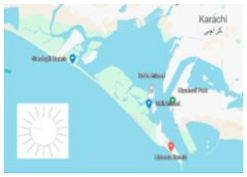

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Chemical and Biological Evaluation of *Albizia lebbeck, Psoralea corylifolia,* and *Trifolium indicum*

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ABSTRACT: *Herbal medicine, rooted in traditional practices, is increasingly recognized for its diverse therapeutic applications. Albizia lebbeck, Psoralea corylifolia, and Trifolium indicum are valued in traditional medicine for their potential health benefits, including anti-inflammatory, antioxidant, and metabolic regulatory properties. This work delves into the rich spectral fingerprint of methanolic extracts from Albizia lebbeck, Psoralea corylifolia, and Trifolium indicum, aiming to elucidate their unique biochemical composition. Employing advanced spectroscopic techniques, including Flame and Atomic Absorption Spectroscopy (AAS) and Fourier Transform Infrared Spectroscopy (FTIR), the minerals and bioactive compounds present in these herbal extracts were meticulously characterized. The study extends beyond conventional spectroscopic analysis by correlating the spectroscopic signatures with the biological potential of the extracts. This study unveiled biological evaluations including time and concentration-dependent antioxidants, anti-inflammatory, antihyperglycemic, analgesic, and anti-anxiety properties of these botanicals through rigorous in vitro and in vivo assessments. Each herbal extract was found to have characteristic chemical composition and biological potential. The present work not only contributes to the fundamental understanding of the spectral features of these herbs but also positions them as promising candidates in the realm of disease management.*

Keywords: Herbal medicines, Spectral analysis, Bioactivity profile, biological activities

INTRODUCTION

Inflammation is a complex phenomenon producing pain along with redness, also termed as a non-specific internal response of the body (Leelaprakash and Dass, 2011), where infiltration of leukocytes along with capillary infiltration leads to vascular permeability and activation of fibroblast, fibrosis, infiltration, and proliferation of monocytes, macrophages, and neutrophils in acute and chronic inflammation. It is distinguished due to the longer residence of macrophages and lymphocytes, which mainly produce fibrosis and tissue necrosis (O'Byrne and Dalgleish, 2001; Wong et al., 2012). Prostaglandins (PGs), histamine and kinins are released in response to inflammation, which direct the blood flow towards affected areas by producing alterations in the structure of the membrane (Leelaprakash and Dass, 2011). Inflammation normalizes the tissue's functions by promoting the proliferation and differentiation of stem cells in the affected tissue as well as by promoting angiogenesis, the formation of new blood vessels (Gurtner et al., 2008).

A significant number of the population of the world is facing anxiety-related problems, causing stomach issues, motor sympathetic hyperactivity, stress, and fear. Exposure to these conditions for a longer period makes a man mentally and physically ill (Shri, 2010). Oxidants are produced because of different chemical reactions (outside and inside the body) and diseases like inflammation. Reactive oxygen species (ROS) are natural byproducts of cellular metabolism having unpaired electron, making them highly reactive and capable of damaging cellular components, including proteins, lipids, and deoxyribonucleic acid (DNA), while low levels of ROS are necessary for normal cellular functions. There are several types of ROS, including superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (•OH), and singlet oxygen ($^1O_2$) (Valko et al. 2006). ROS have been implicated in the pathogenesis of various diseases, including cancer, neurodegenerative diseases, cardiovascular disease, diabetes (Sies, 2015), inflammation, damaging blood vessels and atherosclerosis (Griendling and FitzGerald, 2003). Antioxidants counteract the harmful effects of oxidants by donating electrons to free radicals, hence neutralizing free radicals and preventing them from damaging cells (Sies, 1993). There are several types of antioxidants, including vitamins C and E, beta-carotene, selenium, and flavonoids present in a variety of foods, having ability to prevent the onset of various chronic diseases by reducing oxidative

stress (Block et al., 2002). Phytochemicals are naturally occurring compounds found in plants, having crucial role in the prevention and treatment of chronic diseases such as inflammatory, cardiovascular disease, cancer, diabetes, and neurodegenerative diseases (Liu et al., 2014). For example, resveratrol, has shown to reduce the risk of heart (Silva et al., 2013) with improved insulin sensitivity and glucose metabolism (Goh et al., 2014). They impart characteristic properties such as anti-inflammatory, anti-oxidant, analgesic, anti-anxiety, anti-bacterial, anti-hyperglycemic, anti-viral and anti-cancer to the herbal plants. (Yasmin et al., 2020b).

