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Experiment Findings · March 2017

DOI: 10.13140/RG.2.2.25716.96640

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Evaluation of Memory Enhancing Effect of a Compound Isolated from *Emblica Officinalis* Fruit

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(Received: Dec 2016 Revised: Feb 2017 Accepted: Mar 2017)

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ABSTRACT

Introduction and Aim: The investigation was carried out to separate bioactive constituent from *Emblica officinalis* fruit and to evaluate the memory enhancing activity of the isolated compound.

Materials and Methods: Successive extraction of the plant was made using different non-polar to polar solvents to extract out active principles according to their solubility. Memory enhancing activity was confirmed using scopolamine and sodium nitrite induced memory deficits (amnesia), elevated plus-maze (EPM) and Morris water maze (MWM).

Results: Memory deficits in male albino mice induced by scopolamine hydrochloride and sodium nitrite were recorded as transfer latency time (TLT) in EPM model; escape latency time (ELT) and time spent in the Q4 target quadrant (TSTQ) in MWM model. Scopolamine and sodium nitrite markedly decreased the TLT over 2 min, ELT, TSTQ over 90 sec and consecutively impaired learning and memory. Bioactivity-guided fractionation of the memory enhancing methanolic extract has led to the isolation of a bioactive polar compound. Structure elucidation of the compound by UV, IR, ^{1}H NMR, ^{13}C NMR, MS techniques indicated it being biphenyl dicarboxylic acid.

Conclusion: The memory deficits were significantly reversed by the biphenyl dicarboxylic acid thus confirming its memory enhancing the effect. The biphenyl dicarboxylic acid was found to be responsible for memory enhancing activity of the plant.

Key Words: Bioactivity-guided fractionation, Amla, Amnesia, Nootropics.

INTRODUCTION

Emblica officinalis Gaertn (Euphorbiaceae), a deciduous tree has been reported to contain constituents with variable biological effects (1). The plant is indigenously known Amla in Hindi and Usiri in Telugu. It is used both as medicine and as a tonic to build up lost vitality and vigor. Amla is regarded as the best among rejuvenating herbs, useful in a cough and skin disease and best among the sour fruits (2). The fruit of *E. officinalis* is reported to contain 20 times more vitamin C to that of orange and has been used for its diuretic, cooling, laxative, expectorant activities and in hair preparations. The fruit is widely used in Ayurvedic preparations (Chyavanprash, Kanchnar and Triphala Guggulu). It is also used in glandular swellings and weight reduction (3). It also possesses anti-tumor, immunomodulatory, expectorant, cardiotonic, anti-pyretic, anti-oxidative, anti-viral, anti-emetic, anthelmintic, anti-tussive and gastroprotective properties (4). Phytoconstituents reported from the fruit of the plant include furanolactone, ellagitannins, hydrolysable tannins, alkaloids, coumarins, organic acid gallates, norsesquiterpenoids, polyphenolic

compounds: benzenoids: organic acid gallates, hydrolysable tannins, ellagitannins and flavonoids (5).

The methanolic extract of *E. officinalis* when evaluated as memory enhancer using elevated plus maze and Morris water maze exhibited significant memory improving effect in mice (6). Bioactivity-guided fractionation of this extract using gradient elution column chromatography led to the separation of a fraction F-5.1.4.1 containing compound 1 which was found to be responsible for memory enhancing the activity of the methanolic extract. The present study was carried out to separate the bioactive component of the fraction F-5.1.4.1 and to investigate its memory enhancing the effect.

MATERIALS AND METHODS

The experimental protocol was approved by the Institutional Animal Ethics Committee (1279/ac/09/CPCSEA) and experiments were conducted according to the Committee for the purpose of control and supervision of experiment on animal (CPCSEA) guidelines on the use and care of experimental animals.

Plant drug

The fruit of *E. officinalis* Gaertn was collected, from Ghaziabad, U.P. India. The fruit was authenticated by Dr. H.B. Singh, Head and Taxonomist, NISCAIR, CSIR, New Delhi. A voucher specimen no: NISCAIR/RHMD/consult/06/721/38 was deposited for the plant in the same herbarium.

Morphology of amla fruit

The fruits were spherical, fluorescent yellow with the shallow longitudinal furrow. The seed was single, elliptical, hard nut. The fruit was a berry and consisted of thin epicarp and wide fleshy mesocarp. The epicarp consisted of the very thin epidermis of narrow oblong cells and a subepidermal layer of fairly wider tabular cells. The two layered epicarp was 20 µm thick. The mesocarp was wider part of the fruit; it was fleshy and juicy. It consisted of thin walled compact parenchyma cells throughout. The cells towards the periphery were smaller, and the size increased progressively towards the centre. The cell walls were mucilaginous and flaccid. Several vascular bundles were scattered in the mesocarp. The bundles were diffuse in distribution and not much prominent. The vascular bundle had a mass of thick-walled xylem elements and a patch of phloem adjoining the xylem. The vascular bundle was surrounded by a thin layer of small parenchyma cells.

