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ANTIOXIDANT AND ACETYLCHOLINESTERASE INHIBITORY ACTIVITIES OF METHANOLIC EXTRACT OF *GRANGEA MADERASPATANA*

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ABSTRACT

Background: *Grangea maderaspatana* has traditionally been used to treat neurological disorders. The purpose of this study was to look into the phenolic content, antioxidant activity, and acetylcholinesterase enzyme (AChE) inhibitory activities of various fractions of *Grangea maderaspatana* grown in local areas of kurnool.

Methods: The whole plant of *Grangea maderaspatana* was extracted with methanol and total phenolic content was estimated by folin-catechu method. DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay was used to measure antioxidant activity, while Ellman's colorimetric method was used to measure AChE inhibitory activity.

Results: Our findings indicated that the methanolic fraction had the highest total phenol concentration, which is comparable to 25.21.4 mg quercetin/g of fraction. Our findings also showed that the methanolic fraction had the highest antioxidant activity and AChE inhibitory activity when compared with the standard in a dose-dependent manner.

Conclusions: Our findings suggest that *Grangea maderaspatana* methanolic extract could be a promising source of AChEinhibitors for Alzheimer's disease.

Keywords: Alzeimer's disease, DPPH radical, IC 50, HPTLC fingerprinting

INTRODUCTION

Plant-based medicine has been practised throughout the world since time immemorial. According to the WHO, approximately 80% of people rely on traditional and herbal medicine systems for primary health care **[1]**. Phytochemicals found in medicinal plants are the secondary metabolites that serve specific biological functions in the plant host and have potential disease-inhibiting abilities in animals **[2]**. Indian medicine systems like Ayurveda, Siddha and Unani use traditional herbs and plants against various diseases. Recently, there is a thrust in the research and development of traditional plant based drugs in modern medicine due to their remarkable versatility and proven efficacy. It is estimated that 25% of drugs are plant based, suggesting the significant role of plants as a medicine source **[3]**.

Grangea maderaspatana, also known as Madras carpet, is one such weed that grows in sandy lands and waste areas. It contains flavonoids, diterepenes, sesquiterpenoids, steroid, and essential oil, according to reports. It is a medicinal plant that is widely used in Indian traditional medicine to treat a variety of ailments. The herb is useful for eye and ear pain. The root is an appetiser, diuretic, anthelmintic, emmenogogue, galactogogue, stimulant, beneficial in griping, chest and lung problems, headache, paralysis, rheumatism in the knee joint, piles, muscle pain, spleen and liver diseases, and reduces sweating. The plant has stomachic and uterine stimulant properties **[4]**.

According to Hegde *et al*., each plant has a distinct nature in terms of botany, chemistry, and therapeutic potency, and it is critical to study a medicinal plant's pharmacognostic characteristics not only for proper identification, but also to understand its structure and biology **[5]**. According to the literature, only a few studies on *Grangea maderaspatana* have been conducted. As a result, the dried plant material of *Grangea maderaspatana* was studied for its pharmacognostic properties in this study.

MATERIALS AND METHODS

Collection and identification of plant material

G. maderaspatana plant materials (whole plant) were collected from a field near Nandinapalle village in the Kurnool district, AP. The plant was identified and authenticated by the taxonomist Prof. P. Jayaraman of Plant Anatomy Research Centre, Chennai. The plant was dried under shade. Air dried plant material was ground to #10 powder and the plant material was then used for further investigations.

Chemicals, reagents, and solvents

All of the chemicals, reagents, and solvents utilised in the experiment were of analytical quality.

Physicochemical screening

Physicochemical parameters such as moisture content, ash value, , and extractive values were determined in accordance with WHO quality control methods for herbal materials **[6-8]**.