The growing interest in natural sources of antioxidants is driven by the need for alternative therapeutic options that are effective, affordable, and have fewer side effects compared to synthetic drugs. Herbal plants, rich in bioactive compounds, offer a promising source of such natural antioxidants (Embuscado, 2015). *Albizia lebbeck* (commonly known as Siris or Shiris), *Psoralea corylifolia* (commonly known as Babchi), and *Trifolium indicum* (commonly known as Nakhona or Indian clover) are medicinal plants with traditional uses in managing various ailments. However, a comprehensive understanding of their phytochemical composition and biological activities remains limited. Therefore, the present study was designed to evaluate methanolic extracts of *Albizia lebbeck*, *Psoralea corylifolia*, and *Trifolium indicum* for their anti-inflammatory, anti-hyperglycemic, anti-anxiety, analgesic, and antioxidant potentials with detailed phytochemical and mineral investigation to understand their chemical profiles and how these associates with their biological effects. The uniqueness of this research lies in filling existing gaps in literature along with its integrated approach, combining chemical, mineral, and biological evaluations to explore how these factors play their role to the overall effectiveness of the herbal extracts in treating conditions related to inflammation, pain, anxiety, oxidative stress and hyperglycemia.

MATERIALS AND METHODS

Collection of Herbs

Selected medicinal herbs including *Albizia lebbeck*, *Psoralea corylifolia*, and *Trifolium indicum* were obtained in dried form from Ajmal Dawa Khana located near Ichra, Lahore, Pakistan. These herbs were confirmed by the botanist from the Department of Botany, University of the Punjab, Lahore, before further processing. Then, the herbs were purified, ground to fine

powder and stored in glass vials for further experimental work.

Preparation of Herbs Extract

Herbal extracts were prepared by dissolving 10 g of each medicinal herb in powdered form with 200 mL of methanol used as solvent followed by stirring overnight using magnetic stirrer. The mixture was filtered using Whatman filter paper and the residue obtained was again mixed with 100 mL of methanol and left overnight. The mixture was then filtered on next day and the filtrate obtained was mixed with previously obtained filtrate. The extract was obtained after evaporating filtration and stored in glass vials for chemical and biological evaluation.

Chemical Profiling

Fourier Transform Infrared Spectroscopy Analysis

The methanolic extract of selected herbs was subjected to Fourier Transform Infrared Spectroscopy (FTIR) analysis in the region of (4000-650) cm^{-1} (Agilent Technologies Carry 630 FTIR) (Khalil et al., 2013).

Phytochemicals Analysis and Mineral Detection

The phytochemicals constituents including terpenoids, flavonoid, tannins, steroids, phlobatannins, anthraquinones, alkaloids, saponins, cardiac glycosides, reducing sugars and carbohydrates were evaluated in the selected medicinal herbs by the using methods published earlier (Yasmin et al., 2020b)**.**

The wet method was employed for the evaluation of mineral content in selected herbs sample. Briefly, each herb (0.5 g) in powdered form was added into the beaker followed by adding concentrated nitric acid (HNO_3). This solution was heated till the formation of clear solution. After digestion, the obtained solution was diluted with distilled water up to 50 mL. Concentrations of different minerals were estimated in prepared herbs solution by preparing 5, 10, 15, 20 and 25 parts per million (ppm) standard solutions of respective metal followed by the estimation of mineral content by Flame and Atomic Absorption Spectroscopy (AAS) (Rahayu, 2020).

***In Vitro* Biological Profiling**

Antioxidant Activity

The methanolic extract of selected medicinal herbs was subjected to three antioxidant assays for the evaluation of their antioxidant potential. The $FeCl_3$ Iron Reducing Power, Phosphomolybdenum and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) activities were performed as described previously to estimate the antioxidant potential of herbs (Tajammal et al., 2017; Yasmin et al., 2020a; Samra et al., 2022).

