanins and myrecetin (Jagetia, 2017). The selected plant exhibited antioxidant, antihistaminic, antifungal and antiseptic properties. *Eucalyptus globulus* belongs to the family Myrtaceae and contains tannins,terpenes and flavonoids etc. These phytochemical compounds are frequently used to treat different infections and provide a broad range of therapeutic effects (Singh et al., 2018). *Lawsonia inermis* is belongs to the family Lythraceae family and has antiparastic, anticancer, antimicrobial and antibacterial potential (Wang et al., 2007). *Eucalyptus globulus*, *Lawsonia inermis*, and *Syzygium jambolana* are known to be medicinal plants due to their bioactive constituents, which have anti-leishmaniasis properties. The present study aimed to determine the anti-leishmanial bioactive compounds through FTIR analysis of selected plants.

Methodology

Collection and Identification of Plants

The leaves of the mentioned plants were collected from the local market and identified by Taxonomists at Minhaj University. And fresh leaves were washed with tap water to remove impurities, then dried at room temperature in the shade for one week and ground into fine powder using an electric grinder. After grinding 100 g of powder was stored in a container for further analysis. After that, organoleptic evaluation, phytochemical screening, and FTIR analysis were carried out.

Organoleptic Examination

Condition, color, nature and texture and essence were determined organoleptically, and the results were tabulated (Arya &Thakur, 2012).

Extraction

For five to seven days, a 100 ml solvent system consisting of methanol: chloroform: water (80 ml: 10 ml: 10 ml) was used to dissolve 25g of powdered plant leaves from each selected plant. Following filtering, a drying oven was used to concentrate the organic portion under vacuum, and a water bath was used to dehydrate the liquid extract.

Phytochemical Analysis

The qualitative phytochemical analysis was performed using standard procedure (Daniel & Mani, 2016).

Tests	Experiment	Observations	Results
Alkaloids	Crude extract and two milliliter of Wagner's reagent	Reddish brown Precipitate	Alkaloids confirmed
Flavonoids	Concentrated sulphuric acid and two milliliter crude extract	Reddish brown coloration	Flavonoids confirmed
Phenolic	0.5% Ferric chloride solution and crude extract	Bluish black precipitate	Phenolic confirmed
Terpenoids	Two milliliter chloroform+ three milliliter Concentrated sulphuric acid and five milliliter of crude extract	Reddish brown coloration	Terpenoids confirmed
Tannins	Few drops of 1% lead acetate and two milliliter of plant extract	Yellowish precipitate	Tannins confirmed
Anthocyanin	Two milli liter of Hydrochloric acid and ammonia and two milliliter of aqueous extract	Pink-red precipitate was turned blue-violet	Anthocyanin confirmed
Anthraquinone	Three milliliter of extract and three	Pink, Violet or Red coloration	Anthraquinone confirmed

	milliliter of Benzene + Five milliliter Ammonia (10%)		
Steroids	Two milliliter acetic anhydride + Two milliliter sulphuric acid and 0.5 ml of crude extract	Violet to blue or green coloration	Steroids confirmed
Glycoside	Two milliliter chloroform + Two milliliter carboxylic acid + Two milliliter of crude extract	Violet to Blue or Green coloration	Glycoside confirmed
Carbohydrate	Few drops of extract + Fehling's A and Fehling's B reagents	Greenish or brown colored precipitate	Carbohydrate confirmed
Fatty acids	Five milliliter of ether + one milliliter of extract, extract poured on filter paper	Transparence on filter paper	Fatty acids confirmed
Coumarin	Ten percentage sodium hydroxide + chloroform add in extract	Yellow color precipitate	Coumarin confirmed

FTIR Analysis

The use of FTIR Spectroscopy was employed to evaluate both organic and inorganic functional groups and the chemical bonds existing between them. The vibration frequency of each bond was determined by mass of the linked atoms and the bond energy. For analysis, dried extracts from methanolic solvent were utilized and samples were collected in bottles. The extract was assessed using an FTIR spectroscopy with a range at 400–4000 cm^{-1} (Ahmad et al., 2019).