Powder microscopy of amla fruit

Coarse powder of fruit showed the following inclusions as seen under the microscope:

1. Small pieces of the mesocarp tissue with large, thin-walled parenchyma cells were observed. When these pieces were stained with Sudan stain, amorphous masses of reddish-brown substance, the lipid was seen. The lipid occurs in random within the parenchyma cells.

2. When the mesocarp tissue was observed under the polarized light microscope, large masses of calcium oxalate crystals were seen in the mesocarp cells. The crystal masses consisted of thick needles which were closely aggregated into fan-shaped bodies. These crystal masses were seen mostly adhering to the walls of the mesocarp cells. The masses are 150 x 200 µm in size.

3. Fragments of epicarp were seen in the surface view. The epicarp (epidermis) had small polyhedral thin walled compact parenchyma cells.

Extraction

Air dried fruit (20 g) of *Emblica officinalis* Gaertn (Euphorbiaceae) was coarsely powered and extracted with methanol for 18 hours, under reflux by continuous hot percolation method using "Soxhlet apparatus"

(7). The methanolic extract of the plant was further fractionated successively with organic non-polar to polar solvents such as petroleum ether (60-80 °C), benzene, chloroform, ethyl acetate, methanol, and water. In this method, the sample was dried, powdered and placed in a filter paper thimble. The thimble was then placed in a glass extractor. The glass extractor was then placed on a suitable round bottom flask (Perfit, India) containing extracting solvent. Above the extractor, water condenser was placed.

After complete extraction, the flask containing the solvent and biomolecules was removed, and the solvent was distilled under rotatory vacuum evaporator (Perfit, India) under reduced pressure at ≤ 50°C temperature to afford the extract of the medicinal plant. Each time before extracting with the next solvent, Marc was dried in air. The crude extract obtained after evaporation was stored in a desiccator. The extract obtained with each solvent was weighed, and its percentage was calculated regarding air dried weight of the plant drug.

Separation of bioactive natural product from the fruit of *Emblica officinalis* by column chromatography

Column Chromatography is common and useful separation technique in phytochemistry. The slurry method was used for macro-scale separations. The solid stationary phase was combined with a small amount of non-polar solvent in a beaker. Both were thoroughly mixed until a consistent paste was formed, but was then capable of flowing. This homogeneous mixture was poured into the column as carefully as possible using a spatula to scrap out the solid. Once the column was loaded, the stopcock was opened, and the solvent level was allowed to drop to the top of the packing, but the solvent layer was not allowed to go below this point. Allowing this solvent level to go below the stationary phase, (known as letting the column to "run dry,") should always be avoided since it allowed air bubbles and channel formation to occur leading to a poor separation. Once the packing was complete, the sample was loaded directly to the top of the column.

Sample loading

The dark viscous masses of methanolic extracts were adsorbed on Silica gel for the column to form slurries. The slurries were air dried and chromatographed over silica gel columns packed in petroleum ether. Each column was eluted successively with petroleum ether, mixtures of petroleum ether and chloroform (90:10, 75:25, 50:50, 25:75), chloroform and finally with the mixtures of chloroform and methanol (99.5:0.5, 99:1, 98:2, 95:5, 90:10, etc.) in order of increasing polarity. A minimum amount (5-10 drops) of a polar solvent is used to dissolve the mixture. The solution was then carefully added to the top of the column using a pipette without disrupting the flat top surface of the column. Once the mixture was added, and the protective layer of sand was in place, the solvent eluent was continuously added while collecting small fractions at the bottom of the column. Using a pipette, the first bit of solvent was added on top of the packing, sample, and sand to minimize disturbance of the column and to avoid diluting the sample. A collection of small fractions (1-3 ml) is important for the success of column separation. After confirming all the materials had been removed from the column, the colors of the materials or results of TLC indicated which fractions contained the desired compound(s). The same fractions were combined, and the solvent was evaporated. The pure separated compound was left behind. Re-crystallization was used to further purify a solid product.

General procedures

Bioactive fraction was subjected to chromatographic and spectroscopic studies UV (UV-1700 pharma UV-VIS, Shimadzu), Mass (Micromass Quattro II, LCMS), IR (Multi spoke FT-IR synthesis monitoring

system, Perkin-Elmer, Germany). ^{1}H NMR and ^{13}C NMR (Bruker Advance 400, Ultra Shield, ZH079807, Avane, Germany). The fractions were scanned in the range of 0-1400 m/z. All column chromatographic separations were performed on silica gel 60 (230-400 mesh), while thin layer chromatography (TLC) was performed on silica gel-coated aluminum plates using 20×20 cm Fertigfolien/precoated sheets (0.20 mm) Alugram silica gel G/UV_{254} (Machereny-Nagel).