***In Vivo* Biological Profiling**

Experimental Animals

The Sprague Dawley (SD) rats were used for the evaluation of *in-*

vivo biological potential of methanolic extract of selected medicinal herbs. Animals were kept in a departmental animal house under controlled temperature and humidity and cared for in accordance with international instructions. The experiments were approved by the Ethical Committee, School of Chemistry, University of the Punjab, Lahore. Pakistan.

Experimental Groups and Dose Selection

Rats were fasted overnight and divided into 5 groups (Number of rats (n) =5 in each group):

Group 1: Standard Drug (Diclofenac sodium; 20 mg/kg)

Group 2: Control (Carboxymethyl Cellulose (CMC); 10 ml/kg)

Group 3: *Albizia lebbeck* (300 mg/kg)

Group 4: *Psoralea corylifolia* (300 mg/kg)

Group 5: *Trifolium indicum* (300 mg/kg)

Anti-inflammatory Effect

The carrageenan induced paw edema method in rats was used to determine anti-inflammatory potential of methanolic extract of selected herbs by producing inflammation in the rat paw as reported previously (Samra et al., 2022; Samra and Basra, 2023).

Percentage edema inhibition was calculated by using following formula

% edema inhibition = $(V_c - V_t / V_c) * 100$

Whereas V_c = stands for the mean paw volume of control group rat and V_t stands for the mean paw volume of drug treated group rat.

Anti-hyperglycemic Activity

SD rats were fasted overnight and anti-hyperglycemic effects of methanolic extract of selected medicinal herbs were evaluated by oral glucose tolerance test (OGTT) as reported previously (Tajammal et al., 2017). Rats having blood glucose level between 80-100 mg/dl were selected to check the anti-hyperglycemic activity.

Analgesic Activity

Analgesic potential of methanolic extract of selected medicinal herbs was evaluated by acetic acid-induced method as reported previously (Samra et al., 2022; Samra and Basra, 2023).

The % inhibition of writing was calculated using the following formula

% Inhibition of writhing = $(W_c - W_t/W_c) \times 100$

Whereas W_c stands for mean writhing of control group rat and W_t stands for mean writhing of drug treated group rat.

Anti-anxiety Activity

The Elevated Plus Maze (EPM) test was employed for evaluating the anti-anxiety potential of selected herbs as described previously (Samra and Basra, 2023).

The % of time spent in open arm was calculated using following formula

The percentage time spent in open arm = $(T_o/T_t) \times 100$

Whereas T_o stands for the time spent in open arms and T_t stands for the time spent in both open and closed arms.

Statistical Analysis

In this experimental work, all the data of the *in vivo* experiments were presented in the form of Mean ± standard deviation and this statistical analysis was done using one-way ANOVA, which was done with the help of GraphPad prism (version 7.0).

RESULTS

Chemical profiling

Fourier Transform Infrared Spectroscopy and Phytochemicals Analysis

The FTIR spectra of *Psoralea corylifolia* extract revealed the absorption bands at 2923.2, 2855.6, 1609, 1449, 1120 and 1053.9 cm^{-1} showed the presence of $-CH_2$, (Alkanes), -CH stretching (Aldehydes), C=N (Imines), C=C (Aromatic ring), C-N (Aromatic amines) and C-O (Anhydrides) respectively. The methanolic extract of *Albizia lebbeck* presented the different absorption bands at 3336.0, 2924.6 and 2855.6 cm^{-1} which showed the presence of O-H, $-CH_2$ and -CH group (Alkanes) respectively. The absorption band at 1735.1 and 1617.7 cm^{-1} depicted the presence of C=O group (Aldehyde) and C-N group (primary amines) respectively. In addition to this, the absorption band at 1028.7 cm^{-1} indicated the presence of R-O group (Ethers). Moreover, the spectra of *Trifolium indicum* contained the different absorption bands at 3336.9, 1577.6, 1117.3 and 1047.4 cm^{-1} exhibited the presence of O-H group (Alcohol and phenol), C=C stretching (Aromatic), C-O group (ethers), and C-O group (primary alcohol) (Figure 1a-c).