The methanolic extract was subjected to preliminary phytochemical screening to determine the presence of alkaloids, glycosides, flavonoids, phytosterols, tannins/phenolic compounds, carbohydrates, proteins, and fats. The extracts were filtered after being treated with a few drops of diluted HCl. The filtrates were then treated with Wagner's, Hager's, and Dragendroff's reagents, In order to detect the presence of alkaloids. One gram of the extract was dissolved in a few drops of dry acetic acid, 3 ml of acetic anhydride, and then a few drops of strong sulfuric acid in order to qualitatively analyse the presence of phytosterol. Three independent assays were carried out for tannins and phenolics using 5% diluted ferric chloride solution, 1% solution of gelatin with 10% NaCl, and 10% lead acetate solution. Shinoda's test was run for flavonoids. The extracts were dissolved in alcohol, to which a piece of magnesium was added after conc.Hcl was poured drop by drop. The mixture was then heated to obtain a magenta colour. The extracts were diluted in 5 ml of distilled water, filtered, and then tested for carbohydrates using Molisch's, Fehling's, and Barfoed's methods using the filtrate. In order to conduct Biuret and Millon's test to identify proteins, the extracts were diluted in water. The extracts were individually pressed between two filters papers to get the presence of fixed oils. It is clear that solidified oil was present from the oil spots on the paper. Additionally, a test known as a saponification was conducted to see if fixed oils and fats developed as a result of adding 0.5N alcoholic potassium hydroxide and phenolphthalein to the mixture and heating it in a water bath **[9, 10]**.

HPLTC fingerprinting

HPTLC fingerprinting for oleanolic acid and ursolic acid was done on *G. maderaspatana* extracts in chloroform and methanol. 20 mg of each extract were precisely weighed into a volumetric flask, and 10 mL of methanol were then added. Whatman Filter Paper No. 1 was used to dissolve it, filter it, and use the results for HPTLC profiling. The HPTLC chromatographic condition is given in **Table 1**.

Quantification of Quercetin-3-rutinoside by HPLC

Estimation of Quercetin-3-rutinoside in methanol extracts of *G. maderaspatana* was performed by HPLC **[13]**. *G.*

maderaspatana powder (50 mg) was dispersed in 50 ml of methanol. Solution was sonicated (for 15 minutes) and then vortexed (for <1 minutes). Obtained methanolic extract had concentration of 1 mg/ml this solution was used for the HPLC analysis. The HPLC chromatographic condition is given in **Table 2**.

Acetylcholinesterase (AChE) inhibition assay

Using a spectrophotometric technique, AChE inhibitory activity was evaluated **[14–16]**. When acetylthiocholine is hydrolyzed by enzyme and then combines with DNTB (5, 5'-dithiobis-2-nitrobenzoic acid) ion, thiocholine produces a yellow colour, which was used to measure the enzyme activity. At 405 nm, this is detectable. Tris-HCl buffer 50 mM, pH 8, 0.1 percent BSA as an enzyme blank, and galantamine as a reference standard were employed as the positive controls (enzyme activity without extract), respectively. The enzyme (0.2 U/mL) was dissolved in 50 mM of Tris-HCl buffer, pH 8 and 15 mM of the substrate ATCI (Acethylthiocholine Iodide) kinetic reaction was seen for three minutes.

Following equation was used to calculate the amount of enzyme inhibition (I percent) of the enzymatic reaction:

$$I\% = \frac{(E - S)}{E} \times 100$$

where,

E: The enzyme's substrate hydrolysis kinetics without the test substance

S: The enzyme's substrate hydrolysis kinetics witH the test substance

DPPH assay for measuring antioxidant activity

Preparation of DPPH solution

In a conical flask, DPPH (5 mg) was accurately weighed and dissolved in ethanol (10 ml). Further, DPPH solution (2 ml) was diluted with ethanol (38 ml) in such way that the OD of DPPH solution was 0.7 at 517 nm, to get DPPH working solution **[17, 18]**.

Preparation of sample solution

In a micro centrifuge tube, *G. maderaspatana* powder (10mg) was accurately weighed and methanol (2 ml) was added. It was vortexed for 4min and centrifuged at 8000 rpm for 5 min. Supernatant (5 mg/ml) was collected. 200 l of the supernatant was collected in a micro centrifuge tube and diluted with methanol to make a solution containing 1 mg/ml. 200 l of the aforementioned solution was taken in a micro centrifuge tube and diluted to a volume of 2 ml with methanol to produce a solution containing (0.1 mg/ml).This solution was used for DPPH assay. Ethanol (1 ml) was added to the DPPH working solution (0.5 ml), and absorbance at 517 nm was measured. The test sample was made according to **Table 3's** instructions. Samples were incubated for 20 minutes in a

dark atmosphere after reactants were added. At 517 nm, absorbance was measured. For each concentration, the test was run in triplicate.

