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Phytochemical analysis and antioxidant activities of *Indigofera oblongifolia*, local plant extract used in traditional medicine: Antimalarial activities

[Análisis fitoquímico y actividades antioxidantes de *Indigofera oblongifolia*, extracto de planta local utilizado en medicina tradicional: Actividades antimaláricas]

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Abstract: The use of eco-friendly natural products is one of the major areas of research that has anticoccidial properties. This investigation aims to identify and evaluate the bioactive constituents of the *Indigofera oblongifolia* leaf extract (IOLE), as an antimalarial. Fourier Transform Infrared Spectroscopy (FTIR) was used to determine any significant information about the functional groups, as well as assays for total phenolics, tannins, total flavonoids, DPPH, ABTS tests, XRD, and UV-VIS Spectroscopic analysis. The results of FTIR analysis of the extract showed the presence of 5 phytochemical compounds. Moreover, the quantitative analysis revealed that the concentrations of phenols, tannins, and flavonoids were, 219.106 ± 1.0792, 89.438 ± 0.1599, and 19 ± 0.1500 (mg TAE/g DW), respectively. The results obtained indicate that these extracts have a high level of antioxidant activity and the scavenging activity of DPPH radicals. The UV-VIS showed varying absorbances between 300 and 800 nm. The IOLE proved effective against Plasmodium berghei in mice.

Keywords: Plant extract; FTIR Spectroscopy; DPPH; ABTS; UV-VIS Spectroscopy; XRD (6)

Resumen: El uso de productos naturales respetuosos con el medio ambiente es una de las principales áreas de investigación que poseen propiedades anticoccidianas. Esta investigación tiene como objetivo identificar y evaluar los constituyentes bioactivos del extracto de hojas de *Indigofera oblongifolia* (IOLE) como antimalárico. Se utilizó espectroscopía infrarroja por transformada de Fourier (FTIR) para determinar información significativa sobre los grupos funcionales, así como ensayos para fenoles totales, taninos, flavonoides totales, pruebas de DPPH y ABTS, análisis de DRX y espectroscópico UV-VIS. Los resultados del análisis FTIR del extracto mostraron la presencia de 5 compuestos fitoquímicos. Además, el análisis cuantitativo reveló que las concentraciones de fenoles, taninos y flavonoides fueron de 219.106 ± 1.0792, 89.438 ± 0.1599 y 19 ± 0.1500 (mg TAE/g DW), respectivamente. Los resultados obtenidos indican que estos extractos tienen un alto nivel de actividad antioxidante y actividad de eliminación de radicales DPPH. El UV-VIS mostró absorciones variables entre 300 y 800 nm. El IOLE demostró ser eficaz contra Plasmodium berghei en ratones.

Palabras clave: Extracto de planta; Espectroscopía FTIR; DPPH; ABTS; Espectroscopía UV-VIS; DRX

INTRODUCTION

Natural products play a crucial role in traditional medicine, as they form the basis of primary healthcare systems, especially in underdeveloped countries (Yuan *et al*., 2016). In addition, an increase in the consumption of dietary supplements based on natural products derived from herbal and marine sources is currently observed, highlighting the importance of standardization and comprehensive scientific validation to ensure the safety of these products. As a result, A renewed interest in research into natural products has been found in recent decades, both in the scientific community and in the pharmaceutical sector (Atanasov *et al*., 2021). The Earth's biodiversity offers an incredible variety of different chemical structures that are difficult to replicate synthetically. Due to their chemical diversity, historical achievements, drug-like properties, and potential for novel modes of action, natural products are important resources for drug development (Chopra & Dhingra, 2021).

Plants play an important role in the survival of earth's life. They transform simple molecules into complex structures that produce compounds that are important for human health. People all over the world have used medicinal herbs as folk medicine (Kong *et al*., 2003). Plants have been used by humans since the beginning of time as food, housing, and various common diseases to be treated. Even today, medicinal plants are commonly used in a wide range of products, including pharmaceutical, cosmetic, and nutraceutical products (Petrovska, 2012). With the production of to treat high priced medicines to cure many diseases, the highest demand and widespread use that become a major industry around the world is herbal products. Since many of the recipes used by traditional healers provide excellent treatment for a variety of diseases, the rural people in both countries African and Asian countries often use herbs to treat diseases. the first chemical compound to be identified was Quinine alkaloid in the bark of the Cinchona tree (*Cinchona officinalis*) that effectively suppressed malarial fever (Ahsan *et al*., 2005).

