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Full Length Original Research Paper

In vitro antioxidant and anti-proliferative activities of seed extracts of *Nymphaea mexicana* in different solvents and GC-MS analysis

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Abstract:
A first attempt was made for the GC-MS profiling, anti-oxidant analysis cum DNA protective properties and anti-proliferative activities of a wild aquatic plant, *Nymphaea mexicana* found in Himalayan region and consumed for its peculiar taste and aroma. Three different solvents were used viz; methanol, ethanol and water. Extracts showed a dose dependent relationship with highest antioxidant potential in ethanol, however highest TPC was found in methanol (0.110 ± 0.05 GAE/g) as compared to ethanol (0.095 ± 0.05 GAE/g) and water (0.073 ± 0.05 GAE/g). Plant extracts showed efficient DNA damage protection (at concentrations > 30 µg/mL) and maximum efficiency against DNA damage was seen in ethanolic solvent. The antiproliferative activities of the plant were noteworthy at a concentration of 20 mg/ mL but were significantly lower than standard (5-flourouracil). The plant is known for its specific taste and aroma hence GC-MS profiling were carried out and relative percentage of important compounds found was determined. GC-MS analysis confirmed some major aroma rendering compounds along with some major anti-oxidants.

Keywords: Nymphaea mexicana, Antioxidant, Antiproliferative, DNA damage, GC-MS

1. Introduction

Nymphaea mexicana (commonly called sun lotus, yellow water lily)is a wild growing aquatic plant found abundantly in the eutrophic lakes of Asian subcontinent and belongs to family Nymphaeaceae. Plant is known for its strong aroma and almost all parts of the plant are eaten as vegetable and also used as medicinal herb. Although sufficient literature is not available regarding this particular species, the genus Nymphaeaceae comprises of about 50 species wide spread in tropical and temperate regions of the world and known for their medicinal value e.g. Nymphaea rhizome powder is a good demulcent, promotes hair growth and is used for treating liver disorder and diarrhea (1). Species belonging to Nymphaeaceae are considered to be a good source of antioxidants since, hydrolysable tannin geramim anthocyanin and flavonoid glycosides have been reported (2,3). The presence of some unusual anthocyanins, 2S,3S,4S-trihydroxypentanoic acid (5-deoxyribonic acid), and myricetin 3-O-(30 0- O-acetyl)-a-rhamnoside besides these 2,3-didexyribonolactone, Octadecanoic acid, Vitamin E, γ-tocopherol and several others were identified (4).

Living cells involved in active metabolism are continuously generating free radicals as a result of biochemical processes as well as due to environmental pollutants, radiation, chemicals and toxins (5). These free radicles are highly reactive species that pair with biological molecules such as proteins, DNA etc. leading to

various chronic diseases such as cancer, diabetes, cardiovascular diseases and aging etc. (6). Free radicles are scavenged by antioxidants which interfere with the oxidative processes by acting as electron donors and hence preventing the formation of peroxides which are harmful to human health. Natural antioxidants have gained interest among consumers as well as the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with lower risks of cardiovascular disease and cancer(7). Increased prevalence of diabetes, cardiovascular diseases and cancer in the past few decades have raised public awareness related to diets rich in antioxidants and polyphenols (8-9). There has been an increased demand for antioxidants from natural sources as compared to synthetic ones due to economic and social considerations.

Hence present work was aimed to evaluate the antioxidant potential, protection against DNA damage and anti-proliferative activity of *N. mexicana* for the first time to the best of our knowledge. Different solvents were used since nature of solvent affects the extractability of phytochemicals. Also due its strong aroma GC-MS was performed.

2. Materials and Methods

2.1. Materials

Nymphaea mexicana was harvested in the month of November from Dal Lake, Jammu & Kashmir, India. Seeds were taken and were cleaned manually to remove all extraneous material like mud and dirt. Seeds were separated from the ovary and stored at -18 °C for further analysis. All chemicals used were of analytical grade. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and calf thymus DNA were procured from sigma Aldrich, whereas Folin-ciocalteu reagent, gallic acid, sodium phosphate buffer, potassium ferricyanide, trichloroacetic acid, ferric chloride, ascorbic acid, phosphate ferricyanide, trichloroacetic acid, ferric chloride, ascorbic acid, H_2O_2, KH_2PO_4, thiobarbituric acid, phosphate buffer, hydrogen peroxide, BHT, FeSO4, phosphate buffer 1.5 % agarose gel, Ethidium bromide and Tris,Tris/boric/EDTA gel buffer were procured from Hi-Media laboraties

2.2. Sample preparation

0.3 g of seeds of *Nymphaea mexicana*was dissolved separately in 20 ml each of methanol, ethanol and water, and then stirred for 2 hours on a magnetic stirrer followed by the centrifugation for 10 minutes at 3500 rpm. 3/4th of the supernatant was dried in a rotatory vacuum evaporator (EquitronRoterva) and then re-dissolved in their respective solvents to make the stock sample of varied concentrations (10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml & 50µg/ml). 1/4th of the supernatant was kept for determination of total phenols.