Calculation

The following formula was used to calculate The percent inhibition (% IC) of DPPH radical,

%IC= [(ODDPPH control-ODtest)/OD DPPHcontrol] X 100

Calculation of 50% inhibition conc. (IC_{50}):

The concentration which scavenges 50% of the radical (IC_{50}) is calculated by plotting % IC in Y-axis and sample doses in X-axis.

Statistical Analysis

According to regression analysis of the relationship between scavenging activities (in percent) and different concentrations of the extract, the amount of effective concentration of the extract required to inhibit free radicals by 50%, or Inhibitory Concentration (IC50), was calculated. The antioxidant assay was run in triplicate, and the results were shown as mean + SD.

RESULTS & DISCUSSION

Phytochemical screening

Table 4 contains a compilation of the findings from the various analyses. An indication of the earthy matter, inorganic composition, and other contaminants present with the medicine was provided by the ash values of the drug. The primary function of the extractive values is to identify medicine that has been exhausted or that has been adulterated with. *Grangea maderaspatana* powdered portions were extracted using several solvents in succession (except water extract which was prepared by decoction). **Table 5** lists the various extracts that were collected along with their yield percentage, colour, and consistency.

qualitative analysis

The results of the various qualitative chemical tests performed on the extracts obtained through successive solvent extraction processes to identify the presence of various phytoconstituents, such as steroids, carbohydrates, alkaloids, glycosides, phenolics, and tannins, among others, are shown in **Table 6**.

HPTLC fingerprinting

Utilizing thin layer chromatography, quick screening of oleanolic acid and ursolic acid was investigated (TLC). Toluene:Ethyl acetate:Formic acid (8:2:0.1) led to a better separation. The visualizer recorded the TLC run data, and Figure 1 shows a picture of the chromatographic plate along with the results. After the anisaldehyde-sulphuric acid reagent was sprayed, all four bands were recognised **(Figure 1)**. In **Table 7**, Rf values and the AUC for each of the four bands were listed. Figure 2-5 shows the HPTLC chromatogram of the standard oleanolic acid, standard ursolic acid, and the methanol and chloroform extract of *G.*

maderaspatana . Oleanolic acid and ursolic acid are present in 4.0 percent and 9.5 percent, respectively, of the chloroform extract of *Grangea maderaspatana* , whereas the methanol extract contains Estimation of Quercetin-3-rutinoside in methanol extracts of *G. maderaspatana* by HPLC method 3.0% and 6.5% of oleanolic acid and ursolic acid respectively.

HPLC programming for mobile phase is given in **Table 8**. To assign the peaks in chromatogram, standard compounds (Quercetin-3-rutinoside) was injected in same HPLC gradient system. The corresponding retention time for the standard compound Quercetin-3-rutinoside was 22.22 min. The HPLC chromatogram of *G. maderaspatana* methanol extract and Quercetin-3-rutinoside were given in **Figure 6 and 7**.

DPPH radical scavanging of methanolic extract of G. maderasapatna

The DPPH radical inhibition percentage for the methanolic extract of *G. maderaspatna* was determined. The average IC50 was determined, and **Table 9** shows the results. The methanolic extract of G. maderasapatna used in in vitro assays to determine its antioxidant capacity demonstrated DPPH activity. Antioxidant-DPPH interaction results in 1, 1-diphenyl-2-picryl hydrazine, a persistent free radical. The decrease in absorbance at 517 nm was used to gauge the capacity to neutralise the stable free radical DPPH. The IC50 value of the extract is the amount of extract required to block the DPPH free radical 50% of the time. A lower IC50 value indicates that the sample is more effective in scavenging. **Figure 8** depicts the percentage inhibition.

AchE Inhibition of methanolic extract of G. maderasapatna

Table 10 and Figure 9 both show the methanolic extract of *G. maderaspatna* 's inhibitory efficacy against AChE at concentrations of 50 to 250 g/mL. The percentage of inhibition of *G. maderaspatna* 's methanolic extract ranged from 27.080.12 to 41.220.26. The presence of phenolic acids, flavonoids, and other antioxidant substances may be the cause of the inhibition. Antioxidant substances might be responsible for inhibiting AChE.

