



INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X

Impact factor: 4.295

(Volume3, Issue1)

Available online at: www.ijariit.com

Preliminary Studies on Antidiabetic, Antimicrobial and Antioxidant Activities of Rare Medicinal Plant *Epaltes divaricata* (Linn.)

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Abstract: Pharmaceutical industries are still in the hunt of effective scavengers for free radicals from the unexplored medicinal plants. About 80,000 species of plants are utilized for treating various diseases in different systems of Indian medicine. Many pharmaceutical companies giving importance in plant-derived drugs mainly due to the current widespread belief that 'Green Medicine' is safe and more dependable than the costly synthetic drugs, which have adverse side effects.

The objective of the study is isolation of Phytochemical active constituents, Antidiabetic, antimicrobial and antioxidant activities of the rare Antidiabetic medicinal plant *Epaltes divaricata* (Linn.) since the selected plant has varied medicinal properties used in Ayurveda. This valuable plant is used in traditional Ayurvedic medicine to alleviate jaundice, diabetes mellitus, urethral discharges and acute dyspepsia. It is also regarded as a diaphoretic, diuretic and a stimulating expectorant.

The methanol extract of *Epaltes divaricata* L. showed excellent antimicrobial activity against bacteria and fungi. Phytochemical analysis was carried out for the same extract by two different standard methods and which confirmed the presence of steroids, triterpenoids and phenolic compounds. Decreasing of postprandial hyperglycemia is a therapeutic approach for treating diabetes mellitus. This can be achieved in current trends through the inhibition of carbohydrate hydrolyzing enzymes such as alpha glucosidase and alpha amylase. Agents with α -amylase and α -glucosidase inhibitory activity are very useful as oral anti hypoglycemic agents for the control of hyperglycemia in patients who have diabetes mellitus. In this study the methanol extract exhibited above 50% of inhibition in all standard concentration.

Keywords: *Epaltes divaricata* L., Clinical bacterial cultures, Fungal strains, α -Amylase, α -Glucosidase, Phytochemical, Antioxidant.

I. INTRODUCTION

As per the World Health Organization (WHO) report, 80% of the world population presently uses herbal medicine for some aspect of primary health care. People in developing countries utilize traditional medicine for their major primary health care needs. Moreover, higher plants [4] produce hundreds to thousands of diverse chemical compounds with different biological activities. It is also believed that these compounds have an important ecological role. They can work as pollinator attractants and as chemical defenses against insects, herbivores and microorganisms. These antimicrobial compounds [13] produced by plants are active against plant and human pathogenic microorganisms.

Diabetes mellitus is a metabolic disorder of the endocrine system. The disease is found in all parts of the world and is rapidly increasing worldwide. People suffering from diabetes cannot produce or properly use insulin, so they have high blood glucose. Type 2 diabetes non-insulin-dependent diabetes mellitus, in which the body does not produce enough insulin or properly uses it, is the most common form of the disease, accounting for 90 % - 95 % of cases. The case of diabetes is a mystery, although both genetic and environmental factors such as obesity and lack of exercise appear to play a role. Diabetes mellitus is an endocrine disorder characterized by hyperglycemia is associated with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. A therapeutic approach for treating diabetes is to decrease postprandial hyperglycemia. This can be achieved through the inhibition of carbohydrate hydrolyzing enzymes such as alpha glucosidase and alpha amylase. Alpha amylase and glucosidase inhibitors are drug-design [9] targets in the development of compounds for the treatment of diabetes, obesity and hyperlipaemia. Plants have long been used for the treatment of diabetes. Ethno botanical information indicates that more than 800 plants are used for the treatment of diabetes throughout the world.

***Epaltes divaricata* L.**

It is an annual plant belonging to asteraceae family and mostly survives near to water sources. It is not confined to wetlands but often found in wet places, even in standing water in ditches, marshes and rice fields. *Epaltes divaricata* L. is used in traditional ayurveda medicine to alleviate jaundice, urethral discharges and acute dyspepsia. It is also regarded as a diaphoretic, diuretic and a stimulating expectorant. The species is recorded from South and Southeast Asian countries like Myanmar, Southern China (Hainan), Vietnam, Srilanka and India. In India it is found in Tamil Nadu, Kerala, Karnataka, and Andhra Pradesh.

