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PHYTOCHEMICAL ANALYSIS, ANTI-OXIDANT ACTIVITY AND ANTI-BACTERIAL ACTIVITY OF SALVADORA OLEOIDES L.

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ABSTRACT

Salvadora oleoides L. is a facultative and mesomorphic xerophyte adapted to arid and semi-arid regions. It is commonly known as meetha jal in Gujarat and Rajasthan. These plant species populations were grown in different ecological regions such as Punjab, Rajasthan, and Kutch. 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 plant. Anti-bacterial activity checked against the pseudomonas bacteria is 5 mm (Zone of inhibition) at concentration of 1 mg/ml.

Keywords: Phytochemical screening, TLC, TPC, TFC, Anti-oxidant assay, Anti-bacterial activity

1. INTRODUCTION:

Indian desert plants are stress-tolerant that can sustain under extreme environmental conditions. Salvadora olioides L. is a versatile plant that provides oil, and bioactive compounds with high ecological importance. The plant extract plays a vital role in producing new medicinal compounds. The fruit, branches, bark, leaves, and stem are used for extraction for the determination of secondary metabolites. Phenol, flavonoid and alkaloid are the major secondary metabolites.

Salvadora genus belongs to the family Salvadoraceae, this family has 3 genera and 12 species which are distributed in various western regions of Gujarat state. Salvadoraceae family plants grows naturally by seed germination and is one of the dominant tree species in the vast area of Kutch (northern saline desert). It is considered a shrub or tree, 4-10 m in height with a twisted trunk the plant had a deep root system and was usually known as xerophytes and facultative halophytes with high tolerance to salinity. It is commonly known as jhal, badapilu, pilu, vridh pilu and khakan (Razzaq et al., 2022). The leaves of Salvadora oleoides L. help in possess anti-inflammatory, analgesic, and antiulcer activity, relieve cough and it is also effective in controlling blood glucose levels and improving lipid profile in euglycemic and anti-diabetic conditions and for treatment of enlarged spleen and fever (Kumar et al., 2012). These young branches and leaves are also favorite fodder for Camal because of the high-water content of 15-36%

According to the world health organization (WHO), About 70 percent of the world's population relies on plants for their primary healthcare and some 35,000 to 70,000 species have been used as medicaments a figure corresponding to 14-28% of the 250,000 plants species estimated to occur around the world, today's global market, more than 50 major drugs originated from the tropical plant (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 & Singh 2008). The Indian ayurvedic system has included herbals as one of the most powerful healing ingredients, which are recorded in the Vedas Samhita such as Dhanvantari, Sushruta Samhita (Parasuraman et al., 2014).

The present study shows the quantity of secondary metabolite 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 plants were collected in Dec 2022 from Mandvi, a town in the Kutch 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 Qualitative analysis: The secondary metabolites such as alkaloids, carbohydrates, glycosides, saponin, phytosterols, phenols, tannins, flavonoids, proteins, amino acids, diterpenes, triterpenes, steroids, lactones, flavanols and glucosides are tested by using the standard method of Harborne, 1998.

2.3 Quantitative assay: The estimation of secondary metabolites is measured by different quantitative methods. Secondary metabolites help for protection and adaption against some stress conditions.

2.3.1 Total Phenol Content (TPC): Total phenolic content was determined using Folin-Ciocalteu reagent, according to the method described by Rossi and Singleton (Singleton et al., 1965). 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 give dilution up to 10 ml (100µg/ml of stock solution) with the same solvent. Consider it as a stock solution. From this stock, the solution makes different concentrations of 200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml, and 1000 µg/ml, following the same procedure for standard. Gallic acid is used as a standard. 1 ml of Folin-Ciocalteu reagent was added in these concentration samples. The mixture was kept for 5min and add 4ml 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 develops. The absorbance of the blue color developed which was recorded at 765 nm in a UV spectrophotometer. The % of total phenolic was calculated from the calibration curve of Gallic acid plotted by using a similar process.

