

HPTLC analysis and *in-vitro* antioxidant, anti-inflammatory and anti-diabetic activities of ethanol extract of leaves of *Actinodaphne madraspatana* Bedd

Devarajan Saravanan¹ and Indira Viswambaran Asharani^{2*}

¹Ratnam Institute of Pharmacy, Pidathapolur, Nellore, Andhra Pradesh, India

²School of Advanced Sciences, VIT University, Vellore, Tamil Nadu, India

Abstract: The aim of the present study was to develop the HPTLC fingerprint and to evaluate the *in-vitro* antioxidant, anti-inflammatory and anti-diabetic activities of ethanol extract of leaves of *Actinodaphne madraspatana* Bedd (*A. madraspatana*). HPTLC fingerprint analysis of ethanol extract was investigated by win CATS planar chromatography. The antioxidant activity was evaluated by the various antioxidant assays, such as total antioxidant, reducing power, DPPH radical scavenging, hydrogen peroxide scavenging and hydroxyl radical scavenging. The antioxidant activity was compared to standard drug ascorbic acid. *In-vitro* anti-inflammatory activity was evaluated by inhibition of albumin denaturation assay using aspirin as a standard drug. *In-vitro* anti-diabetic activity was evaluated by α -amylase inhibition assay using acarbose as a standard drug. The HPTLC fingerprint of ethanol extract has shown six peaks, which indicate the presence of six different phytochemicals. The *in-vitro* antioxidant, anti-inflammatory and anti-diabetic activities of ethanol extract of plant leaves increased with the increasing of concentration. The result revealed that extract in all the concentration showed the *in-vitro* antioxidant, anti-inflammatory and anti-diabetic activities compared to standard drugs. The ethanol extract of leaves of *A. madraspatana* has a potential effect as an antioxidant, anti-inflammatory and anti-diabetic in all the tested *in-vitro* methods.

Keywords: *A. madraspatana* leaves, Ethanol extract, HPTLC fingerprint, *In-vitro* pharmacological activities

INTRODUCTION

Free radicals, particularly reactive oxygen and nitrogen species are unstable or short-lived electrophile with an odd number of electrons. The most of reactive oxygen and nitrogen species include thiyl radicals (RS[•]), hydroxyl radical ([•]OH), ozone (O₃), hydrogen peroxide (H₂O₂), hypochlorite ion (OCl⁻), superoxide anion ([•]O₂), carbon centered radicals ([•]O₂CCl₃), peroxy radical (ROO[•]), nitric oxide ([•]NO) and nitrogen dioxide (NO₂) are formed in the body by normal metabolism of compounds containing oxygen, nitrogen and sulfur atoms by the enzyme cytochrome P₄₅₀, smoking of cigarettes, exposure to ionizing radiation, herbicides, alcohol, fried items of foods, synthesized by enzyme systems-neutrophils, phagocytic cells and macrophage and the byproduct of cellular respiration etc. The excessive formation of reactive oxygen and nitrogen species can damage essential proteins, DNA and lipids and cause several human diseases like inflammation, carcinogenesis, drug toxicities, fibrosis, lipid peroxidation of cellular membranes, oxygen toxicity, aging and produced in various specific diseases such as degenerative neurologic disease, atherosclerosis and reperfusion injury (Ashok Shinde *et al.*, 2012).

Antioxidants are molecules that act as free radical scavengers, and which can be safely interacting with free

radicals and prevent or slowing the oxidative damage of the body cell and repair the damage cell. Phytonutrients are the plant's food acts as antioxidants, which help from the body's defense against free-radical damage to cells. More than 25,000 phytonutrients are found in plant foods. Some of the common classes of phytonutrients include polyphenols, carotenoids, ellagic acid, saponins, terpenes, lignans, phenol and cyclic compounds. Polyphenols, which are classified into many groups such as flavonoids, stilbenes and tannins with health beneficial properties such as inhibition of hydrolytic and oxidative enzymes, free radical scavenging, anti-diabetogenic potentiality and anti-inflammatory action (Chanda and Dave, 2009).

A. madraspatana is belonging to the family Lauraceae. It is commonly known as 'Putta Thali' in Tamil, 'Ray Laurel' in English, 'Irolimarom' and 'Mungali' in Malayalam, 'Kovangutti' in Telugu (Gupta and Tandon, 2004). It is a medium-sized evergreen tree and Shrub, widely distributed common on the Rock Hill slopes at higher elevations, Aruku Valley, Vishakhapatnam District, Talakona, Dharmagiri, Microwave station, on the way to Thumburu Theertham (Ram *et al.*, 2001; Saldanha and Nicolson, 1976; Gamble, 1967). Leaves, flowers and fruits constitute the drug. It is a precursor of vitamin A (Pullaiah, 2006). The benzene extract of the Heartwood was reported to contain 5, 7, 8-Trimethoxyflavone (Adinarayana and Gunasekar, 1979). The leaves of the plant are used traditionally to cure wounds, cure mania, fickle minded behavior and diabetic (Pullaiah, 2002).

