INTERNATIONAL ASSOCIATION OF BIOLOGICALS AND COMPUTATIONAL DIGEST
International & Peer-Reviewed Journal
E-ISSN: 2583-3995

PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT ACTIVITY AND ANTI-BACTERIAL ACTIVITY OF LYSIMACHIA ARVENSIS VAR. CAERULEA L.

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ABSTRACT

Lysimachia arvensis var. Caerulea L. is an annual, perennial herbaceous shrub, belonging to the family Primulaceae. It is commonly called a blue pimpernel. All parts of the plant have medicinal value; it is native to the Mediterranean region but dispersed worldwide as an introduced plant. The present study analyzed the presence of secondary metabolites and also their amount. The anti-oxidant assay such as DPPH and ABTS determine the reducing agent which is present in the plant. Anti-bacterial activity checked against the Pseudomonas bacteria is a 2 mm zone of inhibition at a concentration of 1mg/ml.

Keywords: phytochemical screening, TLC, TPC TFC TTC anti-oxidant assay, Anti-bacterial activity.

1. INTRODUCTION:

The Indian medicinal plants' use of herbs and herbal extracts for their therapeutic properties can be traced to the earliest myths, traditions, and writings that were used to relieve pain and treat diseases. The well-known traditional medical system (Mamedov et al., 2012). It is a thin tiny plant that creeps on the ground with spreading stems in wild and farmer fields, on roadsides, and in gardens (Rhizopoulou et al., 2015). Lysimachia arvensis, which has spread throughout the world, most likely originated in the Mediterranean region (Chrtek and Osbornova et al., 1986).

The study was to describe the morphological characteristics of the petals of L. arvensis and to comprehend how floral tissues expanded in the field and adapted to the abiotic environment. In the spring, which is the primary flowering season in the Mediterranean region, Lysimachia arvensis blooms by displaying bluish flowers in the open field (Harris 2007; Rhizopoulou et al., 2012).

Around the world, in today's global market, more than 50 major drugs originated from tropical plants (Mamedov, 2016). India is a vast repository of medicinal plants that are used in traditional medical treatment. The various indigenous systems such as Siddha, ayurveda, unani, and Allopathy use several plant species to treat different ailments (Varma and Singh 2008). A practitioner known as an herbalist uses medicinal plants as their main form of treatment for patients or clients. An herbalist may also use other therapies like diet medications, or meditation in addition to herbal medicine (Chakraborty et al.,2015)

The present study shows the screening of secondary metabolites and also determines the antioxidant activity. Phytochemical screening shows the presence of phenol, flavonoids, and alkaloids in the majority. These phytochemicals show antibacterial activity against pseudomonas bacteria. The TLC profile displays the separation of various secondary metabolites.

2. MATERIAL AND METHODS:

2.1 Sample Collection: The fresh leaves of Lysimachia arvensis var. Caerulea were collected in January 2023 from Khiloda, Shamlaji, Aravalli district, Gujarat, India. The plant herbarium is authenticated by the Department of Botany, Gujarat University. The voucher specimen was deposited at the herbarium of the Botany department, at Gujarat University.

2.2 The qualitative assay: The different secondary metabolites such as alkaloids, carbohydrates, glycosides, saponin, phytosterols, phenols, tannins, flavonoids, proteins, amino acids, diterpenes, triterpenes, steroids, lactones, flavonoids glycosides are tested by using the standard method of (Harborne, 1973).

2.3 Quantitative assay: Several quantitative techniques are used to measure the estimation of secondary metabolites. Secondary metabolites aid in adaptation and defence against several stress-related diseases.

2.3.1 Total Phenol Content: Total phenolic content was determined using the Folin-Ciocalteu reagent, according to the modified method of Medina (Medina et al., 2021). The powdered extracts of the plant were dissolved in methanol to obtain a concentration of 10 mg/10 ml. 1 ml solution was taken from this solution and given dilution up to 10 ml (100µg/ml of stock solution) with the same solvent. Consider it as a stock solution. From this stock solution make concentrations of 200 µg/ml follow the same procedure for standard. Gallic acid is used as a standard. 1 ml of Folin-Ciocalteu reagent was added to these concentration samples. The mixture was kept for 5 min and added 4 ml of 20% w/v sodium carbonate solution. The volume was made with double distilled water. The mixture was kept for 30 min until the blue color developed. The absorbance of the blue color developed which was recorded at 750 nm in a UV spectrophotometer.