Results

The lowest and highest percentage yield was exhibited by *L. inermis* and *E. globules* respectively (Table 1).

Table 1: *Percentage Yield of Selected Medicinal Plants*

Sr.No	Type of Leaves Extracts	Initial weight of Plant Powder (gm)	Final weight(gm) of Plant Extract (gm)	Percentage Yield
1.	*Syzygium jambolana*	25	2.34	9.36
2.	*Eucalyptus globules*	25	3.20	12.8
3.	*Lawsonia inermis*	25	2.29	9.16

Organoleptic evaluation

Organoleptic evaluation of mentioned plants are shown in Table 3.

Table 2: *Percentage yield of selected medicinal plants*

Sr.No	Type of Leaves Extracts	Initial weight of Plant Powder (gm)	Final weight(gm) of Plant Extract (gm)	Percentage Yield %
1.	*Syzygium jambolana*	25	2.34	9.36
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Organoleptic evaluation

Organoleptic evaluation of mentioned plants was shown in Table 3.

Table 3: *Organoleptic evaluation of selected plants*

Organoleptic Examinations	Eucalyptus globules	Syzygium jambolana	Lawsonia inermis
Condition	Powder	Powder	Powder
Taste	Bitter	Slightly Astringent	Aromatic bitter
Color	Green	Dark green	Dark green
Odor	Characteristic	Characteristic	Characteristic
Texture	Fine	Leathery	Silky Smooth

Phytochemical Screening

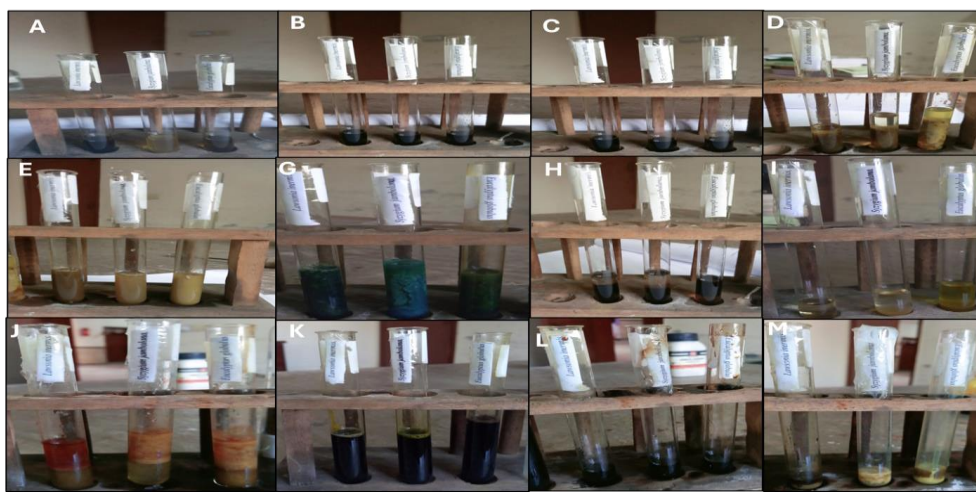
The phytochemical screening showed that alkaloids, proteins and glycosides were not found in the leaf extract of the selected plants while saponins, tannins, anthocyanins, phenolics, flavonoids, anthraquinone, steroids, coumarin, carbohydrates, terpenoids, and fatty acids were present in the selected plants.