2.3.2 Total Flavonoid Content (TFC): The TFC content was estimated by the standard method of Kamtekar et al., 2014. Total flavonoid content was measured using an aluminum chloride colorimetry assay. Place an aliquot of 1 ml and 1 ml of Quercetin standard solution (0.2-1 mg/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. On 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. The samples were performed in triplicates.

2.4 Thin Layer Chromatography (TLC): Thin Layer Chromatography is one of the chromatographic analysis methods. The leaves extract 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 (5 µL) of solution of the extract plant sample were applied separately to each plate. Ethyl acetate: Hexane (10%: 90%), Ethyl acetate: Hexane (30%: 70%), Methanol: Chloroform (10%: 90%), Methanol: Chloroform (15%: 85%), Methanol: Chloroform (50%: 50%). For elution, 5 mobile phases were analyzed but Ethyl acetate: Hexane (20%: 90%) shown the best separation.

2.5 Anti-oxidant activity: Antioxidants are molecules that are stable enough to donate electrons to the growing free radicals to neutralize them and reduce their damaging potential (Tsao et al., 2004). Antioxidants can be defined as "elements that, when present at low concentrations compared to the concentration of an oxidizable substrate, significantly delay or reduced the oxidation of that substrate" (Antolovich et al., 2002). Antioxidants act as hydrogen donors, radical scavengers, peroxide decomposers, singlet oxygen quenchers, synergists, enzyme inhibitors, and metal-chelating agents (Lobo et al., 2010). Some plants are known to have antioxidant properties due to the presence of certain phytochemicals (Engwa, 2018). All in vitro models and in vivo methods have been developed to assess antioxidant activity. However, the interpretation of the results of these model systems should be treated with caution. This is because different methods rely on different mechanisms and therefore have significantly different antioxidant activities (Tsao et al., 2004). Here, a study

of the antioxidant activity of Salvadora plants was carried out using different antioxidant methods.

2.5.1 DPPH assay: DPPH radical scavenging assay assesses the antiradical properties of a different compound. Total antioxidant capacity (measured as free radical scavenging activity) was evaluated using a stable free radical DPPH following the method described by (Germano et al., 2002) 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 50 to 250 mg/ml. Ascorbic acid is used as a standard. Due to its light sensitivity, the DPPH solution was newly made by dissolving 4 mg DPPH powder into 100 ml 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 shift in color is a clear indication of the extract's ability to scavenge free radicals. The absorbance of these incubated color-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 recorded absorbance. This formula is derived by leh and duh, 1994.

% Inhibition= [{Acontrol - Asample}/Acontrol] × 100

Where Acontrol indicates absorbance of control containing 3 ml of DPPH and 1 ml of methanol. Asample 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 measurement.

2.5.2 ABTS radical scavenging assay: The ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radicle 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= [{Acontrol - Asample}/Acontrol] × 100

Where Acontrol = absorbance of the ABTS solution without extract; Asample = 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. For the antibacterial activity of particular parts of plants, the Agar well diffusion method was used. First, 6.25 g 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 in to 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 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℃ for 24 hours. The inhibition zone was measured with a zone scale. (Dhruv Pandya et al., 2019).

3. RESULTS AND DISCUSSION:

3.1 Qualitative analysis of phytochemicals was performed to analyze the presence of certain Phytochemical constituents in leaves of Salvadora oleoides L.