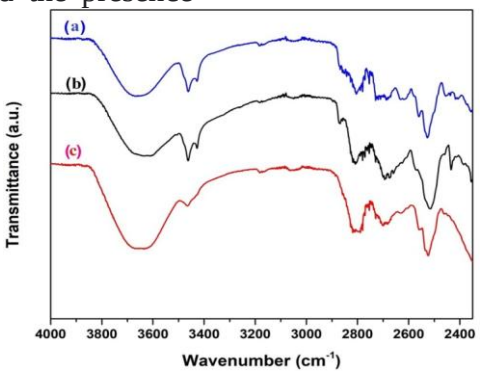

Fig. 1. FTIR Spectra of medicinal herbs. FTIR spectra of (a) *Psoralea corylifolia*, (b) *Albizia lebbeck*, and (c) *Trifolium indicum* depicting key molecular vibrations representative of their chemical characteristics.

Furthermore, biochemical tests revealed the presence of terpenoids, reducing sugars and carbohydrates in all herbs, while steroids and cardiac glycosides were only found in *Albizia lebbeck* and *Trifolium indicum* and alkaloids were best found in *Psoralea corylifolia*. Moreover, saponins and flavonoids were found to be effectively present in the extract of *Trifolium indicum* (Table 1).

Table 1: Phytochemical analysis of methanolic extract of herbs.

Sr. No	Phytochemicals	Medicinal herbs		
		Albizia lebbeck	***Psoralea corylifolia***	***Trifolium indicum***
1	Terpenoids	+	+	+
2	Tannins	-	-	-
3	Saponins	-	-	++
4	Flavonoids	-	-	++
5	Reducing sugars	+	+	+
6	Carbohydrates	+	+	+
7	Steroids	+	-	+
8	Cardiac glycosides	+	-	+
9	Phlobatannins	-	-	-
10	Alkaloids	-	+	-
11	Anthraquinones	-	-	-

Where, (-) shows the absence, (+) shows the presence and (++) show more presence of respective phytochemical.

Mineral Detection by Flame and Atomic Absorption Spectroscopy

All the herbs were subjected to mineral detection and found to have Ni (Nickel), Fe (Iron), Mg (Magnesium), Ca (Calcium), Cu (Copper), Co (Cobalt) and K (Potassium) in different concentrations. Ni, Fe and Ca were found in maximum concentrations of 102.3, 199 and 97.9 ppm respectively in *Psoralea corylifolia* while, Mg, Co and K were present in maximum concentrations of 115.2, 167.7 and 47.5 ppm in *Albizia lebbeck* and Cu was found in maximum concentration of 28.8 ppm in *Trifolium indicum*, as represented in Table 2.

Table 2: Quantitative investigation of minerals in ppm by Flame and Atomic Absorption Spectroscopy in medicinal herbs

Sr. No	Minerals	Medicinal herbs (ppm)		
		Albizia lebbeck	***Psoralea corylifolia***	***Trifolium indicum***
1	Nickel	9.701	102.3	85.5
2	Iron	66.85	199	179.2
3	Magnesium	115.2	30	38.8
4	Calcium	26.34	97.9	31.5
5	Copper	4.263	28.6	28.8
6	Cobalt	167.7	148.5	135
7	Potassium	47.5	42.68	31.05

In Vitro Biological Profiling

Antioxidant Potential

The ability of the phytochemicals presents in the herbal extracts to reduce Fe^{3+} to Fe^{2+} was evaluated by $FeCl_3$ iron reducing power activity. All herbs were found to show an increasing trend in their reducing power with decrease in their IC_{50} values with respect to time. After 30 minutes of incubation, *the Trifolium indicum* showed the lowest IC_{50} value and hence the maximum antioxidant potential among all herbs. The antioxidant potential of methanolic extracts of herbs to reduce Mo (VI) to Mo (V) was determined by Phosphomolybdenum activity. It was observed that after 30 minutes of incubation, methanolic extract of *Psoralea corylifolia* depicted the maximum antioxidant potential and lowest IC_{50} value among all herbal extracts (Table 3).

Table 3: Antioxidant activities of methanolic extracts of medicinal herbs.