2.3.2 Total Flavonoid Content: The TFC content was estimated by the standard method of kamtekar et al., 2014. Total flavonoid content was measured using an aluminum chloride colourimetric assay. Place an aliquot of 1 ml and 1 ml of Quercetin standard solution (0.02, 0.04, 0.06, 0.08, 0.1 µg / ml) in a test tube, 4 ml of distilled water and 0.3 ml of 5% sodium nitrite solution, respectively. After 5 minutes, 0.3 ml of 10% aluminum chloride was added. In the 6th minute, 2 mL of 1M sodium hydroxide was added. Finally, the volume was reduced to 10 ml with distilled water and mixed well. The orange yellowish color was developed. The absorbance was measured at 510 nm using a spectrophotometer. The blank was performed using distilled water Quercetin using as standard.

2.3.3 Total Tannin Content: Total tannin content measured by standard method of folin-denis reagents. Plant samples (200 µl from stock 1 mg/ml) and a series of standard Tannic acids (0-1000 µl from stock 1 mg/ml in methanol) made 1 ml final volume using methanol. 0.1 ml Folin-denis reagent was mixed with extract, here the folin-denis reagent was diluted with water in a ratio of (1:10). After the addition of water 7.5% (w/v) sodium carbonate was added and made final volume up to 3 ml using distilled water. The absorbance of standard and samples for tannin was recorded at 750 nm using UV-Vis Spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto Japan). The results of TPC are expressed as mg of Tannic acid equivalents per gram (mg GAE/g) extract (Vala and Maitreya, 2019).

2.4 Thin Layer Chromatography (TLC): Thin Layer Chromatography is one of the chromatographic analysis methods. The plant sample was analyzed by TLC methods. This technique uses glass or aluminum plates coated with silica gel G-60 (1-1.5 mm thick layers). Aliquots (50 µL) of solution of the extract plant sample were applied separately to each plate. Leaves extract was run in 14 different solvent systems which are used as the mobile phase. Ethyl acetate: Hexane (20%: 80%), Ethyl acetate: Hexane (30%: 70%), Methanol: Chloroform (20%: 80%), Methanol: Chloroform (30%:70%), Methanol: Acetone (10%: 90%), Methanol: Pet ethyl (20%:80%), Methanol: Pet ethyl (30%:70%) give the best separation. The finalization of the solvent system develops the TLC Plated and derivatization of P-Anisaldehyde reagents. After spraying the TLC plates were observed under the UV short wavelength (245 nm) and UV Long wavelength (365 nm). The Rf values of spots were calculated by following this formula (Akhtar et al., 2019).

2.5 Antioxidant activity: Antioxidants are chemical or natural compounds that can help to prevent cell damage (Sen., 2001). Antioxidants are molecules that are stable enough to donate electrons to the growing free radicals to neutralise them and reduce their damaging potential (Tsao et al., 2004). Antioxidants act as hydrogen donors, radical scavengers, peroxide decomposers, singlet oxygen quenchers, synergists, enzyme inhibitors, and metal-chelating agents (Lobo et al., 2010). Here, a study of the antioxidant activity of Lysimachia arvensis plants was carried out using different antioxidant methods.

2.5.1 DPPH assay: Total antioxidant capacity (measured as free radical scavenging activity) was evaluated using a stable free radical DPPH following the method described by (Patel and Ghane, 2022) with slight modifications. However, 1 mg leaf extract with 1 ml methanol. The standard and plant extract series were produced in triplicates with 1 ml extract in each test tube at concentrations ranging from 0 to 600 mg/ml. Ascorbic acid is used as a standard. Due to its light sensitivity, DPPH solution was newly made by dissolving 4 mg DPPH powder into methanol (DPPH is water-insoluble) and maintained in a dark area. 3 ml DPPH solution was added to the produced series of extracts with various concentrations and incubated for 20-30 minutes. After a time of incubation, the purple solution that resulted from the addition of DPPH will be pale yellowish. This colour shift is a clear indication of the extract's ability to scavenge free radicals. The absorbance of these incubated colour-altered extracts was measured using a spectrophotometer at 517 nm, and the results were computed using the ascorbic acid standard curve. Using the equation below, the radical scavenging activity was determined using absorbance data,

% Inhibition= [{A control - A sample}/A control] × 100

Where A control indicates absorbance of control containing 3 ml of DPPH and 1 ml of methanol. A sample is the absorbance of the sample. Due to the high concentration, the sample also absorbs at this wavelength, so it is required to perform the blank measurements.