*Corresponding author: e-mail: ivasharani@gmail.com

Therefore, the objective of the present study was to develop the HPTLC finger-print and to evaluate the *in-vitro* antioxidant, anti-inflammatory and anti-diabetic activities of ethanol extract of leaves of *A. madraspatana*.

MATERIALS AND METHODS

Plant material

The leaves of *A. madraspatana* were collected from Talakona forest near to Tirupathi and were authenticated by Dr. K. Madavachetty, Professor, Department of Botany, S. V. University, Tirupati, Andhra Pradesh. A voucher specimen (ACD) has been kept in the Herbarium of the Department of Pharmaceutical Chemistry, Ratnam Institute of Pharmacy.

Preparation of ethanol extract

The leaves of plant samples were washed with tap water and dried under room temperature for three days. Approximately, about 150grams of leaves were pulverized to a powder in a mechanical grinder. The dry powder of leaves of *A. madraspatana* (100grams) was extracted with ethanol (500mL, 46 hours) at a temperature between 60-65°C by using a Soxhlet extractor. The ethanol extract was filtered and concentrated to dry mass by using vacuum distillation.

Drugs and Chemicals

2,2-Diphenyl-1-picryl-hydrazyl (DPPH), α -amylase, and 3,5-dinitrosalicylic acid were procured from Hi Media Laboratories Pvt. Ltd., Mumbai. Ammonium molybdate, gallic acid, hydrogen peroxide and FC reagent (Folin-Ciocalteu reagent) were purchased from SD Fine Chemicals Pvt. Ltd, Mumbai. Acarbose and 1,10-phenanthroline were procured from Sigma Aldrich, USA. Egg albumin was obtained from Qualigens Fine Chemicals Pvt. Ltd, Mumbai. All other reagents, chemicals, and solvents used in the study were of analytical grade.

Chromatographic conditions

The sample was spotted in the form of bands of width 6 mm with a 25 μ L Hamilton's syringe on an 0.2mm thickness aluminium TLC plate precoated with silica gel 60 F254 (Merck Ltd, Darmstadt, Germany) was used as the stationary phase, with the help of Linomat TLC Autosampler 5 attached to CAMAG HPTLC system, which was programmed through win CATS software version 1.4.4. Various concentrations of sample (2 μ L and 3 μ L) of ethanol extract of *A. madraspatana* were applied in two tracks as a 6mm bands at a spraying rate of 150nL/s. The plate was developed up to a migration distance of 98mm (distance to the lower edge was 15mm) in 27 \pm 2°C with ethyl acetate: methanol: water (10:1.3:1.0) as a mobile phase in a CAMAG twin trough chamber (20x10cm) previously saturated for 30 minutes. The developed plate was dried in a hot air oven at 60°C for 5 minutes, and the densitometric scanning of the

developed plate was performed with a CAMAG TLC scanner 3 equipped with win CATS software (version 1.4.4) at λ_{\max} 366 nm using deuterium lamp as a light source (Gayathri Gunalan *et al.*, 2012).

Quantitative estimation of ethanol extract

Total phenolic content

Total phenolic content in the ethanol extract of leaves of *A. madraspatana* was determined by using of FC reagent (Rekha *et al.*, 2012). 1.0mL of ethanol extract in methanol (1mg/mL) was mixed with 1.0mL of FC reagent (10 fold) and 1.5mL of 20%w/v sodium carbonate solution in a 10mL volumetric flask. The volumetric flask was shaken thoroughly, and volume of the flask was made up to 10mL using distilled water. The volumetric flask was kept in boiling-water bath for 1 minute, cooled and the absorbance of the reaction mixture was measured in the UV-Visible spectroscopy at 650nm against the reagent blank. A standard calibration curve was plotted using different concentrations of gallic acid (20-120 μ g/mL). The total phenolic content was determined as a mg of gallic acid equivalents (GAE)/g of ethanol extract.