Activity	Herbs Extract	IC_{50} (mg/mL) with respect to time (minutes)					
		10	15	30	45	60	120
$FeCl_3$ iron reducing power	*Albizia lebbeck*	0.063	0.063	0.063	0.062	0.059	0.053
	Trifolium indicum	0.014	0.013	0.012	0.011	0.010	0.011
	Psoralea corylifolia	0.024	0.021	0.020	0.020	0.021	0.018
	Ascorbic acid	0.006	0.006	0.006	0.005	0.006	0.005
Phosphomolybdenum	*Albizia lebbeck*	-	0.072	0.091	0.084	0.079	0.079
	Trifolium	-	0.074	0.072	0.069	0.071	0.073

	indicum						
	Psoralea corylifolia	-	0.002	0.0029	0.003	0.005	0.006
	Ascorbic acid	-	0.001	0.001	0.001	0.0020	0.0023
2,2-diphenyl-1-picryl-hydrazyl	*Albizia lebbeck*	-	110.900	59.500	44.500	35.00	23.500
	Psoralea corylifolia	-	5.780	6.190	4.470	5.700	5.900
	Trifolium indicum	-	0.920	0.850	0.890	1.000	1.260

Furthermore, the free radical scavenging potential of methanolic extract of selected herbs was evaluated by DPPH assay and results are shown as IC_{50} values in table 4. The methanolic extract of *Trifolium indicum* showed maximum free radical scavenging potential among all herbs and lowest IC_{50} value, hence showed maximum antioxidant activity at interval of 30 minutes among other medicinal herbs. The antioxidant assays showed that the selected herbs extracts had impressive antioxidant potential, among them *Trifolium indicum* and *Psoralea corylifolia* showed significant antioxidant potential.

***In Vivo* Biological Evaluation**

Anti-inflammatory Potential

The *in vivo* anti-inflammatory potential for the methanolic extracts of selected herbs was evaluated by carrageenan-induced paw edema method in SD rats. Each herbal extract was dissolved in 0.5 % CMC solution and given orally. Negative control group received CMC (10 ml/kg) solution while positive control group was given Diclofenac sodium as standard drug at a dose of 20 mg/kg. A magnificent suppressive effect was observed for herbal extracts in comparison with control group after first hour of carrageenan induction (Fig. 3). The methanolic extract of *Albizia lebbeck* showed remarkable repression in paw volume as compared to other herbal extracts with maximum anti-inflammatory potential equal to standard medicine after third hour of carrageenan induction, while other herbal extracts also showed good anti-inflammatory potential (Fig. 2 a-e). The increasing anti-inflammatory trend was observed for all herbal medicinal extracts after third hour of carrageenan induction as shown in Fig. 3-4.

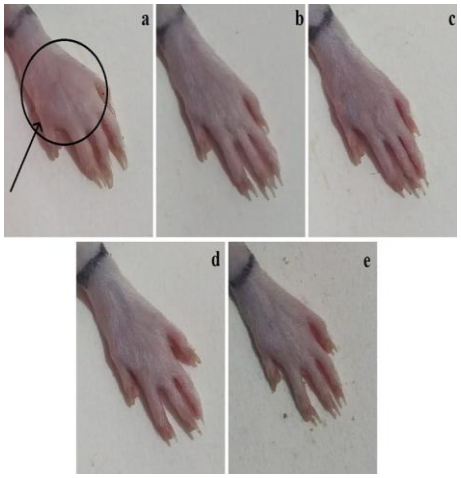

Fig. 2. Edema suppressing effect at 3 hours of carrageenan induction in rat's paw. (a) Inflamed paw (Untreated), (b) Diclofenac sodium treated paw, (c) *Albizia lebbeck* treated paw, (d) *Psoralea corylifolia* and (e) *Trifolium indicum* treated paw edema. Each image illustrates the effect of treatment by representative medicinal herbs on edema
inhibition.

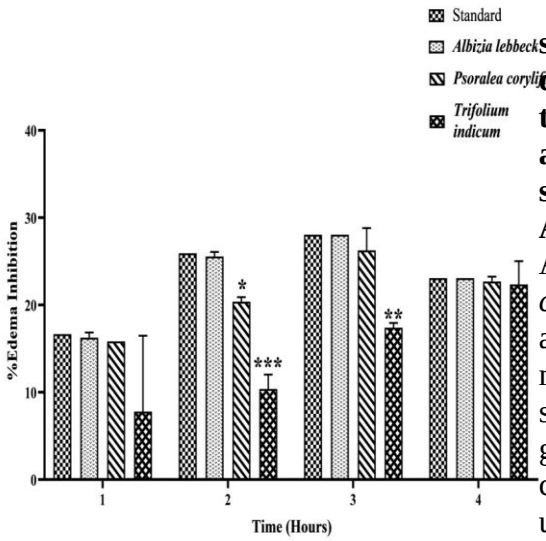

Fig. 3. The percentage of edema inhibition by herbs. Statistically significant differences are $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ when compared with standard and all the data is represented in percentage values as Mean ± standard deviation. Diclofenac sodium was used as a standard drug.