The family Fabaceae, with approximately 650 genera and 18000 different species, ranks as the third largest family of blossoming plants, including the genus *Indigofera*. The family overall is characterised by the pod (legume) type of fruit developing from a single carpal with marginal placentation (Rahman *et al*., 2018). *Indigofera oblongifolia* has shown antimicrobial (Dahot, 1999), hepatoprotective, and lipoxygenase inhibitory activity (Shahjahan *et al*., 2005). Abubakar *et al*. (2006), described *Indigofera pulchra*'s snake-venom-neutralizing bustle. Prakash *et al*. (2007) have also reported the antioxidant and free radical scavenging, as well as the anti-dyslipidemic actions of *Indigofera tinctoria*. *Indigofera emarginella* has been shown to have *in vitro* antimalarial activity against *Plasmodium falciparum*. Chakrabarti *et al*. (2006), have reported the antidiabetic activity of *Indigofera mysorens*. People use the entire plant to treat hepatitis, whooping cough, as an antispasmodic (Hamayun *et al*., 2003), as a tonic, and to prevent hypoglycemia in mice (Sittie & Nyarko, 1998). They also use the plant's leaves, flowers, and tender shoots, which are cooling and demulcent, to treat leprosy and tumorous infections. Apply the leaves directly to the abscesses. People chew the roots to alleviate toothache and lethargy (Sittie & Nyarko, 1998). Esimone *et al*. (1999) reported that the alcoholic extract of the dried shoots has anti-inflammatory action, while Dahot (1999), found antibacterial potency in the leaves, bark, and roots.

The extract reflects and presents the number of bioactive chemicals (Murshed *et al*., 2020). Tests against *Plasmodium berghei* showed that the activities of IOLE could suppress the parasite as effectively as chloroquine (Mendes *et al*., 2018).

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Indigofera oblongifolia (I. oblongifolia) due to its analgesic and anti-inflammatory effects is used to treat pain. *I. oblongifolia*, or in Arabic hasr, is a Fabaceae plant native to Asia and Africa. This plant contains the new compounds indigoferic acid and indigene, which are alkylated xanthenes and the fatty acid ester of p-hydroxy (E)-cinnamic acid, respectively. In the plant, sitosterol and 3-hydroxybenzoic acid were also discovered (Ahsan *et al*., 2005). Hepatocytes are protected *by Indigofera oblongifolia* from carbon tetrachloride-induced hepatotoxicity by suppressing of oxidative stress-induced nuclear DNA, protein oxidation, and membrane lipid (Abdel Moneim, 2016). The number of bioactive chemicals are been reflected and presented by this in the extract (Murshed *et al*., 2020). The activities of IOLE were tested against *Plasmodium berghei* and were able to suppress the parasite as effectively as chloroquine (Mendes *et al*., 2018).

This study aims to analyze the bioactive chemical compounds and antioxidant activity of the methanolic extract evaluation of *I. oblongifolia* leaves and its therapeutic effect on malaria.

MATERIAL AND METHODS

The preparation of the botany extract

Fresh leaves of *I. oblongifolia* leaves were collected from the southern regions of Saudi Arabia. The botanical identity was confirmed by taxonomists in King Saud University's herbarium to confirm their botanical identity. *I. oblongifolia* was extracted with 70% methanolic extract according to the method described in (Begashaw *et al*., 2017). The leaves were thoroughly cleaned to remove unwanted particles, dried at room temperature, and then pulverized using an electric blender. Subsequently, 5 g of extracted pulverized leaves with 200 mL of methanol (25 mg/mL) and shaken at room temperature for 24 hours. Whatman filter sheets were used to filter the leaf extract. The extracted material was then dried and concentrated at 40°C under condensed pressure in a rotary vacuum evaporator (Yamato RE300, Tokyo, Japan) for further use.

Infrared spectroscopy

The absorption, emission, or reflection measurement of infrared radiation by matter is known as infrared spectroscopy. infrared spectrometer is used to categorize compounds or functional groups according to whether they are solid, liquid, or gaseous and generates an infrared spectrum. To visualize the infrared spectrum, an infrared light absorption (transmission) diagram with frequency or wavelength on the horizontal and vertical axes can be used (Zeitler *et al*., 2007).

Indigofera Oblongifolia leaf extract was mixed with powder potassium bromide in a weight/volume ratio of 1:99 (1: 99 wt%, 102648742, PerkinElmer, Waltham, USA) to produce a disc translucent sample (Al-Quraishy *et al*., 2020). The NICOLET 6700 optical spectrometer from Thermo Scientific, equipped with Fourier transform infrared spectroscopy (FT-IR), was used for the analysis.

Evaluation of the phenolic content

The total phenolic content (TPC) of the leaf extract was determined by method (Mwamatope *et al*., 2020). A volume of 100 microliters of extracted leaf was mixed with 100 microliters of the Folin–Ciocalteu reagent and 300 microliters of a solution of 20% sodium carbonate. At room temperature the sample was kept and incubated in the darkness for thirty minutes. Using a UV-visible spectrophotometer (SHIMADZU, UV-1800), we were able to determine a wavelength of 765 nm. The total phenol content of the samples was calculated using the linear equation $y = 0.0021x + 0.0021$ with an R2 value of 0.9995. This equation was derived from a standard curve generated using gallic acid in a range of values ranging from 25 to 400 g/mL. The entire phenolic content was expressed in mg/g dry weight.