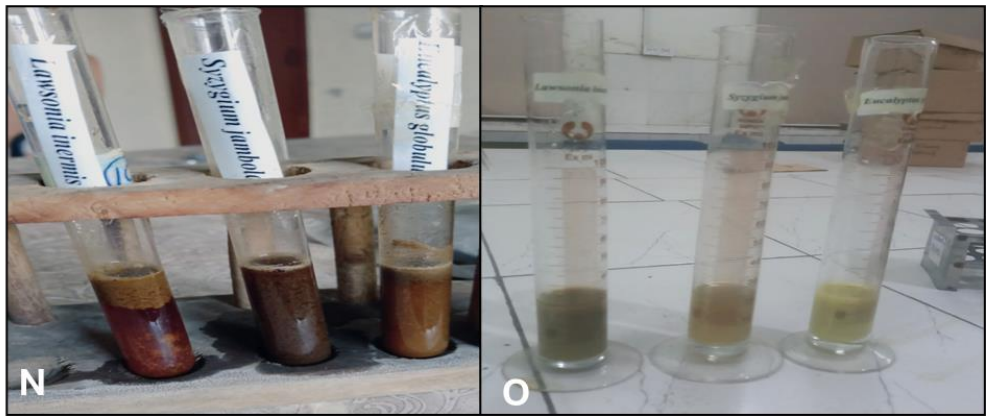
Table 4: *Phytochemical compounds of the leaves extracts of Selected Plants*

Sr. No	Phytochemical Components	*Eucalyptus globules*	*Lawsonia inermis*	*Syzygium jambolana*
1.	Alkaloids	-	+	++
2.	Flavonoids	+++	+	+++
3.	Phenolic	++	+++	++
4.	Terpenoids	+	+	+
5.	Tannins	++	+++	+
6.	Anthocyanins	+	+	+
7.	Anthraquinone	+	++	+
8.	Steroids	+	+	+++
9.	Glycosides	-	-	_
10.	Proteins	-	-	_
11.	Saponins	+++	+	+
12.	Carbohydrates	+	+	+
13.	Fatty acids	+	+	+
14.	Coumarin	+	+	+

+ Present, ++ Moderately Present, +++ Appreciable Present, -Absent

Figure 1: *Phytochemical tests of different phytochemical compounds in crude extract of leaves of E. globules, S. jambolana and L. inermis*





(A) Glycoside, (B) Protein, (C) Flavonoids, (D) Anthocyanin, (E) Anthraquinone, (F) Carbohydrate, (G) Terpenoids, (H) Fatty Acid**,** (I) Coumarin, (J) Phenolics, (K) Steroids, (L) Tannins, (M) Alkaloids (O) Saponins

FTIR spectroscopy

Eleven main peaks were found in the powdered leaves of *S. jambolana*, suggesting the existence of various functional groups. The lowest and highest peaks at 663.83 cm^{-1} correlated with strong C–Br stretching of halogen compounds and the amide group, identified by N–H stretching, was observed at 3296.55 cm. At 1228.95 cm^{-1}, a physiologically active fraction was detected, signifying the presence of nitro (NO_2) molecules and C–N stretching.