2.3. Assay for antioxidant activity

2.3.1. DPPH scavenging activity

DPPH radical scavenging activity of different extract solutions (viz; methanolic, ethanolic and aqueous) was determined according to the method of (10) Gaulejac et al. (1998) with some minor changes. 100µL of sample extracts in different solvents of varied concentrations (10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml & 50µg/ml) were added to 2.9 ml of 6×10^{-5}mol/l methanolic solution of DPPH. The absorbance was measured at 517 nm with a spectrophotometer **(**HITACHI U 2900**)** after the solutions were allowed to stand in

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the dark for 30 min. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

Percentage inhibition was calculated by using the formula:

% inhibition = $A_{control517}$- $A_{sample517}$/$A_{control517}$× 100

Where $A_{control}$ 517 is the absorbance of the control and $A_{sample517}$ is the absorbance of the extract.

2.3.2. Total phenolic content

Supernatants prepared in section 2.2 using different solvents were used for TPC determination which was done according to the Folin-Ciocalteu's spectrophotometric method described by (11) with some modifications. 100 µL of each sample supernatant in different solvents was mixed with 2.5 ml of 10-fold diluted Folin-Ciocalteu's phenol reagent and allowed to react for 5 min. Then, 2 ml of 7.5% Na_2CO_3 solution was added to each of the samples, and the final volume was made up to 10 ml with de-ionized water. After 1 hour of reaction at room temperature, the absorbance at 760 nm was determined. The measurement was compared to a standard curve of Gallic acid (GA) solution, and the total phenolic content was expressed as milligrams of Gallic acid equivalents (GAE).

2.3.3. Reducing power

Reducing power of the plant extract was done according to the method of Oyaizu (12). 100 µL of each sample extract in different solvents at different concentration levels (10µg/ ml, 20µg/ ml, 30µg/ ml, 40µg/ ml, 50µg/ ml) were mixed with sodium phosphate buffer and potassium ferricyanide. The mixture was incubated at 50 °C for 20 minutes followed by the addition of trichloroacetic acid and then centrifuged at 3000 rpm for 10 minutes. The upper layer was mixed with deionized water and $FeCl_3$, and then the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power

2.2.4. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of extracts was assayed by the method of Wu et al., (13) with certain modification. The reaction mixture containing 25 mM of calf thymus DNA (1 mL), 10 mM ferric chloride (200 µl), 100 mM ascorbic acid (200 µl), 2.8 mM H_2O_2 (200 µl) in 10 mM KH_2PO_4 (pH 7.4) and various concentrations (10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ ml, 50 µg/ ml) extracts in different solvents (methanol, ethanol & water). The reaction mixture was incubated at 37 °C for 1 h. Then 1 mL of 1 % thiobarbituric acid and 1 mL of 3 % tricholoroacetic acid were added and heated at 100°C for 20 min. The TBARS was measured spectrophotometrically at 532 nm. The results were expressed as percentage inhibition of DNA oxidation.

Percentage inhibition was calculated by using the formula:

% inhibition = $A_{control532}$- $A_{sample532}$/$A_{control\ 532}$ × 100

Where $A_{control532}$ was the malondialdehyde produced by Fenton reaction treated alone, and A_{sample} 532 was the malondialdehyde produced in presence of extract.

2.3.5. Lipid peroxidation (microsomal)

Lipid Peroxidation was induced and assayed in rat liver microsomes using the method of Chang et al., (14) with slight modifications. Liver of albino rats was homogenized with a homogenizer in ice-cold Tris HCL buffer (20 mM, pH 7.4) to produce. The homogenate was centrifuged at 100 000 g for 60 min. at 4 °C, and the pellet was used for in vitro lipid peroxidation assay. An aliquot of microsomes (1 ml) was incubated with different concentrations of plant extract (10, 20,30,40,50 µg/ml, respectively) in the presence of 20 mM ferric nitrate, 100 mM ascorbic acid and 30 % H2O2 at