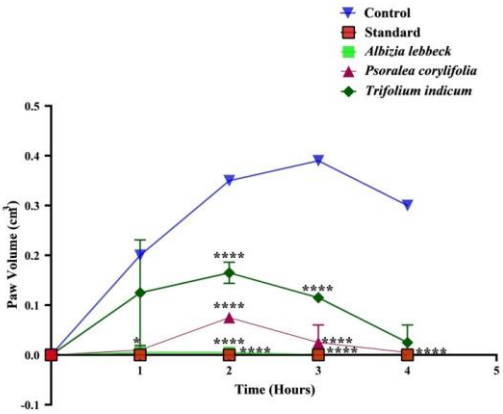

Fig. 4. Reduction in rat's paw volume with respect to time Diclofenac sodium was used as a standard drug. $^{*}P < 0.05$ and $^{****}P < 0.0001$ are the statistically significant differences when compared with the control group and all values are represented as mean ± standard deviation

Anti-hyperglycemic Activity

Among the tested herbs *Psoralea corylifolia* showed magnificent anti-hyperglycemic effect at 210 min, while *Trifolium indicum* also showed good effect on reducing glucose level in the blood as compared with Diclofenac sodium used as reference drug. The decreasing trend in anti-

hyperglycemic effect observed at 210 min was *Psoralea corylifolia* > *Trifolium indicum* > Standard > Control > *Albizia lebbeck* as shown in Fig. 5.

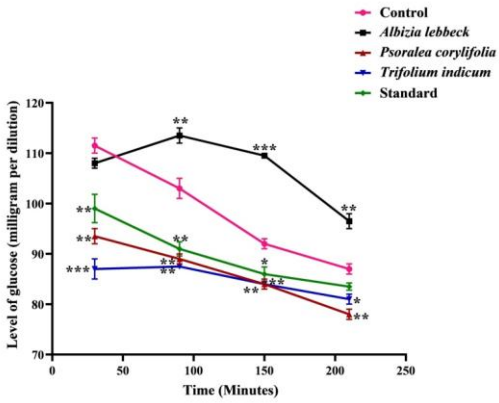

Fig. 5. Antihyperglycemic potential of herbs. Diclofenac sodium was used as a standard drug. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ are the statistically significant difference when compared with control group and all values are represented as mean ± standard deviation.

Analgesic Activity

Analgesic effect for the selected herbs to check their efficacy in injury was evaluated by acetic acid-induced writhing test. *Albizia lebbeck* showed highest inhibition in writing at a dose of 300 mg/kg as compared to reference medicine which was administered at the dose of 20 mg/kg (Fig. 6).

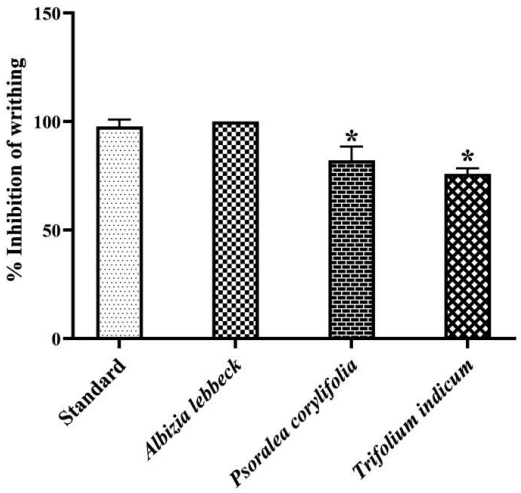

Fig. 6. Analgesic potential of herbs. Diclofenac sodium was used as standard drug while, $^{*}P < 0.05$ is the statistically significant difference when compared with standard and all values are represented as mean ± standard deviation.

Anti-anxiety Potential

The results showed the rat group treated with extract of *Albizia lebbeck* and *Psoralea corylifolia* remained in the open arm for maximum period as compared to standard group, which showed that *Albizia lebbeck* had the highest *Psoralea corylifolia* showed significant anti-anxiety potential as compared with Diclofenac sodium used as standard (Table 4).