Table 1: HPTLC Chromatographic condition

System	**CAMAG Linomat 5**
Method	**Quercetin-3-rutinoside**
Column	**Merk –TLC/HPTLC silica gel 60 F254 on Aluminum sheets (10×10 cm)**
Injection volume	**10µL**
Mobile phase	**Toluene:Ethyl acetate:Formic acid (8:2:0.1)**
Spray agent	**p-anisaldehyde reagent**
Absorbance	**510nm**

Table 2: HPLC Chromatographic condition

System	**Alliance-Water 9PDA 2996)**
Method	**Quercetin-3-rutinoside**
Column	**Xterra RP column (250×4 mm, 5 µm merk)**
Solven system	**Gradient; (A) 0.1% Aqueous Formic acid (B) Methanol**
Detection	**270 nm (PDA detector)**
Flow rate	**0.7 ml/min**
Run time	**35 min**

Table 3: Preparation of test sample for DPPH assay

Quantity of sample	Volume taken (in µl)			Total volume
	G.maderaspatna powder (0.1mg/ml)	Ethanol	DPPH working solution	
1µg	10	990	500	1.5ml
2µg	20	980	500	
3µg	30	970	500	
4µg	40	960	500	
5µg	50	950	500	
6µg	60	940	500	
8µg	80	920	500	
10µg	100	900	500	
12µg	120	880	500	

Table 4: Physico-Chemical Parameters of powder of *G. maderaspatana*

S. No	Parameters	Values (% w/w)
1	Loss on drying	8
2	Ash value	
	Total ash	11.60
	Acid insoluble ash	1.80
	Water soluble ash	4.50
3	Extractive value	
	Water soluble extractive	10
	Alcohol soluble extractive	10

The values given here are expressed as percentage of air dried material. Each value is average of three determinations

Table 5: Preliminary phytoprofile of *G. maderaspatana*

S. No	Solvent	Colour	Consistency	% yield w/w
1	Petroleum ether	Light green	Slight sticky	4.82
2	Chloroform	Darak green	Slight sticky	1.37
3	Ethyl acetate	Brownish green	Sticky	2.50
4	Methanol	Brown	Slight sticky	6.20
5	Water	Dark brown	Sight sticky	5.34

Table 6: Phytochemical screening of extracts of *G. maderaspatana*

Chemical constitutents	Pertroleum ether extract	Chloroform extract	Ethylacetate extract	Methanol extract	Water extract
Carbohydrate	-	-	-	+	+
Protein	-	-	-	-	-
Phenolics & Tannins	-	-	-	+	+
Saponins	-	-	-	+	+
Flavanoids	-	-	-	+	+
Terpenes	+	+	+	+	-
Steriods	+	+	-	-	-
Alkaloids	-	-	-	-	-

+ positive, - negative

Table 7: Result of HPTLC analysis of extracts of *G. maderaspatana*

	Sample			
	Methanoloic extract	Oleanolic acid	Ursolic acid	Chloroform extract
Rf value	0.50	0.48	0.48	0.49
AUC	1517.5	24373.6	11179.7	2142.7

Table 8: Gradient system of solvent-A and solvent-B

Time (Min.)	Solvent A (%)	Solvent B (%)
0	95.0	5.0
12.0	60.0	40.0
13	95.0	5.0
15	95.0	5.0

Table 9: Percent inhibition of DPPH radical by test samples at different concentrations

Quantity of sample (µg)	Percentage inhibition of DPPH radical
1	12.56 (1.35)
2	26.41 (1.95)
4	46.83 (1.30)
5	55.35 (0.87)
6	63.47 (0.42)
8	76.79 (1.18)
10	84.48 (0.23)
12	88.95 (0.55)

Values are expressed in Mean (SD) (n=3)

Table 10: % AchE Inhibition of methanolic extract of *G. maderasapatna*

S. No	Conc. (µg/ml)	% Inhibition AchE
1	50	27.08±0.12
2	100	29.24±0.26
3	150	32.36±0.29
4	200	40.18±0.18
5	250	41.22±0.26