Drugs and chemicals

Piracetam, scopolamine and sodium nitrite were procured from Sigma-Aldrich, Poole U. K., Cadila Health Care (Ahmadabad) and Center Drug House (New Delhi) respectively. All other chemicals used in the study were purchased from S. D. Fine Chemicals Ltd. (Boisar, India).

Experimental animals

Swiss albino male mice (30±2 g) procured from Central Drug Research Institute, Lucknow, India was housed in the animal house provided with 12 hours light and 12 hours dark cycles at 25±2°C and had free access to water and standard laboratory diet (Ashirwad Industries, Chandigarh, India).

Assessment of motor coordination using rota-rod

The ability of the mouse to hold on to the horizontally rotating rod (diameter 2.5 cm, 4 rotations per minutes) was used to assess motor co-ordination (8). Mice which demonstrated impairment of muscle co-ordination (ataxia) with or without drug treatment were not included in the study. 15-20 % exclusion rate was noted.

Evaluation of memory enhancing activity

The memory enhancing activity of the isolated compound was evaluated in mice on scopolamine hydrochloride and sodium nitrite induced amnesia, as interoceptive behavioral models and elevated plus-maze and Morris water maze as exteroceptive behavioral models (where stimulus existed outside the body).

Elevated plus-maze model (EPM)

The EPM served as the exteroceptive behavioral model to evaluate learning and memory in mice (9). The apparatus consists of two open arms (16cm × 5cm) and two covered arms (16cm ×5cm× 12cm). The arms extended from a central platform (5cm × 5cm), and the maze is elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency time (TLT) is the time taken by the mouse to move into any one of the covered arms with all its four legs. TLT was recorded on the first day. If the mouse does not enter into one of the covered arms within 120 sec, they were gently pushed into one of the two covered arms and the TLT was assigned as 120 sec. The mouse was allowed to explore the maze for 10 sec and then return to its home cage. Memory retention was calculated after 24 hrs of acquisition trial on the second day as inflation ratio (10) using the following formula:-

$$\text{Inflation ratio} = L_1 - L_0 / L_0$$

Where L_0 is the initial transfer latency time in second and L_1 is transfer latency time after 24 hrs.

Morris water maze (MWM) model

MWM was employed to evaluate learning and memory (11). It consisted of a circular water tank (diameter 150 cm and height 45 cm), filled with water maintained at 25°C. The water was made opaque with a white colored dye. The tank was divided into four equal quadrants with the help of two threads, fixed at the right angle to each other on the rim of the pool. A platform (10 cm^2) of 29 cm height was located in the center of one of these four quadrants. The position of platform and clues were kept consistent throughout the training session. In the present study, the TQ was

Q 4. Each animal was subjected to four consecutive trials on each day with an interval of 5 min, during which mouse was allowed to escape on the hidden platform and was allowed to remain there for 20 sec. In case the animal was unable to locate the hidden platform within 90 sec, it was gently guided by hand to the platform and allowed to remain there for 20 sec. Escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition and learning. In a preliminary study, the trial was administered to familiarize the mice with the task and was not counted. The mouse was subjected to acquisition trial for four consecutive days. Starting position on each day to conduct four acquisition trials was changed as follows:

Day 1	Q1	Q2	Q3	Q4
Day 2	Q2	Q3	Q4	Q1
Day 3	Q3	Q4	Q1	Q2
Day 4	Q4	Q1	Q2	Q3

Retrieval trials

On day 5, the platform was removed and time spent by mouse in each of four quadrants was noted. The time spent by mouse in Q 4 target quadrant (TSTQ) searching for the hidden platform was noted as an index of retrieval. For evaluation of amnesia, amnesic treatments (scopolamine or sodium nitrite) were administered in mice 30 min before acquisition trial conducted on four consecutive days (day 1 to day 4) and ELT was noted as an index of acquisition and learning. Normal saline (0.9% sodium chloride) solution was administered 30 min before retrieval trial conducted on day 5 increased time spent by mouse in the target quadrant (Q4) searching for the hidden platform was noted as an index of retrieval.

Experimental protocol

The extracts and compound 1 were suspended in normal saline (0.9 % w/v sodium chloride) solution. The compound 1 (1mg/kg, i.p.) was administered for seven consecutive days. Ten groups each comprising six animals were taken.