Figure 2: *FTIR Analysis of leaves of S. jambolana*

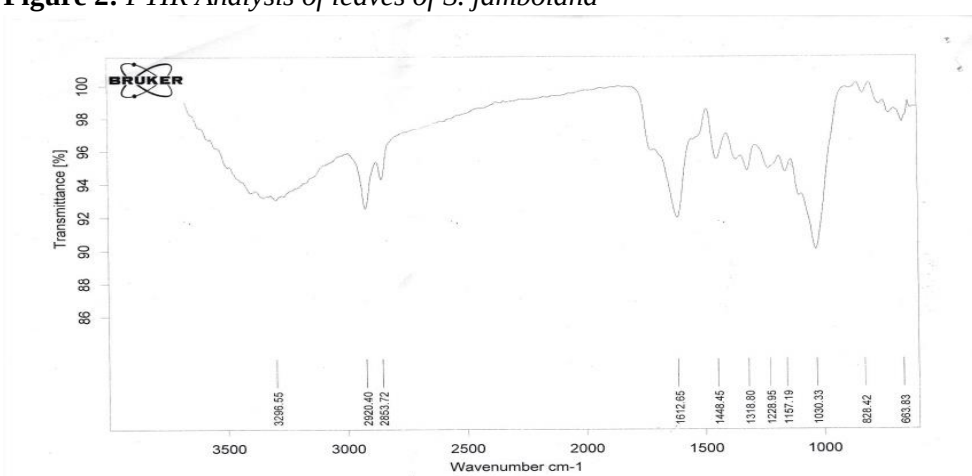
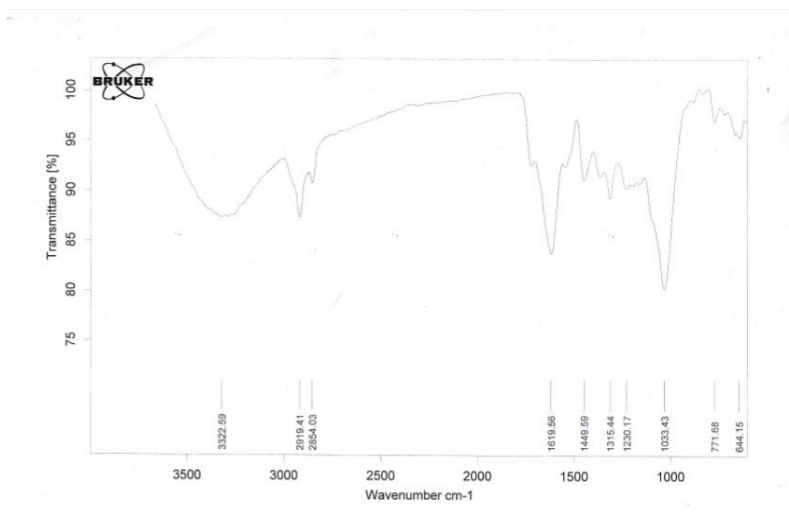


Table 5: *Functional Groups evaluated by FT-IR Spectroscopy for S. jambolana*

Peak Value Wavenumber cm-1	Functional Group	Bond	Peak Details
3296.55	Carboxylic Acid	C=C-CO-OH	Strong and Broad
2920.40	Stretching of Alkane	C-H stretch	Medium
2853.72	Alkyl group	C-H stretch medium	Medium
1612.65	Aromatic Ring	C=C stretch strong	Narrow and Strong
1448.45	Carboxylic Acid	C=O bending	Broad ,Medium
1318.80	Phenol	O-H bending	Broad Medium
1228.95	Bio-active fraction	C-N stretch	Broad
1157.19	Tertiary alcohol	C-O stretch	Strong, Narrow
1030.33	Alkene	C-H stretch	Narrow
828.42	Phenyl	C=H bending strong	Strong, Broad
663.83	C-Br	Strong stretching of Halogen Compound	Strong Narrow

FTIR Analysis of *Eucalyptus globules*

Figure 3: *FTIR Analysis of E. globules*



The O–H stretching bond was represented by the broad peak, measured at 3322.59 cm^{-1}, while the occurrence of C–Br stretching representing an alkyl halide group was indicated by the lowest peak, measured at 644.15 cm^{-1}.

Table 6: *Functional Groups Identified by FT-IR Spectroscopy for E. globules*

Peak Value Wave number cm-1	Functional Group	Bond	Peak Details
3322.59	Carboxylic group	O-H stretching	Broad, Strong
2919.41	Aromatic Ring	C-H stretch	Strong
2854.03	Alkyl group	C-H stretch medium	Strong, Narrow
1619.56	Conjugated Carbonyl	C=O stretch	Broad, Strong
1449.59	Aromatic ring	C=C bending	Strong
1315.44	Phenol	C=O bending medium	Narrow, Strong
1230.17	Carbonyl group	C-O stretch	Weak, Narrow
1033.43	Alcohol, Phenol	C-O-C stretch	Strong, Medium
771.68	Alkenes group	C-H bend or C-O group	Weak
644.15	Alkyl halide group	C-Br stretching	Weak

FTIR Analysis of *Lawsonia inermis*

The maximum peak at 3265.70 cm^{-1} was identified as a phenolic groups. Alkanes were detected at 2854.13 cm^{-1}. An alkyl halide group, detected at 625.79 cm^{-1}, represented the lowest peak.