All values in the table represent mean ±SD (n=3)

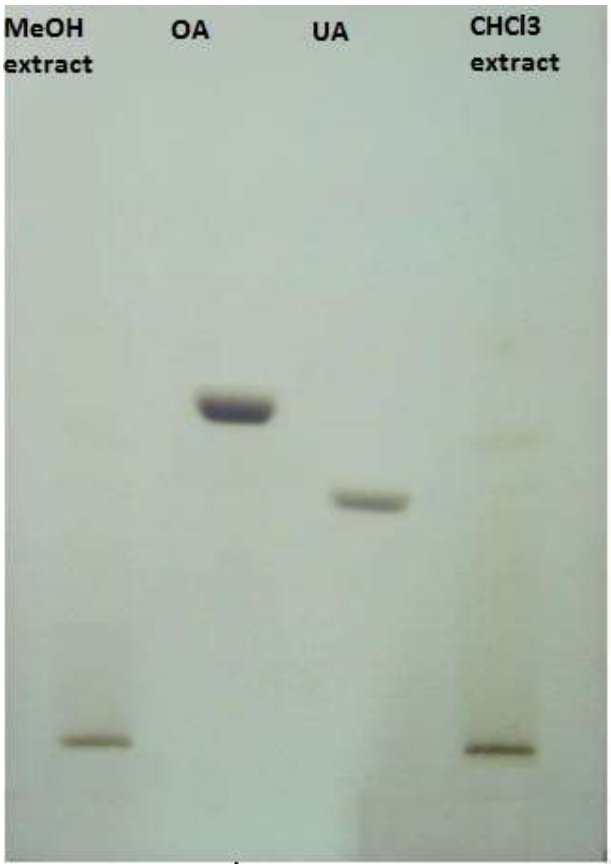

Figure 1: HPTLC plate of *G. maderaspatana* extracts after derivatization (MeoH extract - *G. maderaspatana* methanolic extract, OA- Oleanolic acid (standard 1), UA - Ursolic acid (standard 2), CHCl3- *G. maderaspatana* chloroform extract)