2.5.2 ABTS assay: The ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity was measured by Patel and Ghane, 2022. Plant extract (0-200 µg/ml) and standard Trolox (0-200 µl from stock 4 mg/ml) were taken into a test tube and made the final volume of 1 ml with the help of methanol. 3 ml ABTS reagent was added and then the mixture was incubated for 30 min. The absorption was taken at 730 nm against the blank (methanol without ABTS reagent).

The results were expressed as a percentage of $ABTS^{\bullet+}$ scavenging according to the following formula: % Inhibition= [{A control - A sample}/A control] × 100

Where A control = absorbance of the ABTS solution without extract; A sample = absorbance of the ABTS solution with plant extract.

2.6 Anti-Bacterial activity: The bacterial culture of Pseudomonas was collected from the department of Microbiology and Biotechnology, school of science, Gujarat University. To estimate the antibacterial activity of particular parts of plants we are using the Agar well diffusion methods. First, 6.25g nutrient broth was dissolved in 250 ml distilled water mixed in a conical flask. After that, the media was sterilized with the help of an autoclave. After 30 min of sterilization, the flask was carefully taken out of the autoclave. 20-25 ml of nutrient agar media was poured into the sterilized Petri dish. This whole process was done in a laminar airflow cabinet in between two spirit lamps. Then after, it was allowed to solidify at room temperature for 24 hours. The Pseudomonas bacteria were inoculated on a nutrient agar plate with the help of an inoculating loop. Nutrient agar plates were prepared with methanolic and petroleum ether extract from the stem and leaves. Using the cork borer several wells of 2.5 mm in diameter were punched. An equal volume of methanolic and petroleum ether extract with particular concentrations was poured into wells. Then the plates were incubated at 37°C for 24 hours. The inhibition zone was measured with a zone scale. (Pandya et al., 2019).

3. RESULTS AND DISCUSSION:

3.1 Qualitative analysis of phytochemicals was performed to check the presence of certain Phytochemical constituents in leaves of Lysimachia arvensis L.

Sr.No	Phytochemical	Test	Methanol	Hexane
1	Alkaloids	Dragendroff's Test	-	-
		Mayer's Test	+	-
		picric acid Test	-	-
2		Lead acetate Test	+	-

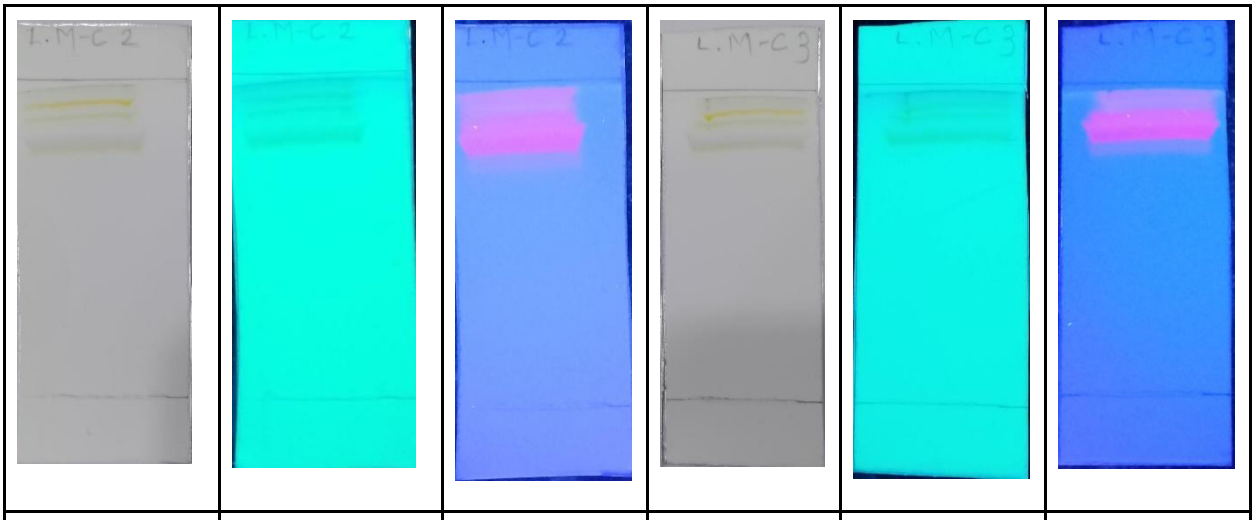
	Flavonoids	Shinoda's test/ Mg- hydrochloride reduction Test	-	-
		Ferric chloride Test	-	-
		Ammonia Test	+	+
		Conc. H2so4 Test	-	-
3	**Phenols**	Gelatin Test	+	+
		Lead acetate Test	+	+
		Ferric chloride test	++	+
		Potassium dichromate Test	+	+
		Iodine Test	-	+
4	**Tannins**	Gelatin Test	+	+
		Braymer's Test	+	+
		10% NaOH Test	-	-
5	**Saponins**	Froth Test	++	+
6	**Terpenoids**	Salkowski Test	+	-
		Copper acetate Test	+	+
7	**Glycosides**	Borntrager's Test	+	-
		Legal's Test	+	+
		Keller-Killiani Test	-	-
8	**Proteins**	Millon's Test	+	+
		Biuret Test	-	-
9	**Phytosterols**	Liebermann Burchard's Test	+	-
		Salkowski Test	+	-