Group I (control) of normal untreated mice was exposed to EPM for measuring TLT on the first day and after 24 hours, i.e., on the second day (14). Group II, III, IV, and V, mice were administered normal saline solution (10 ml/kg, i.p.), scopolamine hydrochloride (0.4 mg/kg, i.p.) sodium nitrite (75 mg/kg, i.p.) and piracetam (400 mg/kg, i.p.) respectively, Group VI, mice were injected compound 1 (1 mg/kg, i.p.) for seven days. For groups II to V, TLT was recorded after 45 minutes and then after 24 hours, i.e., on the second day using elevated plus maze. For group VI, TLT was recorded after 60 minutes and then after 24 hours. Group VII, VIII, IX and X, mice were administered piracetam (400 mg/kg, i.p.) and scopolamine hydrochloride (0.4 mg/kg, i.p.), piracetam (400 mg/kg, i.p.) and sodium nitrite (75 mg/kg, i.p.), compound 1 (1 mg/kg, i.p.) and scopolamine hydrochloride (0.4 mg/kg, i.p.), compound 1 (1 mg/kg, i.p.) and sodium nitrite (75 mg/kg, i.p.) 60 and 45 minutes respectively prior to first day exposure on elevated plus maze. TLT was recorded on the first day and the second day.

Group I (control) of normal untreated mice was subjected to MWM for measuring ELT during acquisition trials conducted on four consecutive days and TSTQ during retrieval trial conducted on day 5. Group II, III, IV and V, mice were administered normal saline (10 ml/kg, i.p.), scopolamine hydrochloride (0.4 mg/kg, i.p.), sodium nitrite (75 mg/kg, i.p.) and piracetam (400 mg/kg, i.p.) respectively 45 minutes before acquisition trial conducted on four consecutive days. In the case of group VI, compound 1 (1 mg/kg, i.p.) was administered 60 minutes before acquisition trial conducted on four consecutive days. Groups VII, VIII, IX and X, mice were administered piracetam and scopolamine hydrochloride (0.4 mg/kg, i.p.), piracetam (400 mg/kg, i.p.) and sodium nitrite (75 mg/kg, i.p.), compound 1 (1 mg/kg, i.p.) and scopolamine (0.4 mg/kg, i.p.), compound

l (1 mg/kg) and sodium nitrite (75 mg/kg, i.p.) respectively, before acquisition trial conducted on four consecutive days. Groups VII to VIII, piracetam, and groups IX to X, compound 1 (P) were administered 60 minutes before the administration of scopolamine and sodium nitrite. In all the above-mentioned groups, 0.9 % w/v sodium chloride solution (10 ml/kg, i.p.) was administered 45 minutes before retrieval trial conducted on the fifth day.

Statistical Analysis

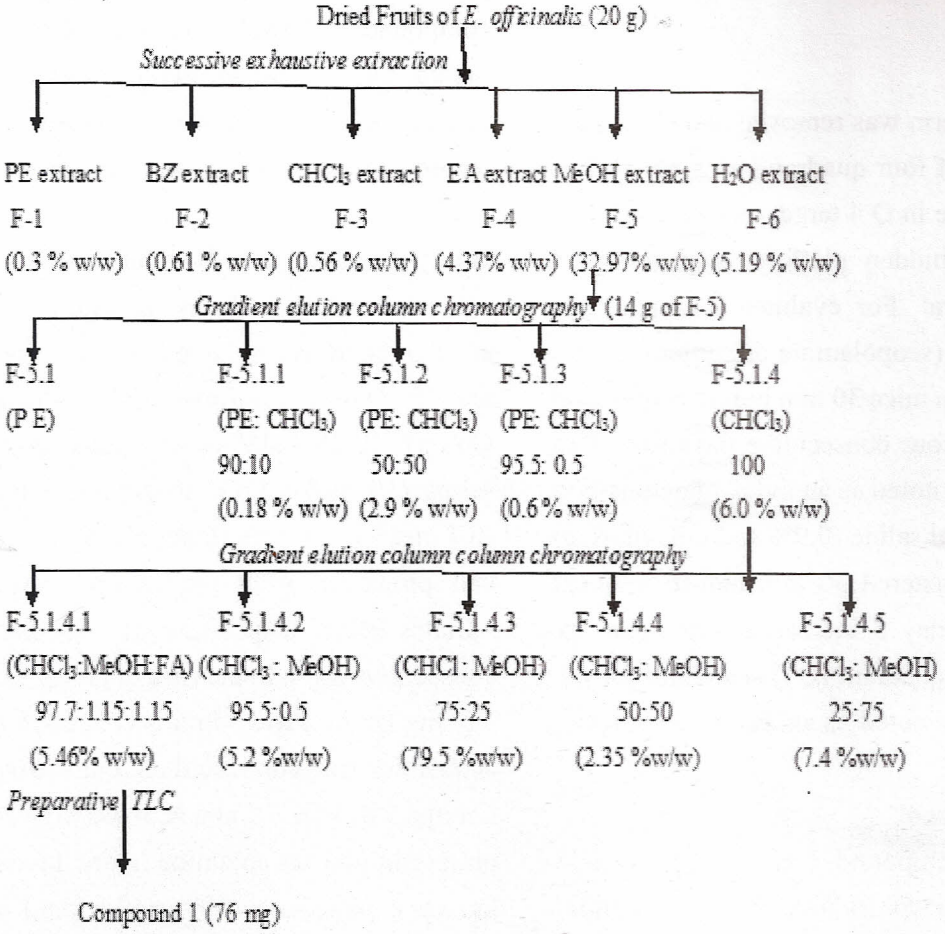
Results were expressed as means ± standard error of the mean (SEM). The data were analyzed with Graph Pad Prism statistical analysis using two-way analysis of variance followed by Bonferroni post-hoc test except in retrieval trial of Morris water maze for which the data were analyzed by one-way analysis of variance followed by Tukey's test. The p value of less than 0.05 was considered to be statistically significant.