Evaluation of the flavonoid content

The approach described by (Mazandarani & Ghafourian, 2017) was used to determine the whole flavonoid content (TFC) in plant materials. An extracted methanol with a volume of 0.5 mL was combined with a 2% AlCl3-containing aqueous solution of the same volume. The wavelength was determined at 420 nm after being stored at 25°C for two hours. The TFC was calculated from a curve calibration generated using diverse concentrations (50–400 g/mL) of the quercetin standard and the following equation: $y = 0.0172x + 0.0507$ with $R2 = 0.995$. This curve was generated using different values of the quercetin standard. The calculated TFC was presented as quercetin in milligrams per gram of dry weight.

Evaluation of the tannin content

According to the method described by Rodrigues *et al*. (2007), the total tannin content (TTC) was determined. with some slight modifications. This approach was used for the leaf extract. An amount of 0.1 mL of the extracted samples was added to a 2 mL Eppendorf tube already containing 1.5 milliliters of Milli-Q water and 0.1 milliliters of the Folin–Ciocalteu phenol reagent and allowed to stand for eight minutes. Then 0.3 milliliters of 35% of solution of sodium carbonate were added to neutralize the solution. The ingredients were then mixed thoroughly and kept in a dark room at ambient temperature for twenty minutes. The measured value for the wavelength was 700 nm. The following equation, $Y = 0.0013x + 0.0052$, with an R2 value of 9937, was used to determine the total tannin content in the leaf extract. For this purpose, a created calibration curve with different concentrations of tannic acid standards. The calculated total content of tannin was expressed in units of mg/g dry weight (DW).

DPPH Radical scavenging assay

Using DPPH (2,2-diphenyl-1-picrylhydrazyl)

(Shahidi *et al*., 2006), *In vitro*, the scavenging activity of IOLE was evaluated. 1 mL of leaf extract with a concentration of 31.25 to 1000 µg/mL was mixed with the same volume of DPPH in methanol at a concentration of 0.135 mM. After thorough stirring, the mixture was stored at room temperature in the dark for 30 min. The optical density of both the extract and the control mixture (1 mL DPPH and 1 mL methanol) was measured at a wavelength of 517 nm. The following formula was used to estimate the activity of percentage of DPPH scavenging of the extract or standard: [(absorbance of control - absorbance of sample)/ absorbance of control] 100 =, DPPH scavenging activity (%) where the absorbance of control is the absorbance of methanol plus DPPH and the absorbance of DPPH radical plus extract.is the absorbance of sample.

%Inhibition = [(A control – A sample)/A control] × 100

Acontrol = Absorbance of negative control at the moment of solution preparation
Asample = Absorbance of a sample after 45 min.

ABTS Radical scavenging activity

The free radical scavenging activity of ABTS was tested as defined by Re *et al*. (1999), with some adjustments. 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was melted in distilled water at a concentration of 7 mM. By mixing the ABTS stock solution with 2.45 mM potassium persulfate (last concentration) and allowing the mixture to be kept in the dark at room temperature for 14 hours before utilization, ABTS radical cation (ABTS+) was generated (Re *et al*., 1999). The ABTS+ solution was diluted with water to an absorbance at 734 nm of 0.70 (0.02). 0.07 mL of the extract and 3 mL of the ABTS radical were used for the reaction. After an incubation time of 5-7 minutes, using a spectrophotometer the absorbance at 734 nm was measured. The activity of antioxidant was calculated using the next equation:

%Inhibition = [(A control – A sample)/A control] × 100

Acontrol = Absorbance of negative control at the moment of solution preparation
Asample = Absorbance of a sample after 5 min

The EC_{50} values were calculated using the graph indicating the concentration of the sample required to scavenge 50% of the ABTS. The EC_{50} is often used to express the concentration of extracts needed to scavenge 50% of the free radicals. ABTS was expressed as mg GAE/L.

UV-VIS Spectroscopic analysis

A UV-visible spectrophotometer (Perkin Elmer, USA Model: Lambda 950) with a slit width of 2 nm and a 10-mm cell was used to analysis the extract of *I. oblongifolia*. The analysis was carried out at room temperature. For proximate analysis, the extract was studied under visible and ultraviolet light with wavelengths between 300 and 800 nanometers. After centrifugation at 3000 rpm for ten minutes, the extract was filtered over Whatman filter paper No. 1 in preparation for UV-VIS spectrophotometer analysis. The sample was diluted with the same solvent at a ratio of 1:10, (Karpagasundari & Kulothungan, 2014).