II. MATERIALS AND METHODS

Chemicals and Reagents

DPPH (1, 1-Diphenyl, 2-Picryl Hydroxyl), NBT (Nitro Blue Tetrazolium), NADH (Nicotinamide Adenine Dinucleotide Phosphate), PMS (Phenazine Metho Sulphate), TCA (Trichloro Acetic Acid), ferric chloride and BHT (Butylated Hydroxyl Toluene) were obtained from Sigma chemical co., USA. Ascorbic acid obtained from SD Fine chem. Ltd., Biosar, India. All the other chemicals were of analytical grade.

Method of extraction

Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form, and are intended for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, and tinctures. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity.

Epaltes divaricata L. plant materials were collected from the banks of Oragadam Lake, Chengalpattu, Tamil Nadu state, South India, during January to February. They were washed thoroughly and kept for shade drying after minced, then finally prepared as fine powder. The powder (65g) was extracted three times by cold percolation method with 195 ml of Hexane, Ethyl acetate and methanol at room temperature for 72 hrs. The filtrates were concentrated under reduced pressure at 40°C, crystals were obtained from methanol plant extract (**Fig-C**) and stored in refrigerator at 2–8°C for use of further experiments.

Preliminary phytochemical analysis

Preliminary phytochemical screening [11] of hexane, ethyl acetate, and methanolic extracts of *Epaltes divaricata* L. was carried out to detect the phytoconstituents [14] using standard conventional protocols.

1. Test for triterpenoids

Salkowski's test: 0.2 g of each extracts was treated with 2 ml of chloroform and filtered. The filtrate was treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicated the presence of triterpenoids.

Tshugajeu test: 0.2 g of each extracts was treated with chloroform and filtered. Excess of acetyl chloride and a pinch of zinc chloride were added, kept aside for some time till the reaction completed and then warmed on water bath. Appearance of eosin red color indicated the presence of triterpenoids.

2. Test for steroids

Salkowski reaction: To 2 ml of plant extracts, 2 ml chloroform and 2 ml concentrated sulphuric acid was added and shaken well. The chloroform layer appeared red and acid layer showed greenish yellow florescence indicating the presence of steroids.

Liebermann–Burchard reaction: 2 ml of plant extracts was mixed with chloroform. About 1–2 ml acetic anhydride and 2 drops of concentrated sulphuric acid were added from the side of test tube. First red, then blue, and finally green colours appeared indicating the presence of steroids.

3. Test for phenolic compounds

Ferric chloride test: To 1 ml of each extracts, few drops [17] of 0.5% ferric chloride solution was added. Formation of bluish black colour indicated the presence of phenolic compounds.

Antimicrobial assay

1. Antibacterial activity

Test organisms

The following clinical and reference bacterial cultures were used to test antibacterial activity by disc diffusion method, (1). *Staphylococcus aureus* ATCC25923, (2). *Klebsiella pneumonia* ATCC 15380, (3). *Enterococcus faecalis* ATCC 29212, (4). *Yersinia enterocolitica* MTCC 840, (5). *Erwinia sp* MTCC 2760, (6) *Vibrio parahaemolyticus* MTCC 451, (7). *Enterobacter aerogenes* MTCC 111, (8). *Escherichia coli* ATCC 25922 and (9). *Proteus vulgaris* MTCC 1771. All the reference cultures were obtained from IMTECH, Chandigarh, India and the clinical isolates were obtained from Department of Microbiology, Christian Medical College, Vellore, and Tamilnadu, India. An inoculum of each bacterial strain was inoculated in 3 ml of Mueller Hinton Broth and incubated at 37°C for 24 hrs in shaker at 120 rpm.

Disc diffusion method

Antibacterial activity was carried out using disc diffusion [15] method. Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (Hi-media, Mumbai). The test culture (100µl of suspension containing 10⁸CFU/ml bacteria) were swabbed (Fig-A) on the top of the solidified media and allowed to dry for 10 minutes. Tests were conducted at three different concentration of the crude extract (5, 2.5 and 1.25 mg/disc) (H1 H2 H3, E1 E2 E3 & M1 M2 M3). The loaded discs were placed on the surface of the medium and left for few minutes [18] at room temperature for compound diffusion. Streptomycin (10µg/disc) was used as a positive control. These plates were incubated for 24 h at 37°C. Zone of inhibition was recorded in millimeters.