In-vitro antioxidant activity

Assay of DPPH radical scavenging activity

The DPPH radical scavenging activity of ethanol extract was determined according to the method of Jun Liu *et al* (2010) and Tefvik Ozen *et al* (2010) with slight modifications. About 0.2mM solution of DPPH in methanol was prepared and 1mL of this solution was added to 3mL of different concentrations of ethanol extract and standard ascorbic acid (150-400 μ g/mL). The mixture was mixed thoroughly and allowed to stand for 30 minutes at room temperature and the absorbance of the resulting solution was measured in the UV-Visible spectroscopy at 517nm against the reagent blank. The ascorbic acid was used as a positive control.

Assay of hydrogen peroxide (H₂O₂) scavenging activity

The H₂O₂ scavenging activity of ethanol extract was determined according to the method of Guang-Rong Zhao *et al* (2006) with slight modification. 1.0mL of 0.1mM H₂O₂ and 1.0mL of different concentrations of ethanol extract and standard ascorbic acid (400-900 μ g/mL) were mixed with 0.5mL of 3% w/v ammonium molybdate, 10mL of 2M H₂SO₄ and 5mL of 1.8M KI. The mixture was kept at room temperature for 30 minutes and the mixture was titrated against a standard solution of 5mM sodium thiosulphate (Na₂S₂O₃) until the disappearance of yellow color.

Assay of hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of ethanol extract was measured according to the method of Nagulendran *et al* (2007) with slight modification. The reaction mixture consisted of 1.5mL of various concentrations of ethanol extract and standard ascorbic acid (150-400 μ g/mL), 150 μ L of 1mM 1,10-

phenanthroline, 100 μ L of 1.0mM FeCl₃, 2.5mL of 0.2 M phosphate buffer (pH 7.8) and 150 μ L of 0.17 M H₂O₂. The reaction mixture was mixed thoroughly and incubated at room temperature for 5 minutes. The absorbance of the reaction mixture was measured in the UV-Visible spectroscopy at 560 nm against the reagent blank.

Total antioxidant capacity

The total antioxidant capacity of the ethanol extract was determined by a phosphomolybdate method (Prieto *et al.*, 1999). The various concentrations of 0.5mL of the ethanol extract and standard ascorbic acid (50-400 μ g/mL) solution were mixed with 6.0mL of reagent (4mM ammonium molybdate, 0.6 M sulfuric acid, and 28mM sodium phosphate) in a tubes. The tubes were closed and incubated in a water bath for 90 minutes at 95°C, cooled and absorbance was measured in the UV-Visible spectroscopy at 695 nm against the reagent blank.

Reducing power assay

The reducing power assay of ethanol extract was determined according to the method of Zhongshan Zhang *et al* (2010) with some modification. 1.0mL of different concentration of the ethanol extract and standard ascorbic acid (100-500 μ g/mL) in a 0.2 M phosphate buffer (pH 6.6) was mixed with 1.0mL of 1% w/v potassium ferricyanide. The reaction mixture was mixed thoroughly and incubated at 50°C for 20 minutes. The reaction mixture was cooled at room temperature, and 2.0mL of 10% w/v trichloroacetic acid solution was added to the reaction mixture to terminate the reaction. Then, the reaction mixture was mixed with 1.2mL of 0.1% w/v ferric chloride solution and the absorbance was measured in the UV-Visible spectroscopy at 700nm against the reagent blank.

In-vitro anti-inflammatory activity

Inhibition of albumin denaturation

Inhibition of albumin denaturation of ethanol extract was determined according to the method of Leelaprakash *et al* (2011) with slight modification. A test solution consisting of 1.0mL of different concentrations of the ethanol extract ranging from 150-400 μ g/mL or standard aspirin (150 and 200 μ g/mL) solution was mixed with 1.0mL of egg albumin aqueous solution (1% w/v) and incubated at 37°C for 20 minutes and then heated the reaction mixture in a water bath at 51°C for 20 minutes. After cooling, the turbidity was measured in the UV-Visible spectroscopy at 660 nm against the reagent blank.

In-vitro anti-diabetic activity

α -Amylase inhibition assay

The α -amylase inhibition assay was performed according to the method of Sangeetha *et al* (2012) and Muhammad Zia-Ul-Haq *et al* (2013) with some modification. In brief, 1.0mL of the different concentration of the ethanol extract

and standard agarbose (150-400 μ g/mL) was allowed to react with 1mL of α -amylase solution (1% w/v) and 2mL of 0.1M of sodium acetate buffer (pH-7.2). After 20 minutes incubation, 1.0mL of 1% w/v starch solution was added. The reaction mixture was kept at room temperature for 30 minutes. Then 1.0mL of 3,5-dinitrosalicylic acid reagent was added into the reaction mixture. They were kept in a boiling water bath for 5 minutes. The absorbance was recorded at 540 nm against the reagent blank. Starch, α -amylase and drug solution were prepared in a sodium acetate buffer.