XRD Spectrum

I. oblongifolia crystal structure extract was executed using X-ray diffraction (XRD, Rigaku Mini Flex 600, Tokyo, Japan) with Cu Kα radiation (40 kV, 15 mA).

Investigating the effects of IOLE on malaria

Female C57BL/6 mice aged 9 to 11 weeks were fed and watered ad libitum. For passage, the mice were administered injections of parasitized erythrocytes containing *P. berghei* via the intraperitoneal (I.P.) route (Wunderlich, 1982). In total, there were five groups of which contained five mice each. The first group, which was not infected, received distilled water daily for seven days. The second set was given 100 mg/kg of IOLE orally every day for seven days. The 3rd, 4th and 5th, groups were each injected intraperitoneally with 105 parasitized erythrocytes of *Plasmodium berghei* (*P. berghei*). One hour later, the

4th group received 100 mg/kg IOLE (every day for seven days) (Murshed *et al*., 2020). The fifth group, on the other hand, was given 10 mg/kg chloroquine phosphate (CQ) (Sigma-Aldrich, St. Louis, MO) (daily for three days), (Abay *et al*., 2015). The Neubauer chamber was utilized, slices were made from all mice on days 5, 6, and 7, and smears blood were prepared from the tails of the mice, which were then stained with Giemsa. The total number of infected erythrocytes was determined in this way. Counting the red blood corpuscles infected with *P. berghei* parasites and determining the quantitative relative population of blood parasites, also known as parasitemia. Taking a blood sample from the tail of the experimental mice and conducting a blood test made it possible to determine the number of parasites present in the blood. Methanol was used to complete the fixation process. After the drying process, a Giemsa stain was added. After the washing process, the slides were examined with a light microscope. The percentage of parasitemia according to the method described in (Wunderlich, 1982) was determined.

Statistical analysis

The presented data in the figures and tables are the average results of three separate replicates, considering the respective standard deviations (mean ± SD). The SPSS program, a one-way analysis of variance (ANOVA), and the Duncan test were used to determine the mean separation values and significance level (p<0.05).

RESULTS AND DISCUSSION

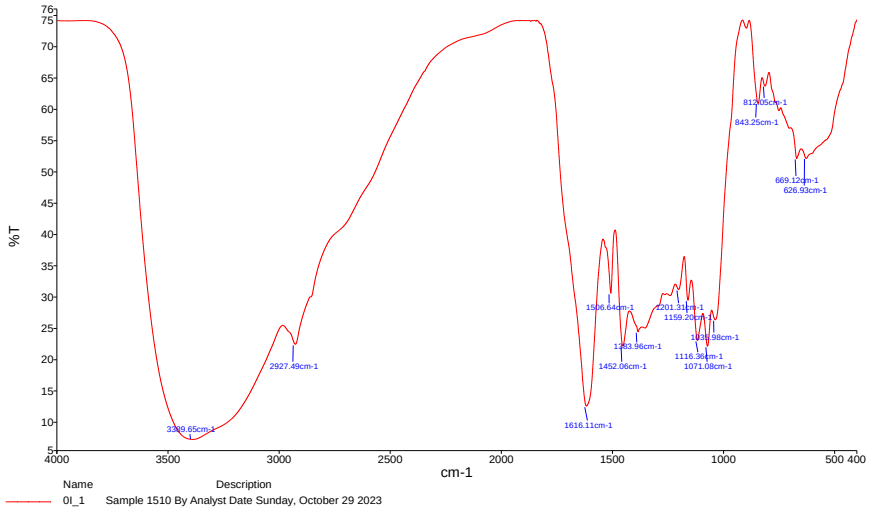

Figure No. 1
Infrared spectroscopy of methanolic extract of *Indigofera oblongifolia* leaves

The Fourier-transform infrared spectroscopy (FTIR) spectrum was utilized to determine the active components functional group, based on infrared radiation range peak value. An FTIR spectrometer analysis of I. oblongifolia showed main bands from 601.79 to 3397.97 Cm−1. N-H, C-H, O=C=O, C=C, N-O, S=O, C-O, C-Cl, and C-Br stretching were displayed in different bands representing many different modules of compounds such as aliphatic primary amine, alkane, α -, β- unsaturated ketone, nitric compounds, sulfonic acid, halogen compounds and alcohols, (Figure No. 1 and Table No. 1), (Pramila *et al*., 2012; Karthikaiselvi & Subhashini, 2014).