2. Antifungal activity

Test fungal strains

The following fungi were used for the experiments: *Botrytis cinerea*, *Curvularia lunata* 46/01, *Aspergillus niger* MTCC 1344, *Trichophyton rubrum* 57/01 and *T. mentagrophytes* 66/01.

Preparation of fungal spore

The filamentous fungi [2] were grown on Sabouraud dextrose agar (SDA) slants at 28°C for 10 days. The spores were collected using sterile double distilled water and stored in refrigerator.

Antifungal assay

The antifungal activity was performed according to the standard reference method (96 well microtiter plate-based method). The extracts were dissolved in water with 2% Dimethyl Sulfoxide (DMSO). The initial concentration of the extract was 1mg/ml. The initial test concentration was serially diluted two-fold (Fig-B). Each well was inoculated and the suspension containing 10⁴ spore/ml of fungi. The antifungal agent, Fluconazole was used as positive control; MIC is defined as the lowest extract concentration, showing no visible fungal growth after incubation time.

Determination of *in vitro* α-amylase, α-glucosidase inhibition and antioxidant assays

1. α-Amylase Inhibition Assay

α-amylase inhibitory activity was determined according to the method described by Jyothi *et al.* 2011 [11]. Briefly, the total assay mixture containing 200 µl of 0.02 M sodium phosphate buffer, 20 µl of enzyme, and the plant extract [3] in the concentration range 200-1000 µg/ml were incubated for 10 minutes at room temperature [5] followed by adding of 200 µl of 1% starch in all test tubes. The reaction was terminated with addition of 400 µl of 3, 5 dinitrosalicylic (DNSA) color reagent, placed in boiling water bath for 5 minutes, cooled at room temperature and diluted with 15 ml of distilled water and the absorbance measured at 540 nm. The percentage of inhibition was calculated by using the formula.

α - Glucosidase inhibition activity

In order to investigate the inhibition activity of MeED (Methanol extract of *Epaltes divaricata* L.), an *in vitro* α-glucosidase inhibition test was performed. α-Glucosidase from yeast is used extensively as a screening material for α-glucosidase inhibitors, but the results do not always agree with those obtained in mammals. Therefore, we used the mouse small-intestine homogenate as a α-glucosidase solution because we speculated that it would better reflect the *in vivo* state. The inhibitory effect was measured using the method slightly modified from Dahlqvist (1964) [7]. After fasting for 20 h, the small intestine between the part immediately below duodenum and the part immediately above the cecum was cut, rinsed with ice-cold saline, and homogenized with 12 mL of malate buffer (100 mM, pH 6.0). The homogenate was used as the α-glucosidase solution. The assay mixture consisted of 100 mM malate buffer (pH 6.0), 2% (w/v) each sugar substrate solution (100 µl), and the sample extract (200-1000 µg /mL). It was pre-incubated for 5 min at 37°C, and the reaction was initiated by adding the crude α-glucosidase solution (50 µl) to it, followed by incubation for 10 min at 37°C. The glucose released in the reaction mixture was determined with the kit described above. The rate of carbohydrate decomposition was calculated as the percentage ratio to the amount of glucose obtained when the carbohydrate was completely digested. The rate of inhibition was given by the following formula:

Inhibition (%) = [(amount of glucose produced by the positive control) – (amount of glucose produced by the addition of sample) / (amount of glucose produced by the positive control)] × 100. -----Formula (1)

Determination of total phenolic content

Total phenolic content was assessed according to the Folin–Ciocalteu method with some modifications. Briefly, 0.1 ml of sample (200–1000 µg/ml), 1.9 ml distilled water and 1 ml of Folin–Ciocalteu's reagent were seeded in a tube, and then 1 ml of 100 g / l Na₂CO₃ was added. The reaction mixture was incubated at 25°C for 2 hrs and the absorbance of the mixture was read at 765 nm. The sample was tested in triplicate and a calibration curve with six data points for catechol was obtained. The results were compared to a catechol calibration curve and the total phenolic content of *E. divaricata* was expressed as mg of catechol equivalents per gram of extract.