STATISTICAL ANALYSIS

Results are expressed as Mean \pm SEM. The difference between experimental groups was compared by paired test using the Graph Pad Instat 3 software.

Calculation of percentage inhibition

The percentage inhibition of different concentration of ethanol extract of leaves *A. madraspatana* and standard drug for various *in-vitro* methods such as assay of DPPH radical scavenging activity, assay of hydroxyl radical scavenging activity, inhibition of albumin denaturation, and α -amylase inhibition assay were calculated by using the following formula

$$\% \text{ inhibition} = (A_0 - A_1 / A_0) \times 100$$

Where; A₀ was the absorbance of control, and A₁ was the absorbance of test or standard.

The percentage inhibition of different concentration of ethanol extract of leaves of *A. madraspatana* and standard drug for assay of hydrogen peroxide scavenging activity was calculated by using the following formula

$$\% \text{ inhibition} = (A_0 - A_1 / A_0) \times 100$$

Where; V₀ was the volume of standard solution of Na₂S₂O₃ consumed (blank), V₁ was the volume of standard solution of Na₂S₂O₃ consumed (test or standard).

RESULTS

HPTLC finger print analysis

The HPTLC fingerprint of ethanol extract of leaves of *A. madraspatana* as shown in Figure 1. The HPTLC finger print of ethanol extract showed the presence of sixspots. The spots with R_f values 0.77, 0.70, 0.57, 0.45 was found to be more percentage area and remaining very less percentage area.

Quantitative estimation of ethanol extract

Total phenolic content

Total phenolic content in the ethanol extract of leaves of *A. madraspatana* was investigated by the Folin-Ciocalteu method using gallic acid as a standard phenolic compound. The total phenolic content was expressed as gallic acid equivalent (mg of gallic acid/g of extract). The results are shown in Figure 2. The calibration curve for gallic acid showed the linearity in the ranges of 20-

120µg/mL with a correlation coefficient of 0.998. The total phenolic content in the ethanol extract was found to be 116.67±1.53 mg/g of dry extract.

In vitro antioxidant activity

Assay DPPH radical scavenging activity

The DPPH radical scavenging effect of ethanol extract and standard ascorbic acid are shown in table 1. The percentage of DPPH radical scavenging activity was increased with the increase in concentration of the ethanol extract and ascorbic acid from 150to 400 µg/mL. The extract, in all the concentrations showed the significant DPPH radical scavenging activity.

Assay of hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging effect of ethanol extract and standard ascorbic acid are shown in Table 2. The percentage of hydrogen peroxide scavenging activity was increased with the increasing concentration of the ethanol extract and ascorbic acid from 400to 900 µg/mL. The extract, in all the concentrations showed the significant hydrogen peroxide scavenging activity.

Assay of hydroxyl radical scavenging activity

The hydroxyl radical scavenging effect of ethanol extract and standard ascorbic acid on hydroxyl radical has been shown in Table 3. The percentage of hydroxyl radical scavenging activity was increased with the increase in concentration of the ethanol extract and ascorbic acid from 150to 400 µg/mL. The extract, in all the concentrations showed the significant hydroxyl radical scavenging activity. table 3: Percentage of hydroxyl radical scavenging activity of ethanol extract of leaves of *A. madraspatana*

Total antioxidant capacity

Total antioxidant capacity of ethanol extract was determined by phosphomolybdenum method. The phosphomolybdenum method is quantitative since the total antioxidant capacity is expressed as the ascorbic acid equivalents. The calibration curve (Figure 3) for ascorbic acid showed the linearity in the ranges of 20-120 µg/mL with a correlation coefficient of 0.997. The ethanol extract of leaves of *A. madraspatana* was containing 308±7.217µg of ascorbic acid/mg of dry extract.

Reducing power assay

Reducing the power of ethanol extract of leaves of *A. madraspatana* as shown in Figure 4. The increased absorbance of the reaction mixture indicated increased reducing power. Reducing power capabilities of the ethanol extract and standard ascorbic acid increased with increases in the concentration are shown in Figure 4. The compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediate of lipid per oxidation processes.

In-vitro anti-inflammatory activity

Inhibition of albumin denaturation

The inhibitory effect of ethanol extract and standard aspirin are shown in Table 4. The inhibitory effect was increased with the increase in concentration of the ethanol extract and aspirin from 150to 400 µg/mL. The extract, in all the concentrations showed the significant inhibition of albumin denaturation.