Figure 4: *FTIR Analysis of leaves of L. inermis*

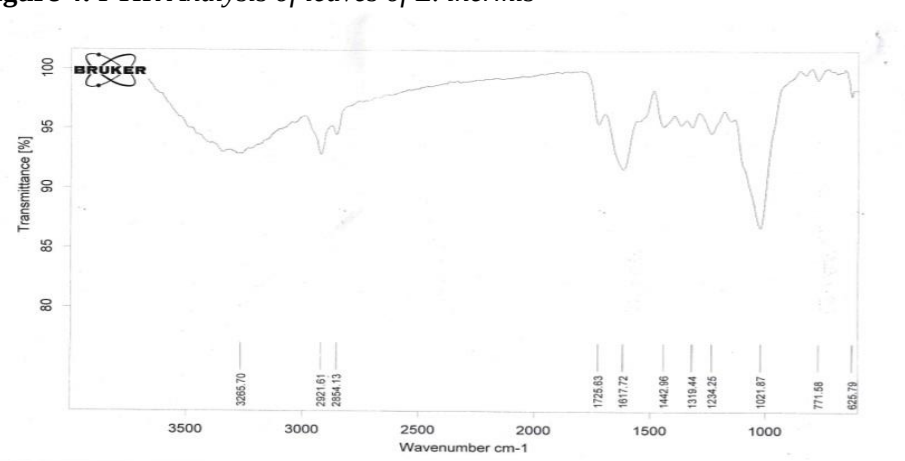


Table 7: *Functional Groups identified by FT-IR Spectroscopy for L. inermis*

Peak Value Wave number cm-1	Functional Group	Bond	Peak Details
3265.70	Amide	N-H stretch	Broad, Strong
2921.61	Carboxylic acids	O-H stretch	Narrow, Strong
2854.13	Alkyl group	C-H stretch medium	Narrow, Strong
1725.63	Aromatic Ring	C=C stretch	Medium, Strong
1617.72	Aromatic Ring	C=C stretch strong	Strong
1442.96	Alkene	C=C bending strong	Narrow, Weak
1319.44	Phenol	O-H bending medium	Strong
1234.25	Carbonyl group	C-O stretch	Broad, Strong
1021.87	Alcohol	C-O stretch	Narrow, Strong
771.58	Phenyl group	C-H bend	Medium, Strong
625.79	Alkyl halide	C-Cl stretch	Weak

Discussion

Plants containing high quantity of bioflavonoids can prevent parasitic invasion and proliferation by interacting with the parasitic cell wall. Because certain *Leishmania* species have become resistant to synthetic treatments, leading to repeated infections, these qualities of medicinal plants are valuable for the development of new drugs. Phytochemicals including alkaloids (González-Coloma et al., 2012) and flavones (Grecco et al., 2010) have been shown in numerous studies to increase anti-leishmanial activity.

For the development of novel chemotherapeutic medications, plants are regarded as a significant source of functional groups (Starlin et al., 2012). The Choice of the solvent for extraction is essential for isolating the maximum number of phytochemical compounds. Methanol is generally considered as one of the best solvents for secondary metabolite extraction (Azzah & Ibtisam, 2019).

Phytochemical study of the methanolic leaf extract of *S. jambolana* exhibited the occurance of anthocyanins, terpenoids, bioflavonoids, saponins, phenolics, tannoids, alkaloids, carbohydrates, steroids, fatty acids, anthraquinones, and coumarins, but not proteins or glycosides. These results were similar to those of Hasanuzzaman et al. (2016), who determined the identification of carbohydrates, alkaloids, bioflavonoids, phenolics, steroids and saponins in the leaf extract of *S. jambolana*. Similary, Ramos & Bandiola (2017) investigated the phytochemical composition of *S. cumini*'s methanolic leaf extract and showed that alkaloids, bioflavonoids, in contrast, carbohydrates, glucosides, tannoids, saponins, polyphenols, fixed oils. Isoprenoids, lipids and steroids were also detected.