RESULTS

Extraction

The methanolic extract of *Emblica officinalis* Gaertn (Euphorbiaceae), fruits were prepared and concentrated to obtain dark viscous mass. The yields of the petroleum ether, benzene, chloroform, ethyl acetate, methanol and water extracts of the fruit were found to be 0.3, 0.61, 0.56, 4.37, 32.97 w/w respectively (Figure 1).

Figure-1: Bioactivity-guided Separation of E. officinalis Fruit fraction. BZ: benzene; $CHCl_3$: chloroform; EA: ethyl acetate; FA: Formic acid; MeOH: methanol; Compound 1: Polar Compound Isolated from Methanolic Fraction; PE: Petroleum Ether



Isolation

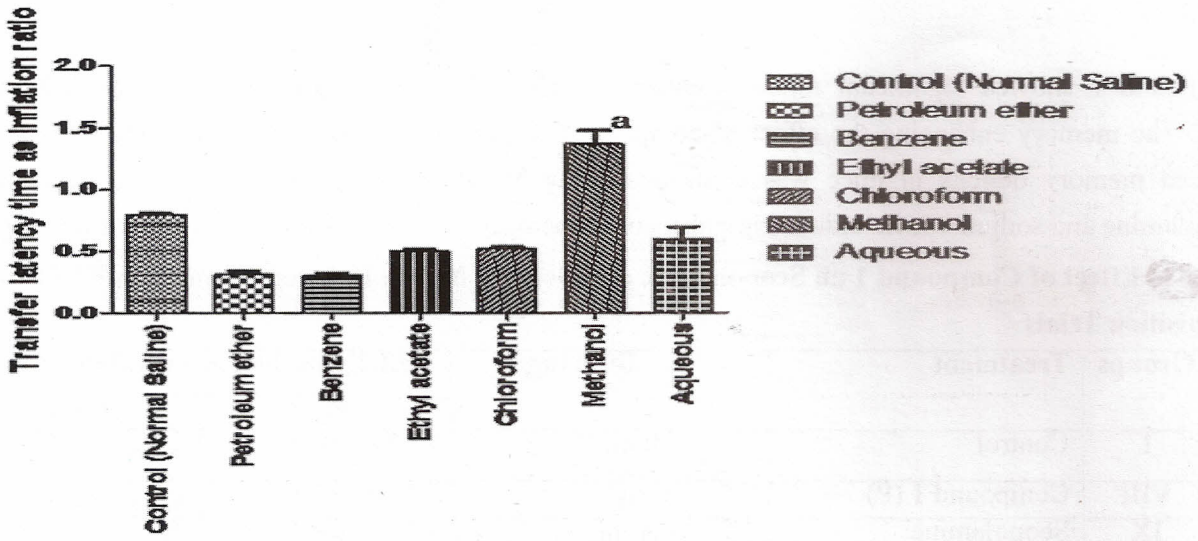
Bioactive sub-fraction F-5.1.4.1 was obtained from methanolic extract after bioactivity guided separation

and was subjected to further separation by column chromatography. Sub-fraction of *E. officinal*is was obtained following the scheme depicted in Figure 1. Chloroform fraction of methanolic extract (14gm) was subjected to silica gel column chromatography (60-120 mesh) and eluted with gradient chloroform-methanol-formic acid (97.7: 1.15: 1.15). TLC of F-5.1.4.1 using chloroform: methanol: formic acid (85:1:1) as mobile phase showed distinct spot (Rf 0.72) in iodine chamber. From F-5.1.4.5, one polar compound named 1 (76 mg) of visible purity was obtained as the dark cream amorphous powder. In the present investigation, it was found to be present to the extent of 5.46% w/w in the fruits of the plant.