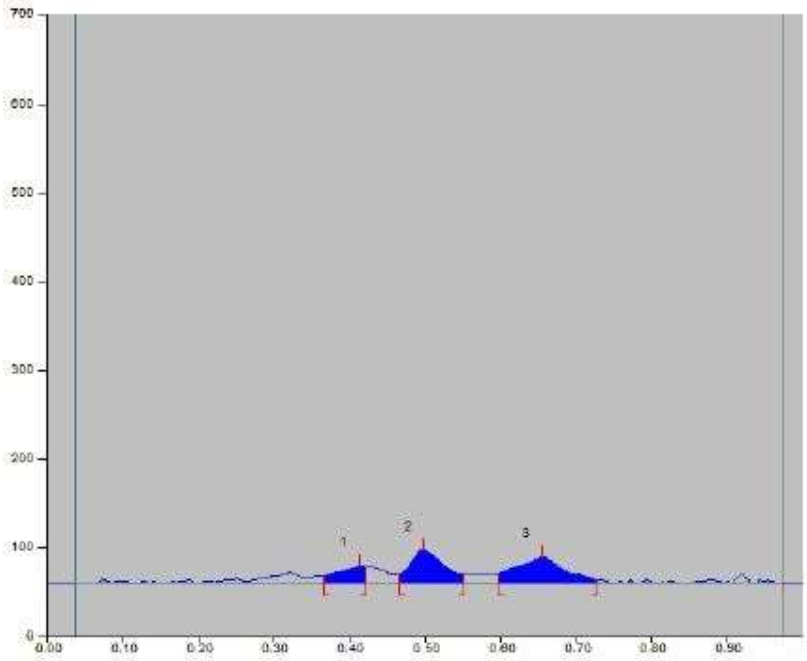

Figure 2: Chromatogram of methanol extract of *G. maderaspatana*

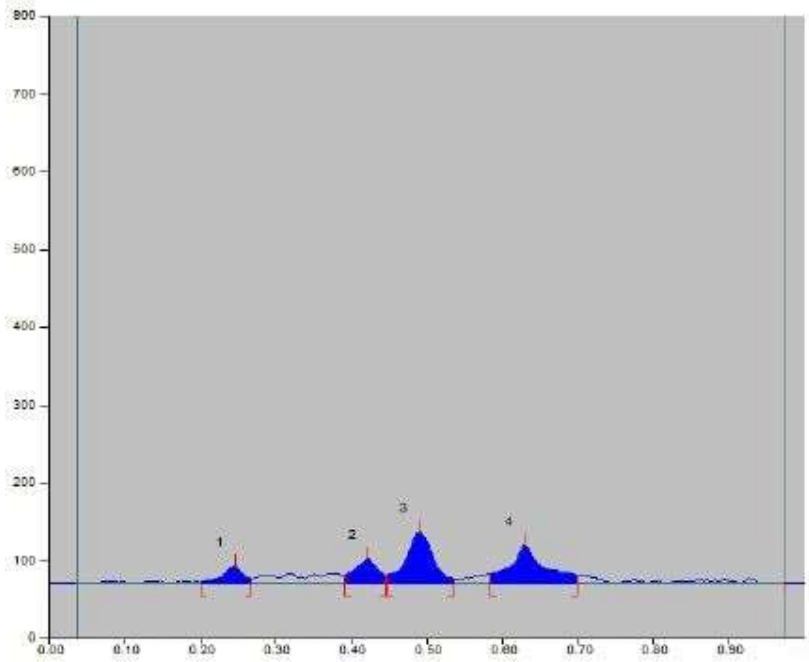

Figure 3: Chromatogram of chloroform extract of *G. maderaspatana*

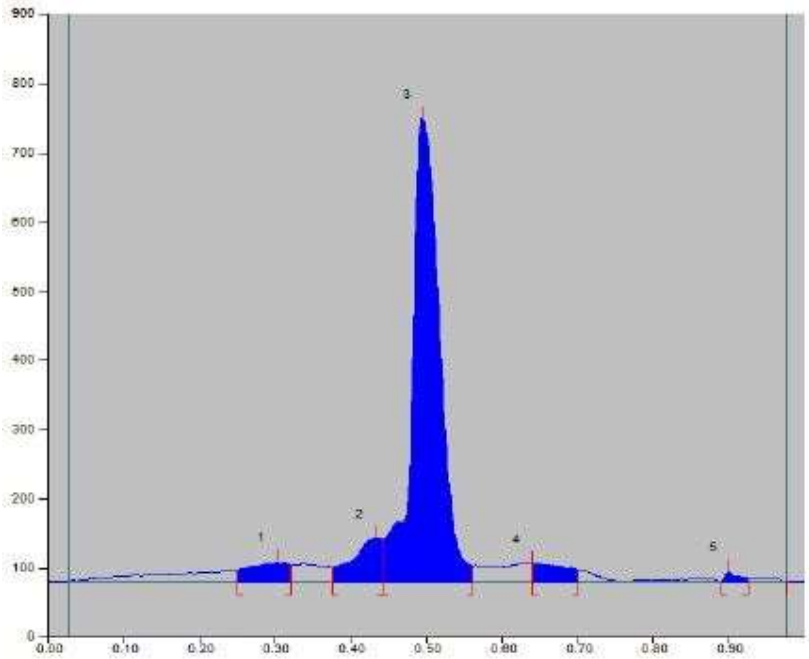

Figure 4: Chromatogram of standard Oleanolic acid

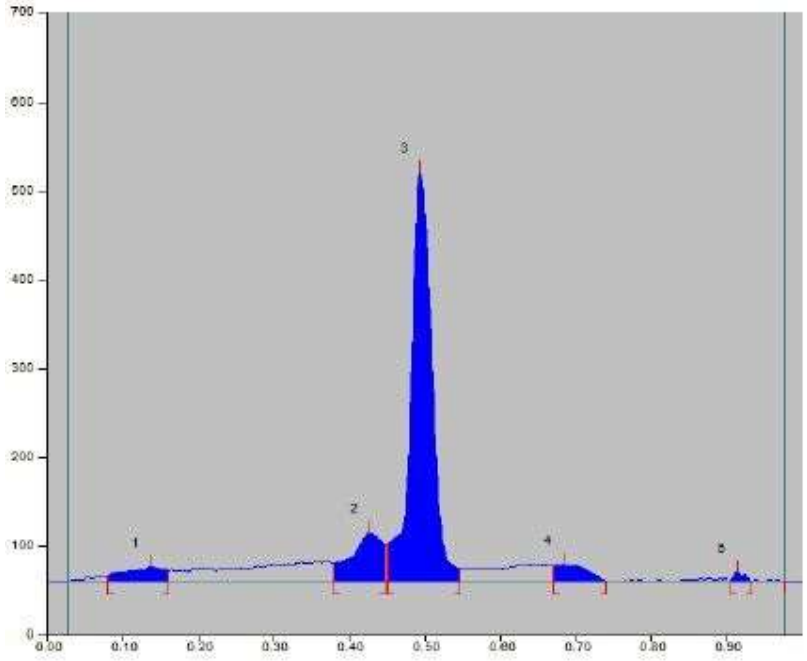

Figure 5: Chromatogram of standard Ursolic acid

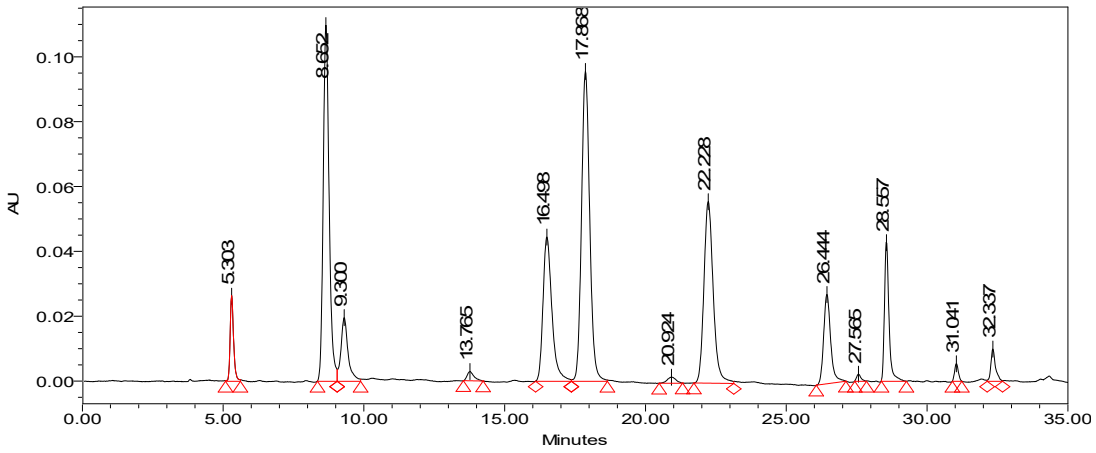

Figure 6: HPLC chromatogram of *G. maderaspatana* methanol extract

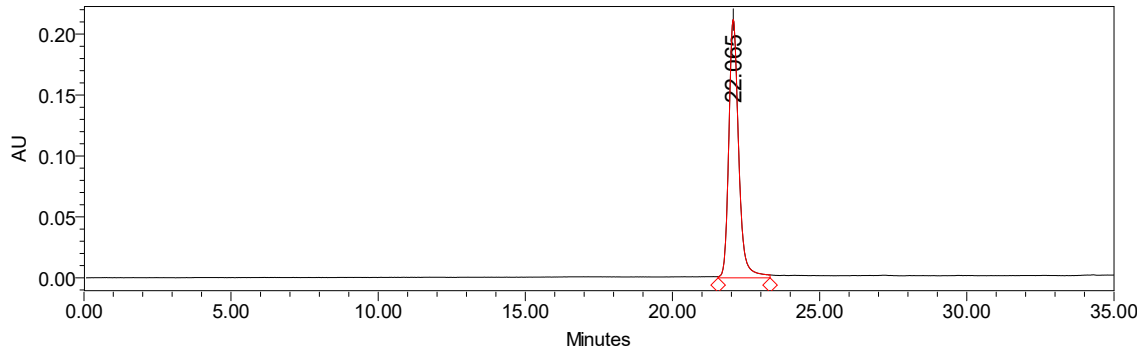

Figure 7: HPLC chromatogram of Quercetin-3-rutinoside