Table No. 1
Infrared spectroscopy (IR) spectrum of *Indigofera oblongifolia* by frequency range

Absorption cm-1	Appearance	Transmittance%	Group	Compound class
3397.97	Medium	7.4	N-H stretching	Aliphatic primary amine
2927.61	Medium	9.2	C-H stretching	Alkane
2365.49	Strong	10.7	O=C=O stretching	Carbon dioxide
1616.22	Strong	8.2	C=C stretching	α.β unsaturated ketone
1506.08	Strong	9.1	N-O stretching	Nitric compound
1455.47	Medium	8.7	C-H bending	Alkane
1349.24	Strong	8.4	S=O stretching	Sulfonic acid
1160.09	Strong	9.2	C-O stretching	Tertiary alcohol
1116.21	Strong	8.7	C-O stretching	Secondary alcohol
1070.87	Strong	8.8	S=O stretching	Sulfoxide
1032.34	Strong	8.9	S=O stretching	Sulfoxide
841.41	strong	10.4	C-Cl stretching	Halo compound
666.06	Strong	10.3	C-Br stretching	Halo compound
647.46	Strong	10.2	C-Br stretching	Halo compound
601.79	Strong	10.4	C-Br stretching	Halo compound

Total flavonoids, tannin and phenolics

The quantities of some secondary metabolites in the IOLE were measured, such as phenols, tannins, and flavonoids. Figure No. 7, shows that the phenols concentration (219.106 ± 1.0792) was high compared to the tannin and flavonoid concentrations (89.438 ± 0.1599, and 19 ± 0.1500 respectively).

Flavonoids, alkaloids, tannins, and phenolic compounds are the greatest important bioactive plant compounds (Mehmood *et al.*, 2015). Several studies have shown that plant extracts consisting of phenolic compounds have inhibitory properties. Natural polyphenolic components extracted from medicinal plants have been shown to inhibit cell invasion by *E. tenella* sporozoite in vitro (Ishaq *et al*., 2022). These researchers similarly noted that extracts containing polyphenolic components might be able to inhibit the enzymes required for the sporulation process of coccidian oocysts. Some flavonoids affect host-parasite interactions, while others interfere with the metabolism or development of protozoan parasites (Kerboeuf *et al*., 2008), including *Leishmania* sp. and *Trypanosoma* sp. (Fotie, 2008).

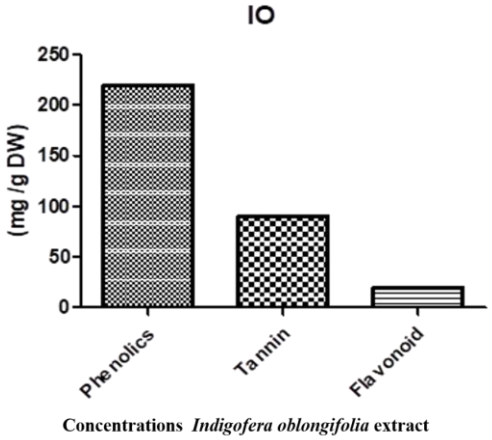

Figure No. 2
Flavonoids, tannin, and total polyphenols in the leaves methanolic extract of *the Indigofera oblongifolia* plant

DPPH scavenging activity

The activity of radical scavenging of the methanol extract of *I. oblongifolia* was determined using the DPPH scavenging assay. The results show the increases ability to scavenge DPPH radicals with increasing concentrations of the leaf extract. It has been found that the properties of radical scavenging of phenolic components, such as polyphenols, flavonoids, tannins, and phenolic terpenes, are primarily responsible for the antioxidant effect of plant products (Rahman & Moon, 2007). Thus, there are more and more reports and data from epidemiological research and laboratory in the literature showing that certain edible plants and their components, which have activities of antioxidant, have a significant protective effect against the development of cancer in humans (Tsao *et al*., 2004; Surai, 2014). The activity of radical scavenging of the extract showed that the ME extract inhibited 90% - 40% at 1000–31.25 µg/mL, with an EC_{50} value of 131.1 µg/mL (Figure No. 5). The results obtained with these extracts were higher than those of the positive control (methanol). The result of this study is comparable to that of a study conducted by (Gupta *et al*., 2014), in which the methanol extract of *S. sisymbriifolium* tested at diverse concentrations (300, 200, 100, 80, 60, and 40 µg/mL), showed a low DPPH activity of radical scavenging with an EC_{50} of 211.4 µg/mL, in contrast to the EC_{50} of 1.85 µg/mL for the positive control quercetin.

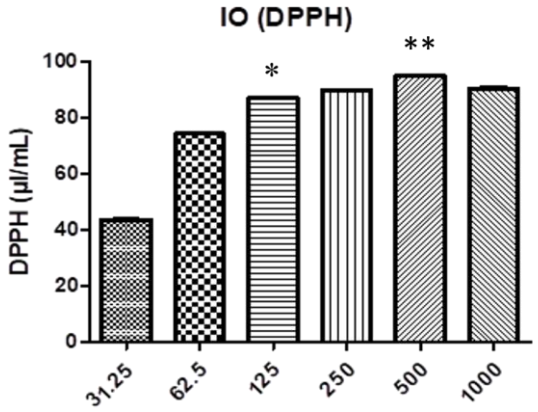

Figure No. 3
DPPH radical scavenging assay of different concentrations of phytochemicals isolated from the leaves of *I. oblongifolia* using methanol as solvent (*$p \leq 0.05$; **$p \leq 0.01$), shows significant differences compared between concentrations. Data are presented as mean ± SD, (n=3).