Evaluation of memory enhancing effect

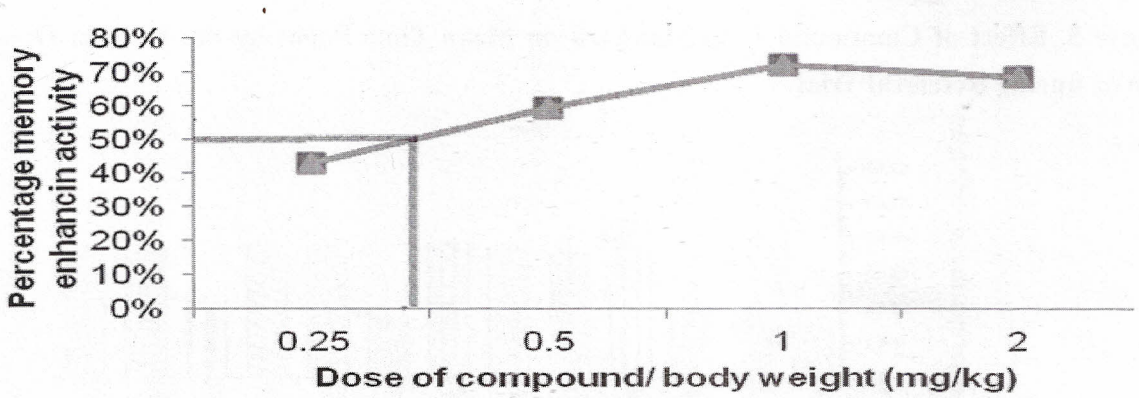
Amongst petroleum ether, benzene, chloroform, ethyl acetate, methanol and water successive extracts, the only methanolic extract was found to be active in Figure 2.

Figure-2: Effect of *Emblica officinalis* Extracts on TLT of the Mouse as Inflation Ratio. a=p≤0.05 versus day TLT in Control



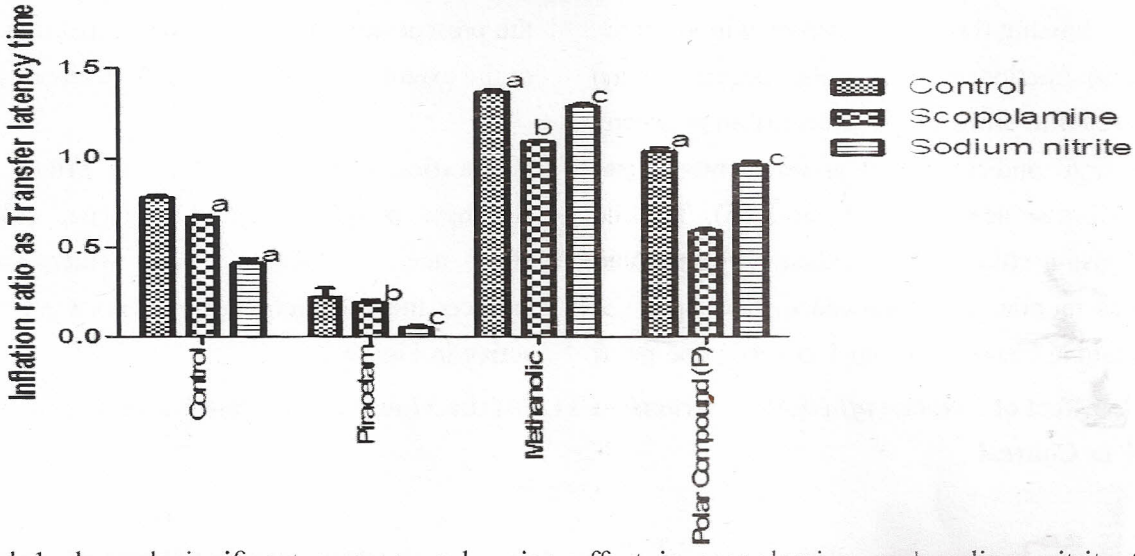
Median Effective Dose (ED50) of compound 1(biphenyl dicarboxylic acid) isolated from *Emblica officinalis* fruits was administered to be intraperitoneally (i. p.) 0.35 mg/kg body weight in mice. 1mg/kg, i.p. the dose of compound 1 isolated from the methanolic extract of *E. officinalis* fruit was found by dose response curve in Figure 3.

Figure-3: Median Effective Dose of Compound 1 Isolated from Methanolic Fraction of *Emblica officinalis* Fruit Extract



The memory enhancing the activity of the compound-1 was evaluated using elevated plus maze model, and the results are depicted in Figure 4.