Table 4: Anti-anxiety effect of herbal plants by elevated plus maze (EPM) test.

Sr. No.	Plant herb	% Time spent on open arm	No. of entries in open arm	No. of entries in closed arm
1	Control	10.25	3.00	3.50
2	Standard	22.00	3.50	3.50

3	*Albizia lebbeck*	42.30	7.00	8.00
4	*Psoralea corylifolia*	26.50	5.50	5.50
5	*Trifolium indicum*	0.50	0.50	1.50

A decreasing trend of anti-anxiety potential observed in herbs was *Albizia lebbeck* > *Psoralea corylifolia* > standard > control > *Trifolium indicum* (Fig. 7).

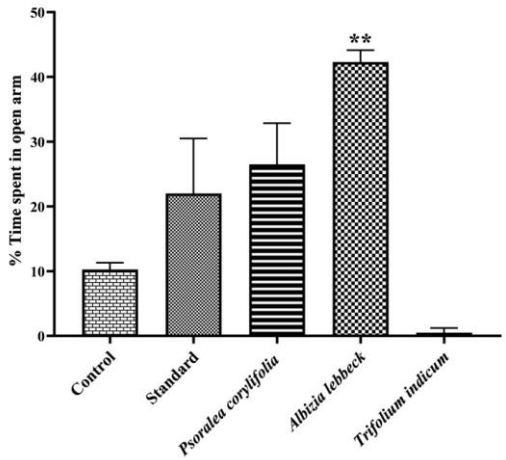

Fig. 7. Anti-anxiety effects of *Albizia lebbeck, Psoralea corylifolia, Trifolium indicum* and standard drug in terms of percentage of time spent in open arm. Diclofenac sodium was used as standard. $^{**}P < 0.01$ is the statistically significant difference when compared with control group and all data is represented as ± standard deviation.

DISCUSSION

The Fabaceae family is known to include medicinal plants with a wide range of potential benefits, including anti-inflammatory, analgesic, anxiolytic, anti-hyperglycemic, antioxidant, antibacterial, anticonvulsant, anticancer, and anti-asthma potentials (Ahmad et al., 2016). Examples include *Acacia nilotica*, *Platypodia elegans*, and *Dolichos biflorus*. These herbs are rich in phytochemicals such as alkaloids, flavonoids, phenols, terpenoids, and steroids, which are responsible for their anti-inflammatory, antioxidant, anticancer, antiviral, antibacterial, and antifungal effects (Jurenka 2009). For instance, garlic (*Allium sativum*) contains sulfur compounds known as allicin, which have notable antibacterial, antifungal, and antiviral properties (Ankri and Mirelman, 1999). Moreover, the Fabaceae family member *Glycine max* (soybean) contains genistein and daidzein, which provide anti-inflammatory potential (Yu et al., 2016). Furthermore, members of the Fabaceae family are rich in minerals such as calcium, magnesium, potassium, and iron, which play crucial roles in maintaining health (Didinger and Thompson, 2021). For example, calcium is essential for bone health, muscle movement, neurotransmitter release, and blood clotting (Heaney, 2002), while potassium helps to maintain