In-vitro anti-diabetic activity

α-Amylase inhibition assay

The inhibitory effect of ethanol extract and standard acarbose are shown in Table 5. The inhibitory effect was increased with the increasing concentration of ethanol extract and acarbose from 150 to 400 µg/mL. The extract, in all the concentration showed the significant inhibition of α-amylase.

DISCUSSION

The HPTLC finger print of ethanol extract showed the presence of six spots when the plate was eluted with mobile phase ethylacetate:methanol:water(10:1.3:1.0), that indicating the presence of six different phytochemicals. The HPTLC fingerprinting of extract will not only help in the quality control and identification of plant but also provide the useful basic information for the isolation, characterization and identification of plant species.

Phenolic compounds are very important plant constituents and act as antioxidants. Flavonols and flavonoids are two polyphenolic compounds; they can scavenge the free radicals due to their hydroxyl groups.

DPPH free radical is widely employed as a substrate to investigate the antioxidant capacity of various antioxidants such as anthocyanins, phenolic compounds, and crude extract of medicinal plants. The reaction based on the reactions of specific antioxidant with DPPH free radical. DPPH is a stable free radical containing odd electron with purple color. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm. The purple color turns to yellow when the odd electrons accept the hydrogen from a free-radical scavenging antioxidants. As a result, there was a reduction of DPPH free radical concentration, which decreases the absorbance, and it was detected at 517nm. Ethanol extract demonstrated the DPPH radical scavenging activity in a concentration dependent manner. The ethanol extract showed the highest percentage of DPPH radical scavenging activity at the concentration of 400 µg/mL with IC₅₀ values 332µg/mL compared to standard ascorbic acid (IC₅₀ values 285µg/mL). The experimental data showed that percentage inhibition values of standard ascorbic acid were high when compared to ethanol extract.

Table 1: Percentage of DPPH radical scavenging activity of ethanol extract of leaves of *A. madraspatana*

S. No	Concentration ($\mu\text{g/mL}$)	% of activity ($\pm\text{SEM}$)	
		Ethanol extract	Ascorbic acid
1	150	23.98 \pm 0.314	27.2205 \pm 0.653
2	200	30.15 \pm 0.551	35.7889 \pm 0.479
3	250	36.15 \pm 0.395	41.2748 \pm 0.551
4	300	44.88 \pm 0.479	51.9854 \pm 0.863
5	350	52.61 \pm 0.707	62.2779 \pm 0.863
6	400	60.92 \pm 0.653	70.7941 \pm 0.958
IC ₅₀ values		332 $\mu\text{g/mL}$	285 $\mu\text{g/mL}$

Table 2: Percentage of hydrogen peroxide scavenging activity of ethanol extract of leaves of *A. madraspatana*

S. No	Concentration ($\mu\text{g/mL}$)	% of activity ($\pm\text{SEM}$)	
		Ethanol extract	Ascorbic acid
1	400	25.76 \pm 3.472	29.55 \pm 2.273
2	500	40.15 \pm 3.472	44.70 \pm 1.312
3	600	60.60 \pm 1.310	62.88 \pm 3.471
4	700	68.18 \pm 2.272	71.97 \pm 3.472
5	800	75.76 \pm 1.312	79.55 \pm 2.272
6	900	84.09 \pm 2.268	87.12 \pm 3.473
IC ₅₀ values		572 $\mu\text{g/mL}$	539 $\mu\text{g/mL}$

Table 3: Percentage of hydroxyl radical scavenging activity of ethanol extract of leaves of *A. madraspatana*

S. No	Concentration ($\mu\text{g/mL}$)	% of activity ($\pm\text{SEM}$)	
		Ethanol extract	Ascorbic acid
1	150	28.50 \pm 0.795	32.32 \pm 0.480
2	200	40.90 \pm 0.480	45.80 \pm 0.382
3	250	46.69 \pm 0.583	53.94 \pm 0.292
4	300	53.31 \pm 0.794	61.00 \pm 0.583
5	350	62.98 \pm 0.380	70.48 \pm 0.480
6	400	73.22 \pm 0.480	80.12 \pm 0.769
IC ₅₀ values		270 $\mu\text{g/mL}$	235 $\mu\text{g/mL}$

Table 4: Percentage inhibition of albumin denaturation by ethanol extract of leaves of *A. madraspatana*