Reducing ability assay

The reducing power of *Epaltes divaricata* L. extract was evaluated according to the method of Oyaizu (1986) [16]. Different amounts of the extract (200–1000 µg/ml) were suspended in distilled water and mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6), and 2.5 ml of 1% K₃Fe(CN)₆. The mixture was incubated at 50°C for 20 min; 2.5 ml of 10% TCA was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicated the ability of reducing power. Butylatedhydroxy toluene (BHT) was used as standard.

DPPH radical scavenging assay

DPPH quenching ability of MeED was measured according to Hatano, Kagawa, Yasuhara, Okuda, (1988). The methanol DPPH solution (0.15%) was mixed [10] with serial dilutions (200–1000 µg/ml) of the extract and after 10 min, the absorbance was read at 515 nm. The antiradical activity was expressed as IC₅₀ (µg/ml), (the antiradical dose required to cause a 50% inhibition). Vitamin C was used as standard. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where A₀ is the absorbance of the control at 30 min, and A₁ is the absorbance of the sample at 30 min. All samples were analyzed in triplicate.

Hydroxyl radical scavenging assay

The assay was performed as described by the method of Elizabeth and Rao (1990) with minor changes [8]. All solutions were prepared freshly. One milliliter of the reaction mixture contained 100 µl of 28 mM 2-deoxy-2-ribose (dissolved in phosphate buffer, pH 7.4), 500 µl solution of various concentrations of different solvent extract (200–1000 µg/ml), 200 µl of 200 µM FeCl₃ and 1.04 mM EDTA (1:1 v/v), 100 µl H₂O₂ (1 mM) and 100 µl ascorbic acid (1 mM). After an incubation period of 1 h at 37°C, the extent of Deoxy ribose degradation was measured by the TBA reaction [12]. The absorbance was read at 532 nm against the blank solution. Vitamin C was used as a positive control. The scavenging activity was calculated using formula (1).

Nitric oxide radical inhibition assay

Sodium nitroprusside in an aqueous solution at physiological pH spontaneously generates nitric oxide; it interacts with oxygen to produce nitrite ions, which can be estimated by the use of GriessIllosvoy reaction. In the present investigation, GriessIllosvoy reagent was modified using naphthyl ethylenediaminedihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and different concentration of the MeHB (200–1000 µg/ml) or standard solution (0.5 ml) were incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then,

1 ml of naphthyl-ethylenediamine dihydrochloride (1%) was added, mixed and allowed to stand for 30 min. A pink colored chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank. Vitamin C was used as positive control. The scavenging activity was calculated using the formula (1).

Inhibition of lipid peroxidation in rat liver homogenate

The inhibition effect of MeED on lipid peroxidation was determined according to the thiobarbituric acid method. FeCl₂-H₂O₂ was used to induce liver homogenate peroxidation. In this method, 0.2 ml of different concentration of MeED (200–1000 µg/ml) was mixed with 1 ml of 1% liver homogenate (each 100 ml homogenate solution contains 1 g rat liver); then 50 µl of FeCl₂ (0.5 mM) and H₂O₂ (0.5 mM) was added. The mixture was incubated at 37°C for 60 min; then 1 ml of trichloro acetic acid (15%) with thiobarbituric acid (0.67%) was added and the mixture was heated in boiling water for 15 min. The absorbance was recorded at 532 nm. Vitamin C was used as positive control. The percentage of inhibition was calculated using the formula. (1).

Superoxide scavenging activity

Superoxide scavenging activities of the *Epaltes divaricata* L. were determined by monitoring the competition of those with NBT for the superoxide anion generated by the PMS-NADH system. Superoxide radicals [6] were generated in 1 ml of 20 mM Tris-HCl buffer pH 8.0 containing 0.05 mM nitrobluetetrazolium (NBT), 0.01 mM phenazinemethosulphate (PMS) and different concentration of extract (200–1000 µg/ml) were pre-incubated for 2 min. The reaction was initiated by the addition of 0.078 mM NADH. Blue chromogen, formed due to NBT reduction was read at 560 nm. Results were expressed as percentage of inhibition of superoxide radicals. Vitamin C was used as a positive control. The scavenging activity was calculated using the formula (1).