ABTS scavenging activity

ABTS activity of radical scavenging was positively correlated with the concentration of methanolic extract. In this test, the sample showed high ABTS activity of radical scavenging. The scavenging activity of the tested extract in a concentration variety of 1000 to 1.953 µg/mL was assessed using the ABTS cation assay. The leaf extracts of *I. oblongifolia* showed inhibitory percentage activity of ≥75% (Figure No. 6). This study showed that the suppression of free radicals and enzyme activity by the extract increased at the maximum tested doses, with the methanolic extract demonstrated substantial ABTS free radical scavenging activity and inhibition; however, activity of enzyme was more pronounced at lower concentrations. These findings imply that *I. oblongifolia* may serve as a natural antioxidant source Table No. 2.

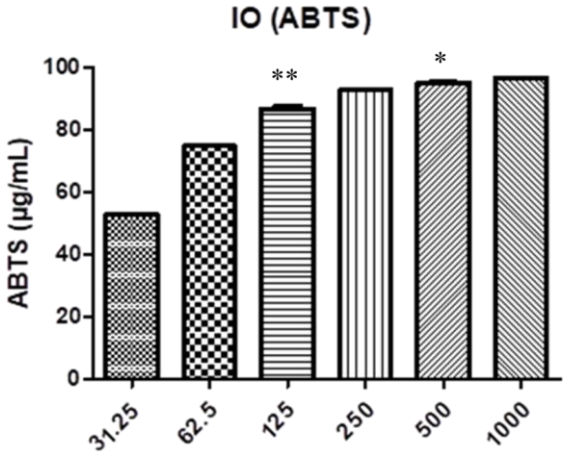

Figure No. 4
Investigation of the ABTS radical scavenging capabilities of different concentrations of phytochemicals isolated from the leaves of *I. oblongifolia* using methanol as solvent (*$p \leq 0.05$; **$p \leq 0.01$), denotes a significant difference when compared between concentrations. Data are presented as mean ± SD, n=3

Table No 2
ABTS radical scavenging activity of the *Indigofera oblongifolia* plant extract

Concentrations	DPPH µg/mL	ABTS µg/mL
31.25	40.46283752 ± 0.24	47.23057993 ± 0.54
62.5	71.22820802 ± 0.11	71.57946574 ± 0.11
125	96.55160401 ± 0.06	96.28104065 ± 1.51
250	129.7412832 ± 0.04	131.9635075 ± 0.03
500	196.0133455 ± 0.23	196.3087145 ± 0.19
1000	317.7796218 ± 0.67	322.3424836 ± 0.02

The values are the mean of three replicates ± SD and are expressed in mg GAE/g dry basis. Means denoted by different letters differ significantly ($p \leq 0.05$)

Ultraviolet–visible spectroscopy (UV-VIS Analysis)
UV-VIS analysis was performed to identify phytoconstituents present in the methanolic extract of *I. oblongifolia*. The UV-VIS spectra were performed to identify the compounds containing biologically active chemical components. The qualitative UV-VIS profile of the methanolic extract of *I. oblongifolia* was recorded at a wavelength of 300 nm to 800 nm based on the sharpness of the peaks and the correct baseline. UV-VIS spectrum of IOLE is shown in Figure No. 2. It can reveal the chemical absorption properties of IOLE. Furthermore, the absorption peaks of IOLE show the chemical makeup and possible bioactive components. These data are essential for medicinal and nutritional studies of the I. oblongifolia plant (Shahidi *et al*., 2006; Njokua *et al*., 2013). The absorbance of the UV-VIS spectrum of IOLE was assigned at around 440 nm. This shift indicates the existence of nucleic acids and aromatic chemicals (Arshad *et al*., 2021). This result was consistent with a previous study (Bechtold & Mussak, 2009).