Figure-4: Effect of Methanolic Fraction of *Emblica officinalis* and Compound1 on Scopolamine and Sodium Nitrite Induced Changes in Inflation Ratio of the Mouse as TLT



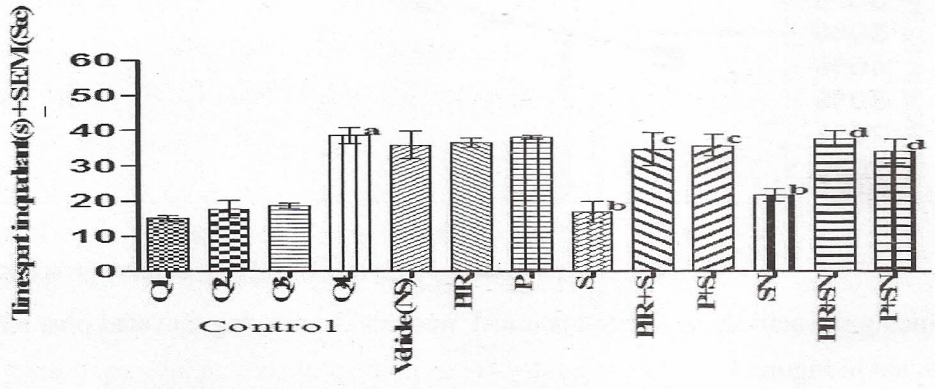
Compound 1 showed significant memory enhancing effect in scopolamine, and sodium nitrite treated mice. The memory enhancing the effect of compound1 in preventing the scopolamine and sodium nitrite induced memory deficits in mice was confirmed using Morris water maze. Effect of compound 1 on scopolamine and sodium nitrite induced impairment of memory in mice is presented in Table 1 and Figure 5.

Table-1: Effect of Compound 1 on Scopolamine and Sodium Nitrite Induced Changes in ELT during Acquisition Trials

Groups	Treatment	Dose (kg^{-1})	ELT (Sec) on Acquisition days	
			Day 1	Day 4
I	Control	10 ml	87.54 ± 0.89	51.03 ± 4.3 [a]
VIII	Compound 1 (P)	1 mg	82.1±2.9	54±3.82
IX	Scopolamine	0.4 mg	89.13± 0.47	85.15 ± 1.77 [b]
X	Sodium nitrite	75 mg	88.60 ± 0.60	77.3 ± 2.2 [b]
XI	Piracetam - *per se*	400 mg	82.75 ± 3.4	50.3 ± 6.3
XII	Piracetam + Scopolamine	400 mg + 0.4 mg	89.42 ± 0.17	69.71 ± 3.5 [c]
XIII	Piracetam + Sodium nitrite	400 mg + 75 mg	89 ± 0.82	51.0 ± 9.13 [d]
XIV	Compound 1 + Scopolamine	1 mg + 0.4 mg	86.05±1.5	73.33 ± 3.6 [c]
XV	Compound 1 + Sodium nitrite	1 mg + 75 mg	78.82 ±5.9	54.5 ± 1.8 [d]

a=p≤0.05 versus day 1 ELT in control group; b=p≤0.05 versus day 4 ELT in control group; c=p≤0.05 versus day 4 ELT in scopolamine treated group; d=p≤0.05 versus day 4 ELT in sodium nitrite treated group.

Figure 5. Effect of Compound 1 v/s Standard on Mean Time Spent by the Mice in Quadrant (s) of MWM during Retrieval Trial



The effect of administration of compound 1 (P) on time spent by mice in quadrant (s) during retrieval trial is exhibited. Histograms of time spent in quadrant (s) for control, vehicle [normal saline (NS)], standard drug [Piracetam (PIR)], amnestic agents [scopolamine (S) and sodium nitrite (SN)] and compound 1 (P) are shown. It may be noted that while the amnestic agents have a significant decreasing effect on time spent in the target quadrant (Q4). the standard and test compound have a reversing effect. Each value represents mean time spent in quadrant (s) ± S.E.M. a=p≤0.05 versus time spent in other quadrants in control group; b=p≤0.05 versus time spent in target quadrant (TSTQ) in control group; c=p≤0.05 versus TSTQ in scopolamine treated group; d=p≤0.05 versus TSTQ in sodium nitrite treated group.In the present investigation, scopolamine and sodium nitrite markedly decreased the TLT over two minutes, ELT and TSTQ over 90 seconds; and these were significantly reversed by the compound 1 thus confirming its memory enhancing the effect.