S. No	Concentration ($\mu\text{g/mL}$)	% of activity ($\pm\text{SEM}$)	
		Ethanol extract	Aspirin
1	150	20.80 \pm 0.800	58.53 \pm 0.611
2	200	29.60 \pm 0.800	70.27 \pm 0.611
3	250	38.80 \pm 0.800	
4	300	45.33 \pm 0.833	
5	350	53.33 \pm 1.001	
6	400	62.80 \pm 0.800	
IC ₅₀ values		326 $\mu\text{g/mL}$	

Table 5: Percentage inhibition of α -amylase by ethanol extract of leaves of *A. madraspatana*

S. No	Concentration ($\mu\text{g/mL}$)	% of activity ($\pm\text{SEM}$)	
		Ethanol extract	Acarbose
1	150	20.16 \pm 0.516	17.12 \pm 0.850
2	200	27.59 \pm 0.850	31.42 \pm 0.676
3	250	33.56 \pm 0.516	37.61 \pm 0.703
4	300	45.94 \pm 0.676	48.31 \pm 0.676
5	350	56.31 \pm 1.086	59.80 \pm 0.676
6	400	66.67 \pm 1.032	68.47 \pm 0.516
IC ₅₀ values		319 $\mu\text{g/mL}$	306 $\mu\text{g/mL}$

*All values are expressed as mean \pm SEM for three determinations

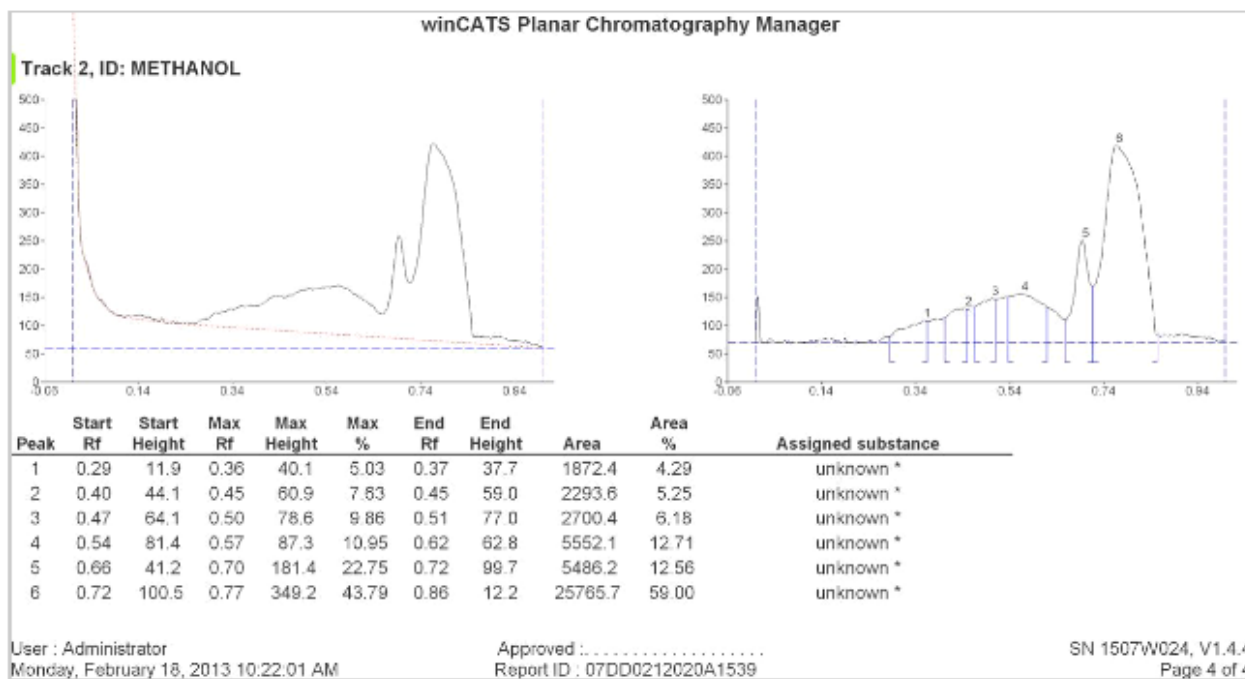


Fig. 1: HPTLC fingerprint of ethanol extract of leaves of *A. madraspatana*

Hydrogen peroxide scavenging activity is based on the loss or decomposition of hydrogen peroxide, when the hydrogen peroxide incubated with the scavenger. The loss of hydrogen peroxide can be determined by replacement titration. Hydrogen peroxide is a weak oxidizing agent who oxidizes the iodide to iodine in the presence of dilute sulfuric acid and ammonium molybdate as a catalyst to produce yellow color. The solution changes to be colorless when liberated iodine reacts with sodium thiosulphate. Ethanol extract demonstrated the hydrogen peroxide scavenging activity in a concentration dependent manner. The ethanol extract showed the highest percentage of hydrogen peroxide scavenging activity at the concentration of 900 µg/mL with IC₅₀ values 572 µg/mL compared to standard ascorbic acid (IC₅₀ values 539 µg/mL). The experimental data showed that percentage inhibition values of standard ascorbic acid were high when compared to ethanol extract.