Statistical analysis

The data for biochemical and physiological parameters were analyzed and expressed as mean ± SD. The IC₅₀ values were calculated from linear regression analysis. Results were processed by computer program Microsoft Excel (2007).

Detection of compounds in TLC

A solid is used as the stationary phase and liquid as the mobile phase. The TLC is a solid liquid chromatography. The physical principal involved in the separation of compounds in a mixture is adsorption. Thus, TLC is an adsorption chromatography. The separation of compounds is due to their different adsorption strength. TLC has several applications. It is mostly used to determine the number of compounds present in a sample. TLC was performed on silica plate (Fig-D) for crystals obtained from methanol extract, using three different solvents such as hexane, chloroform, ethyl acetate (1:1 & 1:3 ratios).

- Hexane - ethyl acetate = 1:1
- Chloroform - ethyl acetate = 1:1
- Hexane - ethyl acetate = 1:3
-

UV analysis for crystals

Crystals collected from the methanol extract was washed with hexane and dissolved in methanol. Then the solution was subjected to UV analysis. This is used for identification of chemical constituents present in this rare medicinal plant. In addition UV spectroscopy is proved to be a reliable and sensitive method for detection of bio molecular compounds.

RESULTS

Preliminary phytochemical analysis

The preliminary phytochemical (Table-1) evaluation of *Epaltes divaricata* L. studied by (both) methods showed the presence of steroids, triterpenoids and phenolic compounds.

Antimicrobial activity

Preliminary screening employing the disc diffusion assay and MIC was utilized to compare the antimicrobial activity of the *E.divaricta* extracts. In antibacterial activity the extracts showed active against gram positive and gram negative bacteria. Table-2 showed the antibacterial activity of *Epaltes divaricata* L. methanol extract. The results suggested that methanolic extract showed significant activity against more in gram positive pathogen, *Enterococcus faecalis* (18 mm), compared with gram negative pathogens *Erwinia sp* and *Escherichia coli* (12mm). Methanolic extract also shows the good antifungal activity (Table-3) against *Curvularialunata*, and *Aspergillus niger* (250µg/ml).

In vitro α-Amylase, α-glucosidase inhibition and anti-oxidant activity

α-Amylase activity

The results for α-amylase inhibition assay are shown in table 4 and all the concentration of standard exhibited above 50% of inhibition.

α-glucosidase inhibition

The results for α-glucosidase inhibition assay are shown in table 4 and all the concentration of standard exhibited above 50% of inhibition.

Total phenolic content

The total phenolic content of methanol extract of *Epaltes divaricata* L. was 228 mg catechol equivalent/gram extract.

Reducing power

Chart-6 shows the reductive capabilities of the extract compared to the standard butylated hydroxyl toluene. The reducing power of the extract of *Epaltes divaricata* L. increased with increasing quantity of the sample.

DPPH radical scavenging activity

The methanol extract of *Epaltes divaricata* L. exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC₅₀) at a concentration of 520.21 ± 1.02 µg/ml. The results are presented in chart-1. The IC₅₀ value of vitamin C was 470.12 ± 2.25 µg/ml.

Hydroxyl radical scavenging assay

The results for hydroxyl scavenging assay are shown in chart.4. The concentrations for 50% inhibition were found to be 510.21 ± 1.51 and 250.10 ± 1.71 µg/ml for the extract and vitamin C respectively.

Nitric oxide radical inhibition assay

The scavenging of nitric oxide by the extract was increased in a dose-dependent manner as illustrated in chart.2. At concentration of 700.02 ± 2.13 µg/ml of extract 50% of nitric oxide generated by incubation was scavenged. The IC₅₀ value of Vitamin C was 510.12 ± 1.74 µg/ml.

Lipid peroxidation assay

Activity of extract on lipid peroxidation is shown in chart.5. The extract showed inhibition of peroxidation at all concentrations; it showed 50% inhibition at 600.32 ± 2.00 µg/ml. The IC₅₀ value of vitamin C was 490.21 ± 1.88 µg/ml.