heart health and blood pressure (Weaver, 2013). Similarly, iron contributes to oxygen-carrying ability in hemoglobin, and magnesium supports water balance and antioxidant functions (Quintaes and Diez-Garcia, 2015). Additionally, *Aegle marmelos*, with its content of niacin, enhances its anti-inflammatory potential (Angajala et al., 2014) and cobalt has a distinct role in boosting anticancer potential (Veeralakshmi et al., 2015) and cobalt has a distinct role in boosting anticancer potential (Veeralakshmi et al., 2015). The present research has conducted chemical and biological profiling of methanolic extracts from Fabaceae family herbs, including *Albizia lebbeck, Psoralea corylifolia,* and *Trifolium indicum*. It has successfully examined the phytochemicals and minerals in these herbs, revealing their antioxidant, anti-inflammatory, antihyperglycemic, anti-anxiety, and analgesic properties. In the present study, $FeCl_3$ Iron Reducing Power, Phosphomolybdenum and DPPH assays showed that *Trifolium indicum* had the maximum antioxidant potential among all herbs. The previously reported work on *Psoralea corylifolia* showed that its ethanolic and acetone extracts showed IC_{50} values of (115.82±0.06 and 172.27±0.04 µg/ml) respectively by DPPH method, while its ethanolic extract showed IC_{50} value of (102.81±0.05 µg/ml) by $FeCl_3$ iron reducing activity (Karale et al., 2022). The present study conducted on the methanolic extract of *Psoralea corylifolia* showed its lowest IC_{50} value of (0.0180 mg/ml or 18 µg/ml) after 120 minutes of incubation by iron reducing power activity, (0.002 mg/ml or 2 µg/ml) after 15 minutes of incubation by Phosphomolybdenum assay and maximum antioxidant potential than its previously reported work (Szakiel et al., 2011). Various members of the family Fabaceae for example *Acacia Arabica, Arachis hypogaea, Cassia tora, Dalbergia sissoo, Acacia catechu , Acacia nilotica* (Ahmad et al., 2016) have been found to show anti-inflammatory activity. The present study shows the maximum edema inhibition by methanolic extract of *Albizia lebbeck* (28.05%) at a dose of 300mg/kg while, *Psoralea corylifolia* showed significant result (26 %). This study, which was conducted on methanolic extract of *Psoralea corylifolia* by glucometer method showed excellent anti-hyperglycemic effect among all herbs and standard and *Trifolium indicum* showed significant anti-hyperglycemic potential at an interval of 210 min. In another study, the methanolic extract of *Albizia lebbeck* was found to have anti-hyperglycemic effect (Patel et al., 2015), while the present results

of *Albizia lebbeck* also coincides with the previously reported work, showing it is good in reducing blood glucose level. Previously reported studies investigated that various members of the family Fabaceae such as *Acacia modesta* Wall, *Sutherlandia frutescens*, *Pterocarpus marsupium* Roxb, *Dalbergia sissoo* have shown analgesic effects (Ahmad et al., 2016). In a study reported, the extract prepared by the mixture of ethyl acetate, methanol and petroleum ether of *Albizia lebbeck* had shown significant analgesic potential with 52.4% inhibition of writhing (Saha and Ahmed, 2009) and the methanolic extract of *Psoralea corylifolia* possesses significant analgesic potential (Kumar et al., 2015). This study is significant in this regard as results demonstrated that *Albizia lebbeck* showed maximum (100 %) analgesic effect in comparison with others, while *Psoralea corylifolia* and *Trifolium indicum* showed significant analgesic potential. *Albizia lebbeck* is found to have shown excellent anxiolytic properties as reported previously (Mishra et al., 2010) and *Psoralea corylifolia* contains anti-depressant potential (Mahajan et al., 2022). The results of the present investigation are significant as it showed that the rats treated by methanolic extract of *Albizia lebbeck* showed powerful anti-anxiety activity with 42.30% time spent in open arm while, *Psoralea corylifolia* was found to have significant anti-anxiety effect (26.50% time spent in open arm). The present results are different from previously reported literature due to differences in methodologies, extraction techniques and plant sources, which may affect the results. Additionally, the age and growth conditions of the plants, as well as the geographical location can influence the phytochemical and mineral composition and ultimately biological activity of the plants (Szakiel et al. 2011). Therefore, it is important to evaluate the biological potential of plant extracts using various methods and compare the results to previous studies to identify any inconsistencies.

CONCLUSION

Chemical and biological analysis of selected medicinal herbs including *Albizia lebbeck*, *Psoralea corylifolia*, and *Trifolium indicum* revealed the presence of bioactive compounds and minerals which are imparting biomedical properties. *Psoralea corylifolia* and *Trifolium indicum* exhibited impressive antioxidant potential. *Albizia lebbeck* found to contain impressive anti-inflammatory effects, while *Psoralea corylifolia* showed better anti-hyperglycemic effects. These herbs also showed pain reducing potential along with anti-anxiety effects. Keeping in view these results, these plants

might be used to reduce glucose level and suppress the inflammation and pain without any anxiety, but further research work is needed to evaluate their pharmacodynamics effects at molecular level.

CONFLICT OF INTEREST

All authors have read the manuscript completely and thoroughly and take responsibility for this experimental work and assure that all questions related to the correctness of this work have been thoroughly resolved. All individuals who contributed to the writing and conducting of this experimental work are named authors and all authors declare no conflict of interest.

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