The table represents the presence of phenol, and flavonoid, Tannin, Saponins, glycoside, Terpenoids present in both extracts of Lysimachia arvensis L. leaves; Alkaloids, phytosterol were present in the methanolic extract.

3.2 Thin Layer Chromatography (TLC):

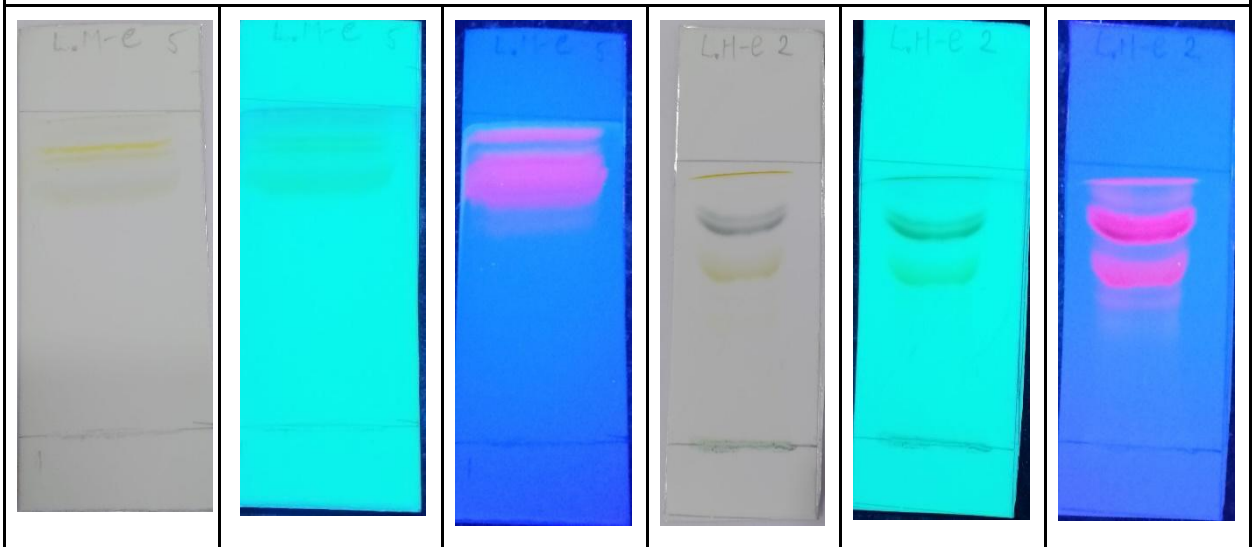
Sr No	**Plant name**	**Solvent system (Methanol: Chloroform)**	**Total band**	**Rf value**
1	Lysimachia arvensis	8:2	4	0.90,0.84,0.80, 0.76
		7:3	3	0.91, 0.87, 0.81
Methanol: Acetone				

2	Lysimachia arvensis	9:1	4	0.93, 0.88, 0.84, 0.73
Hexane: Ethyl acetate				
3	Lysimachia arvensis	8:2	4	0.85, 0.72, 0.57, 0.52



Daylight	UV-256 nm	UV-360 nm	Daylight	UV-256 nm	UV-360 nm

Fig. 1 Methanol Leaves Extract TLC Images of Lysimachia arvensis var. Caerulea L. in Visible Light, Short Wavelength and Long Wavelength of UV.