Superoxide scavenging activity

Superoxide anion scavenging activity of *Epaltes divaricata* L. is given in chart.3. The 50% of superoxide anion radical generation at the concentration of 510.31 ± 1.45 µg/ml. The IC₅₀ value of vitamin C was 300.08 ± 2.51 µg/ml

Detection of compounds in TLC

TLC was performed on silica plate for crystals obtained from methanol extract using three different solvents such as hexane, chloroform, ethyl acetate (1:1 & 1:3). TLC technique was used to separate the different compounds as spots on the silica plate. The TLC analysis revealed different spots for the crystals which collected from the methanol extract of *Epaltes divaricata* L. Presence of many bands indicated the number of compounds.

VU analysis of crystals

Chart.7 shows the UV spectrum of the crystals of methanol extract of *Epaltes divaricata* L., different wave length ranges of peaks were appeared. This proved that the number of compounds present in the crystals of methanol extract.

CONCLUSION

The observed activity may be due to the presence of potent phytoconstituents in the extracts. Scavenging activity for free radicals of DPPH has been widely used to evaluate the antioxidant activity of natural products from plant and microbial sources. Free radical scavenging of total crude extracts from the medicinal plants was quantitatively determined using DPPH. The antioxidant activity may be responsible for their usefulness in the management and treatment of various diseases.

In the present study, antibacterial screening of methanol extract of *Epaltes divaricata* L., showed varying degrees of antibacterial and antifungal activity against human pathogenic bacteria and fungi cultured *in vitro*. Out of three extracts, the methanol extract of plants exhibited significant antibacterial and antifungal activity against Gram-positive, Gram-negative bacteria and fungi. This is a special interest since Gram-positive bacteria are more resistant to this plant extract.

The antioxidant activity reflected by the DPPH radical scavenging assay was clearly observed in the methanol extract of plants. Results from our phytochemical analysis revealed that the Hexane, Ethyl acetate and Methanol extracts of plant, accumulate substantial amounts of triterpenoids, phenolic compounds and steroids which could be correlated with the activities measured. We shall conduct further work to isolate the antioxidant and antimicrobial constituents of the plant and evaluate their minimum inhibitory concentration (MIC).

In this study we evaluated *in vitro* alpha amylase and alpha glucosidase activity of crude methanol extract of *Epaltes divaricata* L. The plant showed significant inhibition activity, so further our *in vitro* studies indicated that plant had a alpha amylase and alpha glucosidase inhibitory activity and it might possess therapeutic antidiabetic effects in the type-II diabetes mellitus (Table-4). The result obtained from the present studies (*in vitro*) will be confirmed by taking up *in vivo* studies in future. The separation of bands by TLC and UV spectrum can be used to isolate and identify the number of compounds, from the crystals of methanol extract for therapeutic uses.

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FIGURES

Figure-A: Antibacterial activity of methanol extract of *Epaltes divaricata* by disc diffusion method.

Figure-B: MIC-Antifungal Activity

Figure-C: Crystals of methanol extract

Figure-D: TLC of Methanol Extract

TABLES

Table-1. Phytochemical analysis of solvent extracts of *Epaltes divaricata*.

Phytochemical analysis	Hexane	Ethyl Acetate	Methanol
1. Triterpenoids	Negative	Positive	Positive
2. Steroids	Negative	Negative	Positive
3. Phenolic compounds	Positive	Positive	Positive

Table-2. Antimicrobial activity of solvent extracts from *Epaltes divaricata*.