Characterization

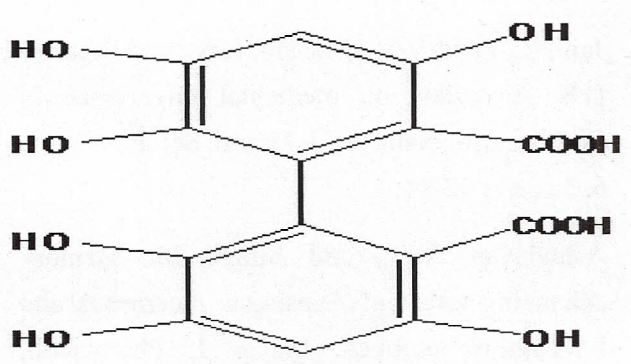
The polar compound 1, a dark cream amorphous solid, exhibited a melting point (M.P.) of 270 °C. The compound was characterized by UV, Mass, IR, ^{1}H NMR and ^{13}C NMR spectroscopy. The UV spectrum of Compound 1 showed maxima at 278 nm. The IR spectrum featured band at 1726.1 cm^{-1} (carboxylic) and 1596.2 cm^{-1} (aromatic). Only, one singlet at δ 6.8 in ^{1}H spectrum indicated the molecule being symmetrical. The two carboxylic carbonyl moieties were highly shielded at 159.13 cm^{-1} because of the presence of phenolic OH at ortho as well as para position. The molecule is symmetrical as only seven signals were got in spite of two phenyl rings. The signals observed in ^{13}C NMR are presented in Table 2.

Table-2: ^{13}C NMR Spectroscopic Data for Compound 1

Carbon number	δC	Carbon number	δC
1	107.61	1'	107.61
2	112.32	2'	112.32
3	148.11	3'	148.11
4	107	4'	107
5	148.11	5'	148.11
6	139.61	6'	139.61

The data of mass spectra indicated molecular ion peak $[M]^+$ at *338 m/z* besides at *361 m/z* due to M^+ *23*. The molecular weight was found to be 338. Elementary analysis indicated a molecular formula of $C_{14}H_{10}O_{10}$. Based on this data, the chemical structure of the compound 1 was characterized as 3, 5, 6, 3', 5', 6'-hexahydroxy-biphenyl-2, 2'-dicarboxylic acid mentioned in Figure 6.

Figure-6: Chemical Structure of Compound 1



DISCUSSION

Biomolecules were extracted successively with organic solvents in Soxhlet extraction. Amongst non-polar to polar successive extracts, the only methanolic extract was found to be bioactive. A significant decrease in TLT of mice noted on the second day as compared to their TLT on first day indicated normal memory in elevated plus maze model. Similarly, a marked decrease in ELT during subsequent trials as compared to first exposure on MWM, denotes

normal learning ability whereas an enhancement in the time spent by the mouse in the target quadrant in search of the missing platform reflects successful retention of memory. The administration of normal saline did not produce any modification in the EPM and MWM performance of control animals.

Acetylcholine, one of the neuro-chemical transmitters is responsible for memory retention (12). Memory deficits in rodents induced by scopolamine and sodium nitrite are recorded as TLT in EPM model, ELT, and TSTQ in MWM model. Scopolamine and sodium nitrite treatments produced a significant learning and memory deficits as indicated by a decrease in EPM and MWM performance and therefore have been widely used as animal models to study the anti-amnestic potential of new drugs (13, 14, 15). Scopolamine, centrally acting, muscarinic cholinergic receptor blocker impairs memory. Sodium nitrite has been reported to induce severe vasodilatation (16) and methemoglobinemia (17) which may be responsible for producing cerebral hypoxia (18) that initiates the generation of free radicals and may damage hippocampus. Hypoxia is noted to release adenosine (19) which consequently leads to inhibition of synaptic transmission (20, 21). Agents that have memory enhancing effect decrease the TLT and ELT but increase the TSTQ (22, 23). In the present investigation, scopolamine and sodium nitrite markedly increased the TLT over two minutes, ELT and decreased TSTQ over 90 seconds; and these were significantly reversed by the compound 1 thus confirming its memory enhancing the effect. It may be speculated that prevention of scopolamine and sodium nitrite induced amnesia with compound 1 may be due to the prevention of memory deficit by its restoration of cholinergic transmission and protecting them from hypoxia.

CONCLUSION

Bioactivity-guided fractionation of methanolic extract of E. officinalis by column chromatography ultimately led to the isolation of compound 1 (biphenyl dicarboxylic acid) which was found to be responsible for the memory enhancing the activity of E. officinalis. The administration of the compound 1 significantly improved learning and memory, prevented scopolamine and sodium nitrite induced experimental amnesia in mice and might be a great potential in memory deficits associated with numerous psychiatric and neurodegenerative states.

ACKNOWLEDGMENT

This work is dedicated to the memory of Late Prof. (Dr.) Manjeet Singh, Ex-Dean Research Punjabi University, Patiala, Punjab, India. The author is grateful to Prof. (Dr.) K. L. Dhar, Ex-advisor, Shoolini University, Solan Himachal Pradesh for his kind help in the characterization of the compound 1.

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