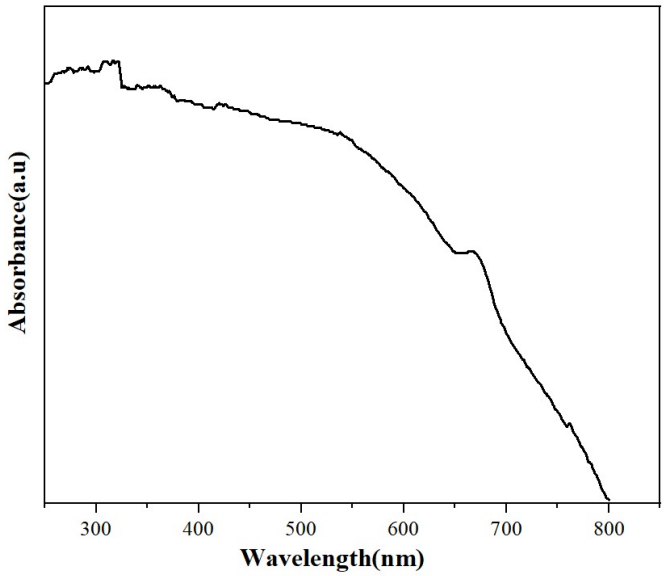

Figure No. 5
UV-VIS spectrum of *Indigofera oblongifolia* extracts methanolic extracts extract

XRD- I. oblongifolia methanolic extracts

The extract samples were amorphous, with the foreign, peak at approximately 25 thetas, indicating the presence of carbon components. As shown in Figure No. 3, the XRD spectrum of IOLE at 21.77 shows the crystalline structure of the compounds. The peak at 21.77 demonstrates a distinct crystalline configuration, which is probably related to the chemical components or phases of IOLE. This peak indicates the presence of components in IOLE that have therapeutic potential.

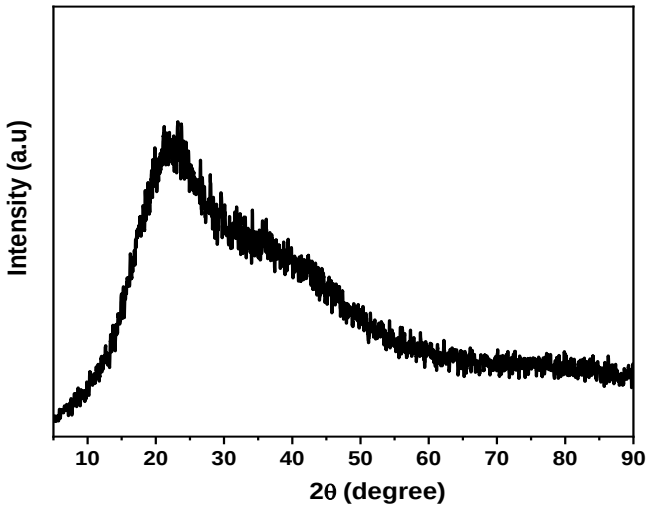

Figure No. 6
XRD- *Indigofera oblongifolia* leaf extract

Plasmodium berghei in mice

On day 5 post-infection parasitemia peaked at approximately 37.9% in mice infected with 105 parasitized erythrocytes of *P. berghei*. However, when the infected mice were administered IOLE on days 5, 6, and 7 post-infection, the parasitemia decreased to approximately 91.8 ± 0.8, 94.9 ± 0.5, and 96 ± 0.21%, respectively, as shown in Table No. 3. This reduction in parasitemia was very related when the mice were treated with chloroquine (CQ), which was subsequently administered to the animals.

Table No. 3
Effect of *Indigofera oblongifolia* leaf extract (IOLE) on the suppression of parasitemia of mice infected with *Plasmodium berghei*

Gruop	Suppression (%)		
	Day 5	Day 6	Day 7
Infected	0	0	0
Infected + IOLE	91.8 ± 0.8	94.9 ± 0.5	96 ± 0.21*
Infected + CQ	93.9 ± 0.6	95.4 ± 0.51	97.1± 0.15*

***Values are mean ± SD**

The dose of IOLE (100 mg/Kg) used in the subsequent tests was determined on the basis of our previous findings on parasitemia, which showed that on 7th day (P.I.), parasitemia in the infected group reached about 37.9%. The group that was treated with 100 mg/Kg IOLE also had a parasitemia of less than 1% (Figure No. 7), just like the group treated with CQ.

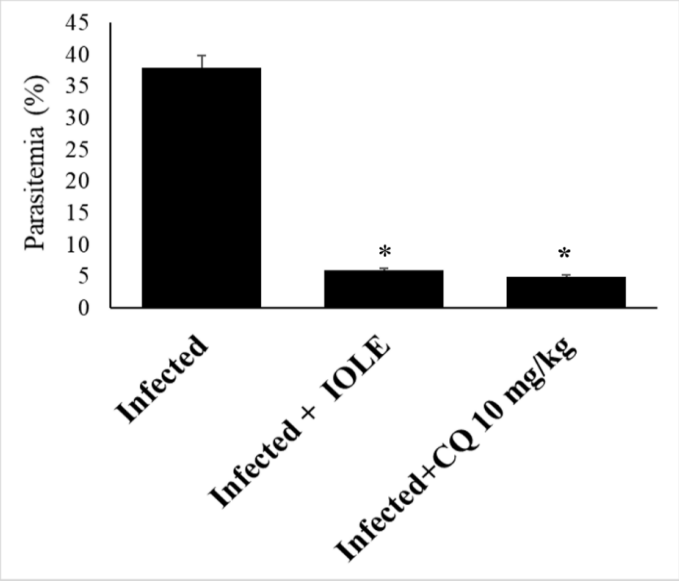

Figure No. 7
***Indigofera oblongifolia* leaf extract (IOLE) reduced parasitemia of mice infected with *P. berghei*. (*) significance at $p<0.01$ against the infected group on day 7 parasitemia**