Daylight	UV-256 nm	UV-360 nm	Daylight	UV-256 nm	UV-360 nm

Fig. 1 Hexane Leaves Extract TLC Images of Lysimachia arvensis var. Caerulea L. in Visible Light, Short Wavelength and Long Wavelength of UV.

3.3 Quantification of Secondary Metabolites:

Phenol, Flavonoid, Tannin, and Terpenes are the most common and useful chemicals of the plant. It acts as an anti-oxidant agent, plays a role in pigmentation, helps in pollination, and protects against herbivory (Anokwuru et al., 2011). The total phenolic content estimated in

the methanolic extract is 10.56±2.54 GAE/g, the total flavonoid content is 36.38±5.83 QE/g and the total Tannin content is 11.03±0.41 TAE/g.

3.4 Anti-oxidant activity:

The highest radical scavenging activity in the DPPH assay is 34.43% shown at 600 µg/ml concentration of methanolic extract of the plant. The highest radical scavenging activity in ABTS assay is 77.90% shown at 200 µg/ml concentration of methanolic extract of plant.

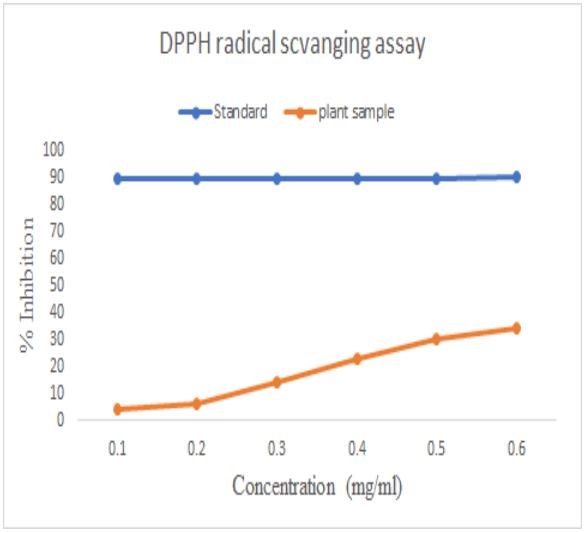


Fig. 1 Comparison of % inhibition of standard (Ascorbic acid) and plant extract

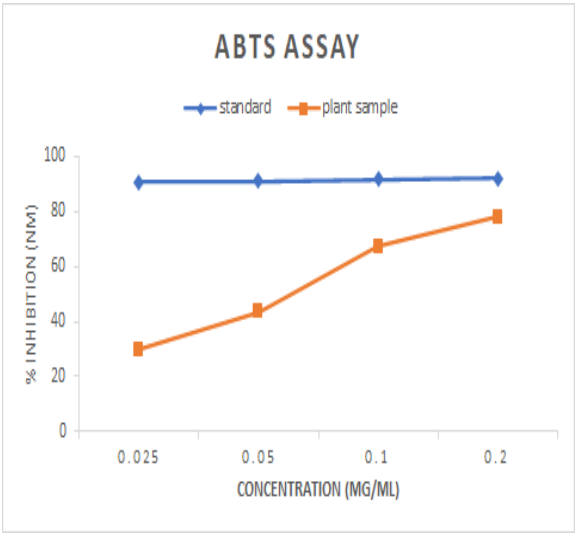


Fig. 2 Comparison of % inhibition of standard (Trolox) and plant extract

3.5 Anti-bacterial activity:

The methanolic extract of leaves is used for estimate the zone of inhibition in bacterial growth area.

Leaves Extracts		**Zone of inhibition (mm)**			
		1mg/ml	**5mg/ml**	**10mg/ml**	**15mg/ml**
Solvent	Methanol	0.6mm	2mm	0.5mm	1mm
	Hexane	0.0mm	0.0mm	0.0mm	0.0mm

The above table shows the maximum inhibition concentration is 5 mg/ml. The maximum zone of inhibition is 2mm in the methanolic extract of leaves.