Sr. No.	Phytochemicals	Test	Methanol Extract	Hexane Extract
1.	Alkaloids	Mayer	-	-
		Wagner	-	+

		Dragendorff	++	-
		Hager's	+	+
2.	Carbohydrates	Molisch	-	-
		Benedict	+	-
		Fehling	+	-
3.	Glycosides	Modified Borntrager	-	-
		Legal	-	-
		Keller Killiani	-	-
		NaOH Reagent	+	-
4.	Saponins	Froth	++	-
		Foam	++	-
5.	Phytosterols	Salkowski	-	-
		Liebermann Burchard	-	-
6.	Phenols	Ferric Chloride	++	-
		Lead Acetate	++	+
7.	Tannins	Gelatin	+	-
8.	Flavonoids	Alkaline Reagent	++	+
		Lead Acetate	+	+
9.	Proteins	Xanthoproteic	+	-
		Millon's	-	-
		Biuret	+	-
10.	Amino Acids	Ninhydrin	-	-
11.	Diterpenes	Copper Acetate	-	+
12.	Triterpenes	Salkowski	+	-
		Tschugergen	+	-
13.	Steroids	Salkowski	-	-
		Liebermann Burchard	-	-
14.	Lactones	Legal	+	+
		Baljit	+	+
15.	Flavanol Glucosides	Mg and HCl Reduction	-	-

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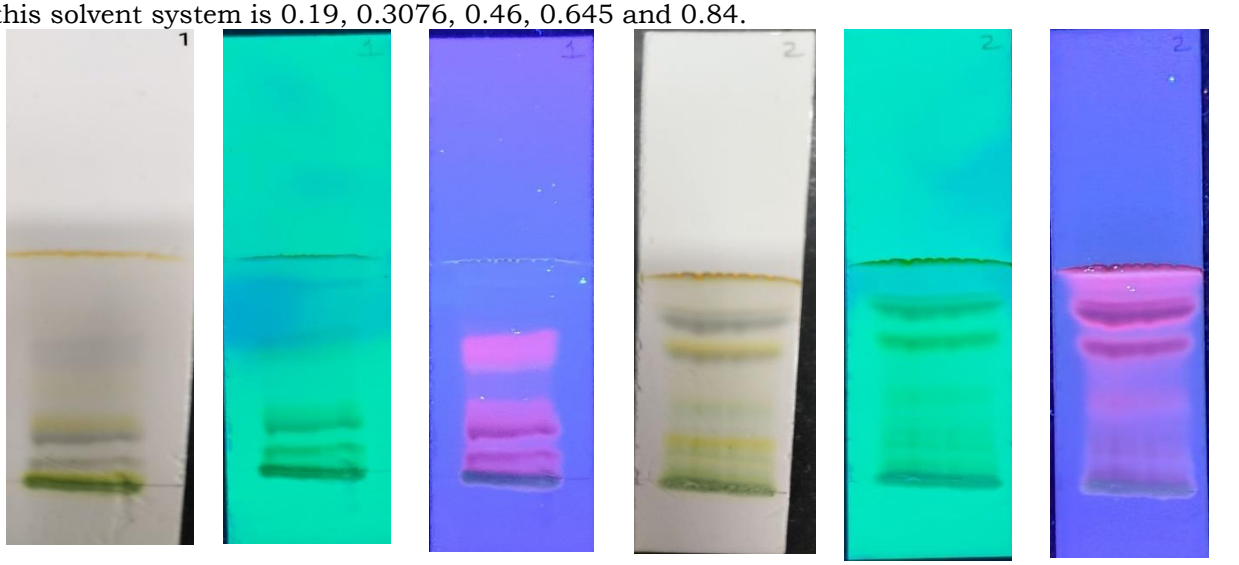
The table represents the presence of alkaloids, phenol, and flavonoid, present in both extracts of Salvadora oleoides L. leaves; carbohydrates, protein, saponins, and tannin were present in methanolic, extracts; Flavanol glucoside, steroid, amino acid and phytosterol not present in any extract.

3.2 Thin Layer Chromatography (TLC):

The best solvent system for hexane extract is Ethyl acetate: Hexane (2:8). The RF values of this solvent system is 0.19, 0.3076, 0.46, 0.645 and 0.84.