The phytochemicals present in IOLE have demonstrated their ability to prevent parasite-induced parasitemia. In this study, this phenomenon occurred, with the suppression rate almost identical to that caused by the reference drug CQ used. It is possible that the active elements, such as quinines, phenolic compounds, and alkaloid compounds, are responsible for the anti-plasmodial impact of the infection (Murshed *et al*., 2024).

This investigation used leaf extracts of *I.*

oblongifolia to combat the blood stages of malaria infection in mice. According to the results of this investigation, administering 100 mg/Kg to female mice can greatly reduce parasitemia caused by *P. berghei* infection. On the control group achieved 89% parasitemia on the seventh day of infection (Figure No. 8) observed this daily increase in parasitemia. It was found by Krücken *et al*. (2005), that the extract from *I. oblongifolia* helped mice recover from *P. berghei* infections and build up a lasting immunity against homologous challenge. The infection-induced parasitemia significantly decreased as a result. Shahjahan *et al*. (2005), suggest that the presence of active chemicals produced by *I. oblongifolia* could explain this. These substances include saponins, which are either steroids or triterpenes; coumarin; quinines; and phenol.

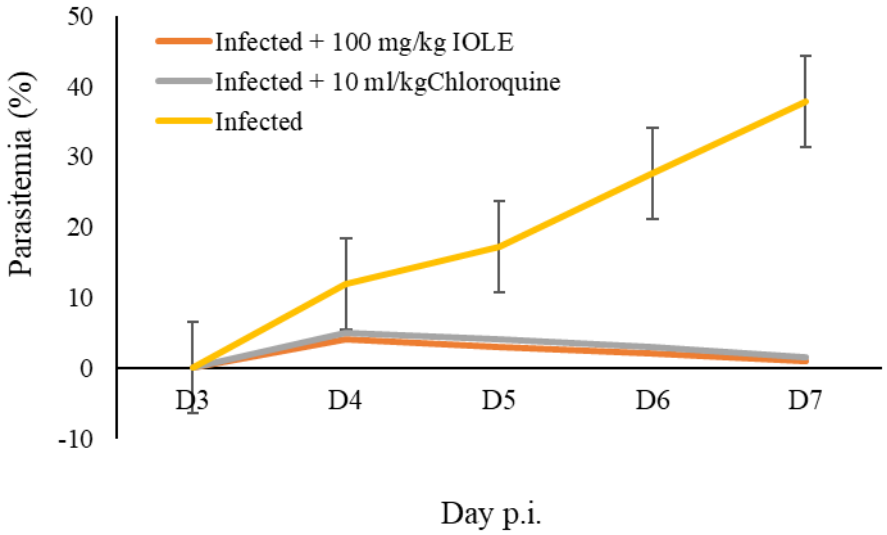

Figure No. 8
Changes in parasitemia after treatment of mice with *Indigofera oblongifolia* leaf extracts. Values are means ± SD. *Significant against the infected

LIMITATIONS

Some limitations of this study include the following: a) The results are not representative of all blood parasites, and the sample consisted of experimental mice that were infected with malaria. Future studies will need to cover diverse parasites using a variety of plants and identifying the bioactive compounds in the plant. b) causality: incomplete information was found in some cases to evaluate causality assessment and herbal products containing different metabolites. Therefore, the causality is attributed to a mixture of several components. However, causality assessment scales do not consider a mixture of components. We need to separate the chemical compounds to find out which substance effective that suppresses Plasmodium. c) There is a significant possibility of undeclared medicinal herbs.

CONCLUSIONS

The results indicate that the plant studied contains components that could be used in medicine. Previous studies have reported ample evidence of the bioactivity of the phytochemicals discovered. The existence of these phytochemicals confers both medicinal and physiological properties to the studied the treatment's plants for malaria. The essences of these plants therefore have the probability to serve as a rich source for the development of effective drugs. In addition to the strong recommendation to use these plants in traditional medicine, it is strongly recommended that further work be passed out to isolate, purify, and characterize the active elements that responsible for the action of these plants. Future research should also be encouraged to explain the potential mechanism of action of these extracts.

Ethical approval
The study followed Saudi Arabia's animal use ethics (Ethics approbation ID: KSU-SE-21-86).

Conflict of interest
No potential conflict of interest.

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