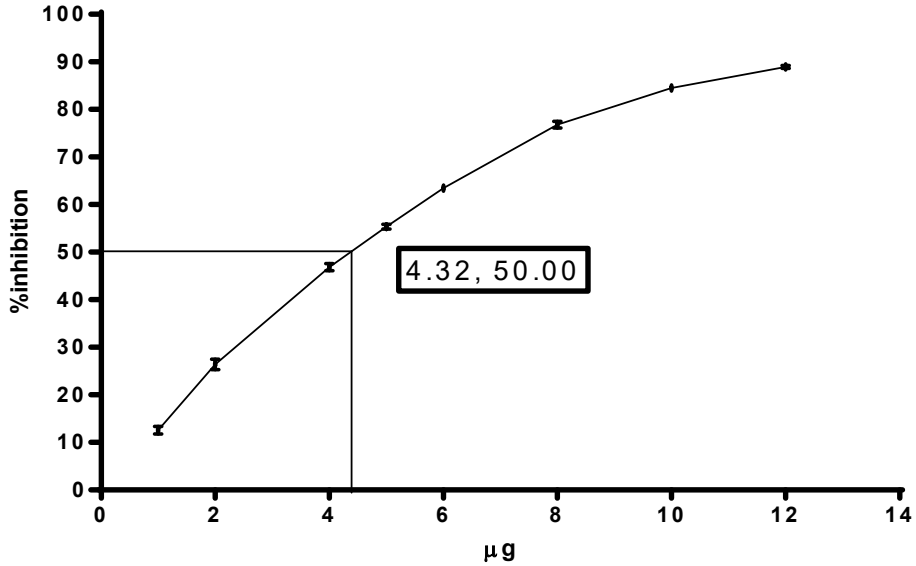

Figure 8: Percent inhibition of DPPH scavenging by methanolic extract of *G. maderaspatna* at different concentrations

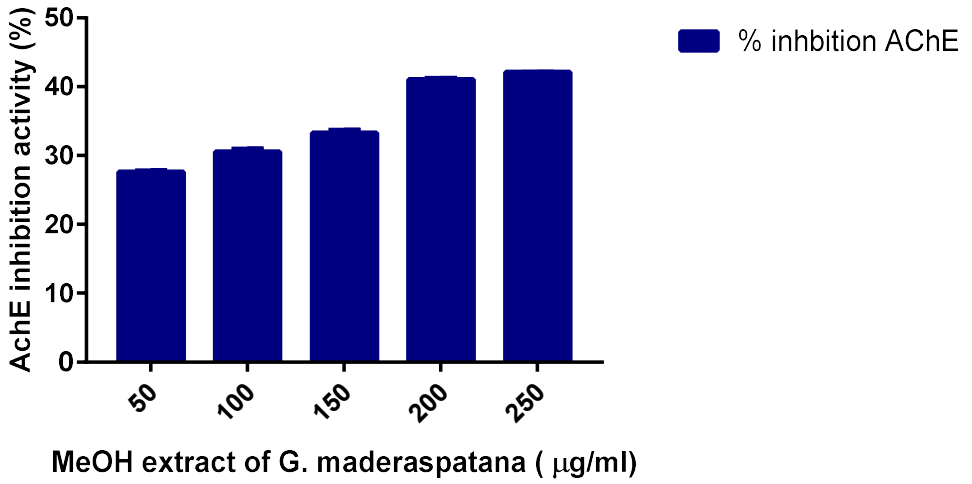

Figure 9: MeOH extract of *G. maderaspatana* inhibits acetylcholinesterase. The extract was tested for its acetylcholinesterase inhibitory activity. Each bar represents the mean percentage of inhibitory activity ±SD. (*P<0.001; n=3)

CONCLUSION

The phytochemical studies described in the current study require additional scientific research to determine their identity down to the component level. To support the use of *G. madarasapatna* by traditional healers, research is required on a variety of biological processes that are similar to those of *G. madarasapatna*. The standardisation of *G. madarasapatna*'s quantitative and qualitative properties will be helped by the pharmacognostic characteristics that were researched. For *G. madarasapatna*, comprehensive differential studies utilising molecular and chemical markers are needed, particularly for their authentication in their medication form.

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CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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