Visible Light | UV 254 nm (Short Wavelength) | UV 365 nm (Long Wavelength) | Visible Light | UV 254 nm (Short Wavelength) | UV 365 nm (Long Wavelength)

Fig. 1 Hexane Extract TLC Images of Salvadora oleoides L. Leaves in Visible Light, Short Wavelength and Long Wavelength of UV.

3.3 Quantification of Secondary Metabolites:

Phenol, Flavonoid, and Terpene is the most common and useful chemical of the plant. It acts as an anti-oxidant agent, role in pigmentation, helps in pollination, and protects against herbivory (Anokwuru et al., 2011). The total phenolic content estimated in the methanolic extract is 0.657±0.2 mg/ml and the total flavonoid content is 0.037±0.008 mg/ml.

3.4 Anti-oxidant activity:

The highest radical scavenging activity in DPPH assay is 37.79% shows at 100 µg/ml concentration of methanolic extract of plant. The highest radical scavenging activity in ABTS assay is 90% shows at 200 µg/ml concentration of methanolic extract of plant.

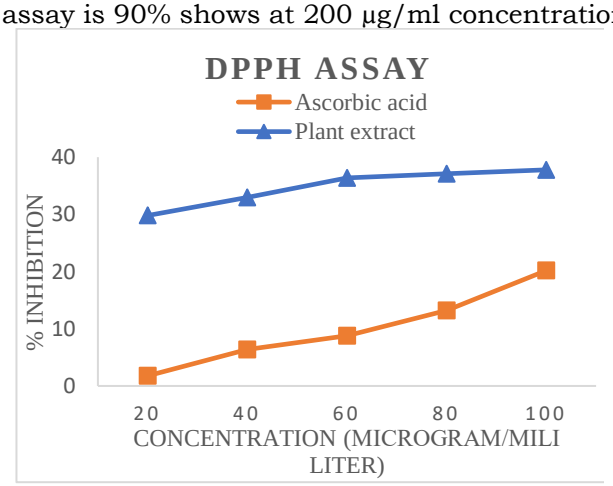


Fig. 1 Comparison of % inhibition of standard (Ascorbic acid) and plant extract

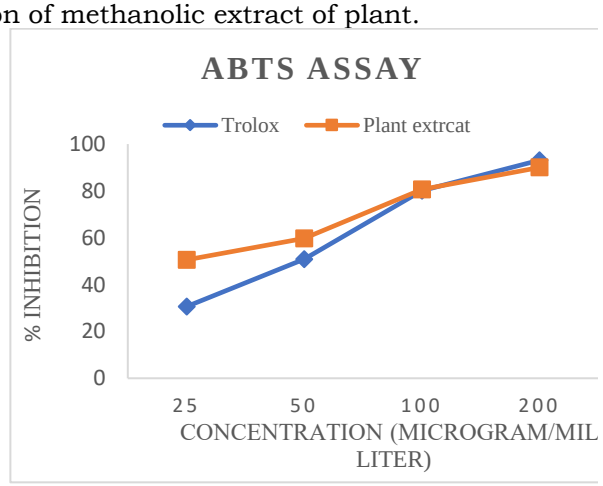


Fig. 2 Comparison of % inhibition of standard (Trolox) and plant extract

3.5 Anti-bacterial activity:

Part of	Extracts	Zone of inhibition(mm)

plant		1mg/2ml	5mg/2ml	10mg/2ml	15mg/2ml
Leaf	Methanol	5mm	2mm	3mm	0.2mm
	Hexane	0.0mm	0.0mm	0.0mm	0.0mm

The above table shows the maximum inhibition concentration is 1 mg/ml. The maximum zone of inhibition is 5 mm in methanolic extract of leaves.