The scavenging activity for hydroxyl radicals can be determined using Fenton's reaction. The hydroxyl radical is highly reactive oxygen species formed by the reaction of various hydroperoxides with transition metal ions. Hydroxyl radicals are attacks DNA, proteins, polyunsaturated fatty acid in membrane and is abstracting the hydrogen atoms from polyunsaturated fatty acids and brings about the peroxidic reaction of membrane lipids. The ethanol extract showed the highest percentage of hydroxyl radical inhibition at the concentration of 400 µg/mL with IC₅₀ values 270 µg/mL compared to standard drug ascorbic acid (IC₅₀ values 235 µg/mL). The

experimental data showed that percentage inhibition values of standard ascorbic acid were high when compared to ethanol extract.

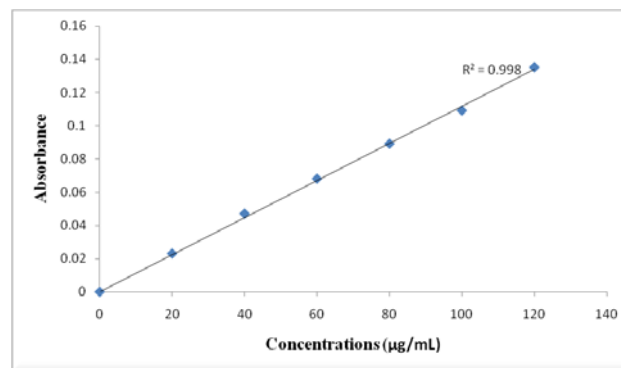


Fig. 2: Calibration curve for gallic acid for determination of total phenolic content

The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the ethanol extract and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phosphomolybdenum method is used to investigate the total antioxidant capacity of medicinal plant extract and also evaluates the both fat soluble and water soluble antioxidants.

The study is based on the ability of the ethanol extract to inhibit the protein denaturation. Inflammation is a very frequent symptom of many chronic diseases and caused by the release of chemicals from the tissues and migration cells. Denaturation of protein is a well-documented cause

of inflammation. Protein denaturation is a process in which proteins lose their secondary and tertiary structure by the application of external stress such as heat or organic solvents, strong acid or base and concentrated inorganic salt. The ethanol extract showed the highest percentage of inhibition at the concentration of 400 $\mu\text{g/mL}$ with IC_{50} values 326 $\mu\text{g/mL}$ compared to standard drug aspirin. Aspirin showed the highest percentage of inhibition at the concentration of 200 $\mu\text{g/mL}$. The experimental data showed that percentage inhibition values of standard aspirin were high when compared to ethanol extract.

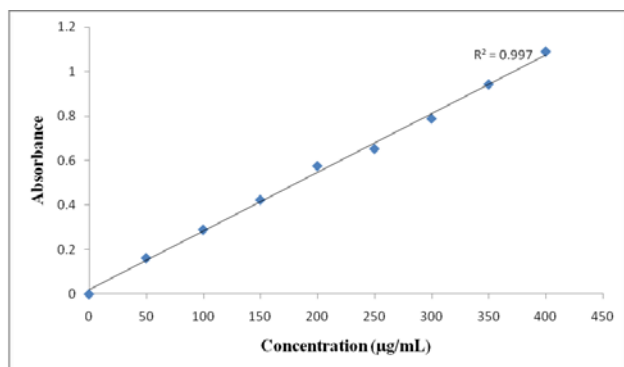


Fig. 3: Calibration curve for ascorbic acid for determination of total antioxidant capacity

α -Amylase is the key enzyme and catalyses the hydrolysis of 1,4-glucosidic linkages of starch into glucose, which can be readily available for the intestinal absorption. Degradation of this dietary starch leads to elevated postprandial hyperglycemia. Inhibition of α -amylase in the digestive tract reduces the rate of digestion of carbohydrates and is to be effective in controlling the diabetes. α -amylase inhibitors such as acarbose and miglitol lowering the postprandial glucose level by decreasing glucose release from the starch and delaying carbohydrate absorption by inhibiting the activity of α -amylase in the small intestine. The ethanol extract showed the highest percentage of inhibition at the concentration of 400 $\mu\text{g/mL}$ with IC_{50} values 319 $\mu\text{g/mL}$ compared to standard drug acarbose (IC_{50} values 306 $\mu\text{g/mL}$). The experimental data showed that percentage inhibition values of standard acarbose were higher when compared to ethanol extract.