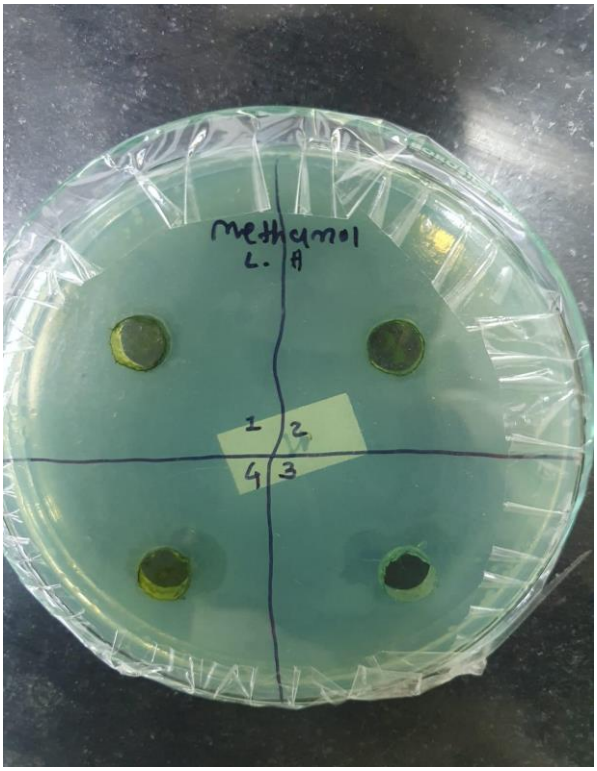


Fig 3: Antibacterial activity in Methanol extract

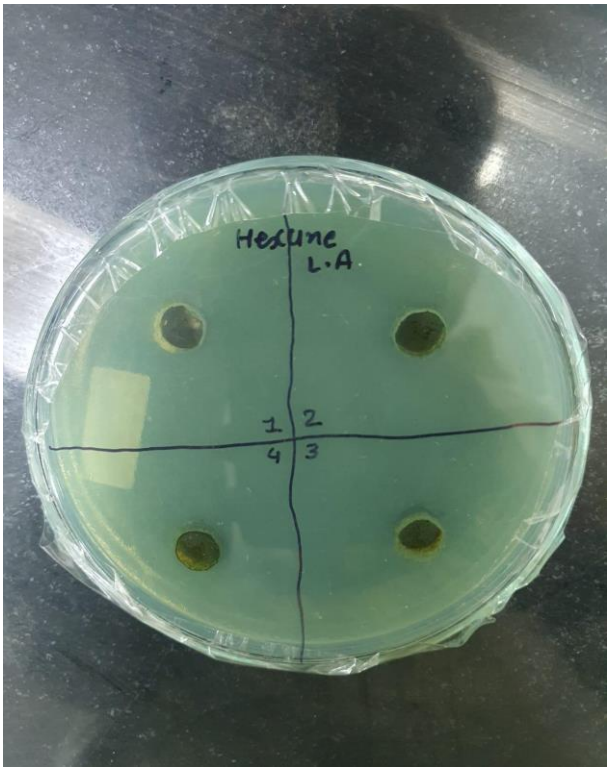


Fig 4: Antibacterial activity in Hexane extract

DISCUSSION

The goal of the current experiment was to identify the TPC and TFC levels in various leaf solvent extracts (Samatha et al., 2012). The highest concentration of phenolics and flavonoids is 4.27mg GAE/g and 0.25 mg QE/g found in methanol extract (Tepal, 2016). The present study of the Lysimachia arvensis plant observed the highest TFC Value at 36.38±5.83 QE/g and the TPC value is 10.56±2.54 GAE/g. Lopez et al., 2008 show the DPPH radical scavenging assay of the methanolic extract value 113.39±8.82% observed in Lysimachia arvensis. The present study estimated the DPPH radical scavenging value of 34.43±0.01% in leaves of Lysimachia arvensis.

CONCLUSION

The Present work shows that many phytochemical and anti-oxidant agents are present in leaf extract of Lysimachia arvensis L. Plants have great medicinal properties which are useful in curing many diseases. Many phytochemicals are useful in preparing the drug and medicinal in the pharma industries. Plants provide different secondary metabolites like alkaloids, saponins, tannins, terpenoids, sterols, steroids, flavonoids, and phenols. In addition, a high correlation between the antioxidant capacities of the plant and their total phenolic contents was found. This study also successfully identified the high capacity of method total phenolic content, total flavonoid contains ABTS activity and DPPH activity shown in Lysimachia arvensis L. This plant also presents various phytochemical studies into mainly major activity shown against microorganisms. Antibacterial activity checked against Pseudomonas bacteria is 2 mm (zone of inhibition) with methanolic extract 1mg/ml.

ACKNOWLEDGMENT

We are thankful to the Head of the Department for providing instrument facilities and support. We are also grateful to Prof. Dr. Bharat Maitreya and Dr. Himanshu Pandya for guiding this research work.
work.

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