Bijauliya et al. (2017) assessed the presence of gallotannins, essential oils, anthocyanins, two flavanol glycosides, and monoterpenoids such as, borneol, terpinene, terpineol terpinolene, and eugenol in S. cumini leaf extract. Similarly, Jagetia (2017) found that a variety of chemicals, such as glucosides, phenolic acids, steroids, bioflavonoids, saponins, tannoids, and cardiac glycosides, were present in methanolic extracts of *S. cumini* leaves. In the FTIR spectrum, the S. jambolana leaf extract had eleven major peak values. The peak at 828.42 cm-1 and 3296.55 cm-1 were identified as the C-H bend and N-H stretching indicating the presence of a phenyl and an amide group, respectively. The presence of key phenolic compounds (phenylic acid, tannins, caffeine and isoprenoids) as well as functional groups such as aldehyde, polysaccharide, halogen compounds, alcohol group's alkenes and ester carbonyl was identified by Selvaganesh &Sadhana (2022) using FTIR analysis. A previous study reported that the *Eucalyptus globulus* leaf extract included glycosides, alkaloids, bioflavonoids, phenolics, terpenoids, tannins, anthocyanins, anthraquinones, steroids, and saponins, but no proteins. These results were consistent with those of Chukwu et al.(2016) and Usman et al. (2021).

Further study of *E. globulus'*specific phytochemical activites, including the presence of phenolic acids, terpenoid polyphenols, and flavonoids and their peaks were coincided with those reported by Danjuma et al.(2022). *E. globulus* leaves were analyzed using FTIR, multiple unique peaks were observed. The band at 3322.59 cm^{-1} represented the hydroxyl group of alcohols, phenols and carboxylic acids. The absorption at 2919.41 cm^{-1} corresponded to methyl group. Vibrations at 1449.59 cm^{-1} were linked to carboxylate ion and carbonyl groups, While the absorption at 1230.17 cm^{-1} suggested ν–C–O stretching, the band at 1033.43 cm^{-1} correlated with ν–C–O vibrations of alcohols, acids, phenols, esters and ethers. The current study's results were consistent with the research of Mopoung & Dejang (2021).

Phytochemical screening revealed that the methanolic leaf extract of *L. inermis* contained flavonoids, tannins, phenolics, terpenoids, anthocyanins, anthraquinones, saponins, alkaloids and steroids, but no glycosides and proteins were found.. These findings were in line with previous study of Yusuf (2016) which showed the ethanolic extract of *L. inermis* leaves included bioactive constituents like flavonoids, terpenoids, alkaloids, steroids, and glucosides but no glycosides. The results also coincided with those of Usman et al. (2018), who identified the presence of steroids, bioflavonoids, and saponins in the *L. inermis* leaf extract. Phenolic groups (O-H stretching) were indicated by a significant peak at 3265.70 cm^{-1} in the FTIR study of *L. inermis*. An aromatic band was detected at 2921.61 cm^{-1}. The peaks at 1725.63 cm^{-1} and 1021.87 cm^{-1} represented the presence of carbonyl and ester groups

respectively. While aromatic bending was represented at 771.58 cm^{-1}. These findings were in line with the study of Fayyadh & Alzubaidy (2021).

Conclusion

Medicinal plants are essential to preserving human health, as most of the world's population primarily uses herbal medicine. According to phytochemical screening and FTIR analysis. *Syzygium jambolana* has higher concentrations of flavonoids and alkaloids than *Eucalyptus globules* and *L. inermis*. However, phenolic compounds were more abundant in *E. globules* and these plants have been used to cure different infectious diseases like leishmaniosis, because of their high level of bioactive components. They also have significant potential for the development of novel drugs.

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