In vitro anti-inflammatory activity

Inhibition of albumin denaturation

The study is based on the ability of the ethanol extract to inhibit the protein denaturation. Inflammation is a very frequent symptom of many chronic diseases and caused by the release of chemicals from the tissues and migration cells. Denaturation of protein is a well-documented cause of inflammation. Protein denaturation is a process in which proteins lose their secondary and tertiary structure by the application of external stress such as heat or

organic solvents, strong acid or base and concentrated inorganic salt. The inhibitory effect of ethanol extract and standard aspirin are shown in table 4. The inhibitory effect was increased with the increase in concentration of the ethanol extract from 150 to 400 $\mu\text{g/mL}$ and aspirin (150 and 200 $\mu\text{g/mL}$). The extract, in all the concentrations showed the significant inhibition of albumin denaturation. The ethanol extract showed the highest percentage of inhibition at the concentration of 400 $\mu\text{g/mL}$ with IC_{50} values 326 $\mu\text{g/mL}$ compared to standard drug aspirin. Aspirin showed the highest percentage of inhibition at the concentration of 200 $\mu\text{g/mL}$. The experimental data showed that percentage inhibition values of standard aspirin were high when compared to ethanol extract.

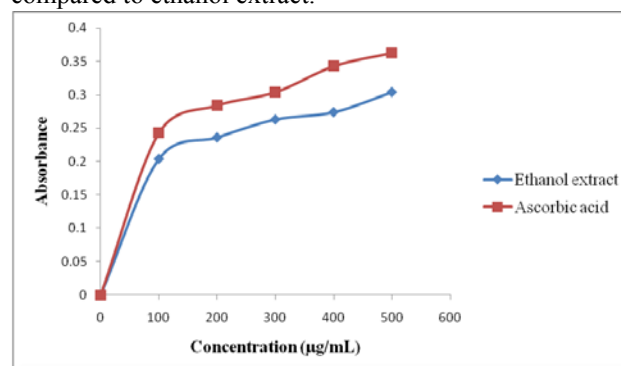


Fig. 4: Reducing the power of ethanol extract of leaves of *A. madraspatana* and ascorbic acid

In-vitro anti-diabetic activity

α -Amylase inhibition assay

α -Amylase is the key enzyme and catalyses the hydrolysis of 1,4-glucosidic linkages of starch into glucose, which can be readily available for the intestinal absorption. Degradation of this dietary starch leads to elevated postprandial hyperglycemia. Inhibition of α -amylase in the digestive tract reduces the rate of digestion of carbohydrates and is to be effective in controlling the diabetes. α -amylase inhibitors such as acarbose and miglitol lowering the postprandial glucose level by decreasing glucose release from the starch and delaying carbohydrate absorption by inhibiting the activity of α -amylase in the small intestine. The inhibitory effect of ethanol extract and standard acarbose are shown in Table 5. The inhibitory effect was increased with the increasing concentration of ethanol extract and acarbose from 150 to 400 $\mu\text{g/mL}$. The extract, in all the concentration showed the significant inhibition of α -amylase. The ethanol extract showed the highest percentage of inhibition at the concentration of 400 $\mu\text{g/mL}$ with IC_{50} values 319 $\mu\text{g/mL}$ compared to standard drug acarbose (IC_{50} values 306 $\mu\text{g/mL}$). The experimental data showed that percentage inhibition values of standard acarbose were higher when compared to ethanol extract.

CONCLUSION

This present study revealed that the HPTLC analysis of ethanol extract of leaves of *A. madraspatana* can provide standard fingerprint and can be used as a reference for the identification and quality control of the drug. Further the ethanol extract of leaves of *A. madraspatana* posses the *in-vitro* antioxidant, anti-inflammatory and anti-diabetic activities. The antioxidant, anti-inflammatory and anti-diabetic activities of ethanol extract may be due to the presence of polyphenolic compounds such as phenols, flavonoids, tannins and steroids. Further study in an animal model to confirm the antioxidant, anti-inflammatory and anti-diabetic activities of ethanol extract is warranted. It may be true that the isolated phytoconstituents from the ethanol extract of leaves of *A. madraspatana* may have better effects on all these activities.

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