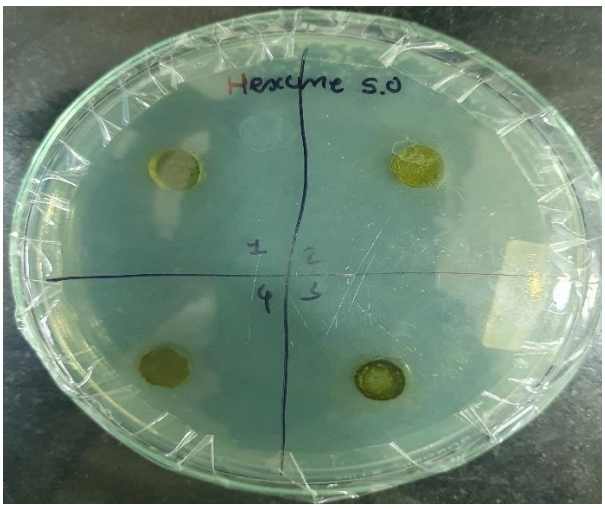


Fig 2: Antibacterial activity in Hexane extract

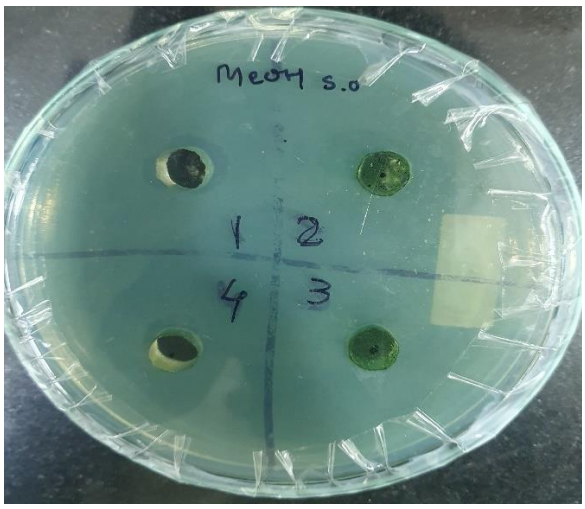


Fig 3: Antibacterial activity in Methanol extract

DISCUSSION

Ibrahim et al., 2022 the flavonoid contains a rich function obtained after purification of highest TFC value 358.88 ± 0.12 mg/g and TPC (180.82 ± 0.82 mg/g) value are observed. The present study observed highest TFC Value is 37.13 ± 0.008 mg/g and TPC value is 522.76 ± 0.2 mg/g. Hence, they are responsible for increasing the structural framework of these living organisms. Plant body produce phytochemicals as secondary metabolites works with dietary fibres and nutrients to defend against pathogenic effects (Lakho et al,2021).

Kanam et al., 2022 shows the DPPH radical scavenging assay of the methanolic extract value 46.90 ± 0.34 % detected in leaves of Salvadora oleoides L. The present study determined the DPPH radical scavenging value 20.17 % in leaves extract. Kumari and Parida, 2016 author observed ABTS activity value 4.5µg/ml and the present study of Salvadora oleoides L. plant ABTS value is 90%. So, this plant has higher antioxidant potential.

Noumi et al., 2011 checked antimicrobial activity against E. coli bacteria showed the minimum inhibition zone is 8 mm and the present study determine the antibacterial activity is 5 mm (minimum inhibition zone) in methanolic extract, thus Salvadora oleoides L. plant has good antibacterial activity.

CONCLUSION

The present work provides qualitative and quantitative analysis of phytochemicals are present in leaves of Salvadora oleoides L. Plants have great medicinal properties which are useful in curing many diseases. Many phytochemicals are useful in preparing the drug and medicinal in pharma industries. Plants provide different secondary metabolites like alkaloids, saponins, tannin, terpenoids, sterol, steroids, flavonoids, phenols. The results indicated that these two traditional extract methods could effectively extract antioxidants from Salvadora oleoides L. 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 ABTS activity and DPPH activity are shown in Salvadora oleoides L. This plant also shown. Antibacterial activity against microorganism which is determine against Pseudomonas bacteria The zone of inhibition is 5 mm in methanolic extract at concentration 1mg/ml.

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