Name of the Pathogens	Zone of inhibition (mm)									STD
	Hexane (mg/ml)			Ethyl acetate (mg/ml)			Methanol (mg/ml)			
	1.25	2.5	5	1.25	2.5	5	1.25	2.5	5	
<i>Staphylococcus aureus</i> ATCC25923	-	-	-	-	10	13	12	12	14	25
<i>Klebsiella pneumonia</i> ATCC 15380	-	-	-	-	-	-	10	11	13	27
<i>Enterococcus faecalis</i> ATCC 29212	-	-	-	-	12	12	10	14	18	24
<i>Yersinia enterocolitica</i> MTCC 840	-	-	-	-	11	11	12	14	14	28
<i>Erwinia sp</i> MTCC 2760	-	-	-	-	10	10	12	11	12	28
<i>Vibrio parahaemolyticus</i> MTCC 451	-	-	-	-	8	10	10	12	14	36
<i>Enterobacter aerogenes</i> MTCC 111	-	-	-	-	-	11	12	10	13	28
<i>Escherichia coli</i> ATCC 25922	-	-	-	-	-	-	8	10	13	25
<i>Proteus vulgaris</i> MTCC 1771	-	-	-	-	12	12	14	14	16	30

Table-3. Effect of *Epaltes divaricata* solvent extracts on antifungal activity.

Name of the Pathogens	Hexane (µg/ml)	Ethyl acetate (µg/ml)	Methanol (µg/ml)	Fluconazole (µg/ml)
<i>Aspergillus niger</i> MTCC 1344	-	500	250	100
<i>Botrytis cinerea</i>	-	1000	500	100
<i>T. rubrum</i> 57/01	-	1000	500	25
<i>Curvularia lunata</i> 46/01	-	500	250	<12.5
<i>Aspergillus flavus</i>	-	1000	500	50
<i>T. mentagrophytes</i> 66/01	-	1000	500	25

Table-4. Preliminary screening for Antidiabetic activities of solvent extracts of *Epaltes divaricata*.

Sample	Concentrations (µg/ml)	% of α-Amylase Inhibition	IC ₅₀ µg/ml	% of α-Glucosidase Inhibition	IC ₅₀ µg/ml	% of Acarbose	IC ₅₀ µg/ml
MeED	200	20.75 ± 2.49	3.91	20.15 ± 2.93	2.85	40.91 ± 1.54	1.24
	400	31.83 ± 1.80		49.93 ± 2.99		61.14 ± 0.29	
	600	40.71 ± 1.19		54.05 ± 2.79		81.75 ± 0.29	
	800	51.09 ± 2.16		62.41 ± 1.00		92.76 ± 0.29	
	1000	60.37 ± 2.87		71.58 ± 1.65		93.93 ± 0.50	

CHARTS

Chart-1. DPPH radical scavenging activity of methanol extract of *Epaltes divaricata*.

Chart-2. Nitric oxide radical inhibition activity of methanol extract of *Epaltes divaricata*.

Chart-3. Superoxide scavenging activity of methanol extract of *Epaltes divaricata*.

Chart-4. Hydroxyl radical scavenging activity of methanol extract of *Epaltes divaricata*.

Chart-5. Lipid peroxidation activity of methanol extract of *Epaltes divaricata*.

Chart-6. Reducing ability activity of methanol extract of *Epaltes divaricata*.

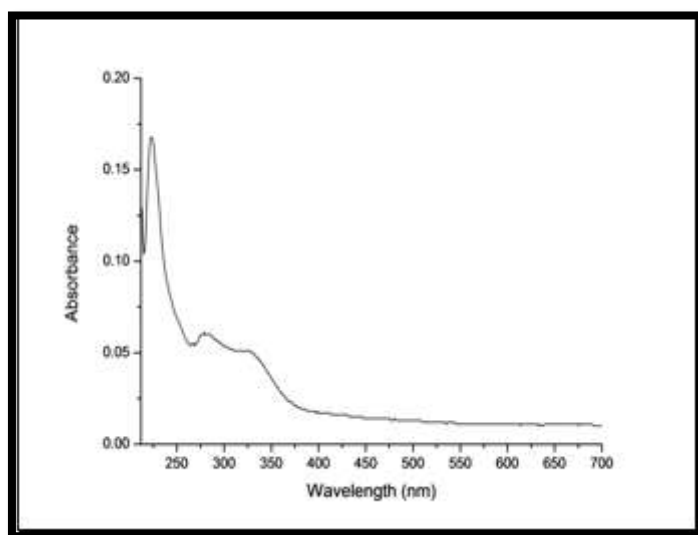


Chart-7. UV analysis for crystals of methanol extract of *Epaltes divaricata*.