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37 °C for 1 hr. the reaction was ended by the addition of 1.0 mL of trichloroacetic acid (TCA; 28 %,w/v) and 1.5 mL of TBA (1 %, w/v), followed by heating at 100 °C for 15 minutes. The absorbance of the malonaldehyde (MDA) - TBA complex was measured at 532 nm. α-tocopherol was used as positive controls. % inhition was calculated using formulae

$$\% \text{ inhibition} = (A_{control} - A_{sample}) A_{control} \times 100$$

2.4. Inhibition of oxidative DNA damage

The hydroxyl radical-mediated DNA strand breaks were measured by the procedure described by Yeung et al., (15) with some minor modifications. Briefly, 0.5 µg DNA was incubated with 1 µl of 1 mM $FeSO_4$, 1 µl of 10% H_2O_2, 3 µl of extract in in three different solvents (viz; methanol, ethanol & aqueous) at three different concentrations (30 µg/ml, 40 µg/ml, 50 µg/ml) and the final volume was made up to 15 µl with 50 mM phosphate buffer (pH 7.0). The mixture was incubated in water bath at 37 °C for 30 min. After incubation, the sample was immediately loaded in a 1.5 % agarose gel along with 3 µlEthidium bromide, containing 40 mMTris, 20 mM sodium acetate and 2 mM EDTA, and electrophoresed in a horizontal slab apparatus in Tris/boric/EDTA gel buffer. The gel was then photographed under UV light.

2.5 Antiproliferative effects of Nymphaea Mexicana on human cancer cells

Cell proliferation inhibitions were investigated according to Mosmann, (16) with minor modifications. Colo-205 (human colon cancer cell line), T47D (human ductal breast epithelial tumor cell line) and MCF7 (human breast adenocarcinoma cell line) were used for the MTT human cancer assay. The cells were plated in 96 well plate at 5000-7000 cell density per well. Cells were grown overnight in 100 µL of 10 % FBS. After 24 hours cells were replenished with fresh media and the extracts of the sample solution were added to the cells. The extracts (50µg/mL each) were added to wells in triplicates. Cells were incubated with the extract for 24 hours. After 24 hours 20 µl of MTT dye (5 mg/mL) were added to each well and further incubated for 3 hours. Before read-out, precipitates formed were dissolved in 150 µL's of DMSO using vortex for 15 minutes. All the steps after MTT additions were performed in dark and 5-flourouracil was used as positive control. Absorbance was measured at 590 nm.

2.6. GC-MS analysis

2.6.1. Preparation of extract

Mix 2 g of sample extract with 50 ml of methanol for 72 hours at room temperature. The supernatant was filtered and concentrated under reduced pressure in a rotary vacuum evaporator (Rotary Equitron).

2.6.2. Instrument and chromatographic Conditions

The concentrated pure extract was vacuum dried in a vacuum oven at 60 °C to get a powdered residue. The residue was dissolved in 20 ml methanol and collected in corked glass test tubes. The extracts were analysed on a Shimadzu QP2010 Plus GC-MS system with 2010 GC. An Omega SPTm column (0.25 mm ID, film thickness 0.25 µm) was used with nitrogen as carrier gas. The injector temperature was 270 °C with split ratio of 10.0. The GC oven temperature was programmed to hold at 100 °C for 2 minutes and then increased to 200 °C at 15 °C/min and hold for 2 minutes and finally increased to 240 °C at 20 °C/min and hold for 18 minutes. Ion source temperature was 230 °C and the interface temperature was set at 280 °C. Mass spectra were collected over the range of

m/z 40-650. Each compound was identified using WILEY library (8 L).

2.7 Statistical analysis

Results were expressed as mean of triplicate analyses. A one-way analysis of variance and Duncan's test were used to establish the significance of differences among the mean values at the 0.05 significance level. The statistical analyses were performed using SPSS software.

3. Results and Discussion

3.1. Antioxidant activity

3.1.1. DPPH

The anti-oxidant potential of the extract was measured by DPPH radical scavenging assay. On pairing DPPH with protonating substance the radical gets scavenged and absorbance is reduced resulting in the discoloration (Purple to yellow). The degree of discoloration indicates the scavenging potential of the antioxidant compounds present in extract in terms of hydrogen donating ability. The percentage inhibition of DPPH free radicles increased significantly with increase in concentration of the extract in a dose dependent manner (Fig 1). At a concentration of 50µg/ml in aqueous, methanol and ethanol solvents were 50.9 %, 52.8 %and 53.3 % but were lesser in comparison to that of a-tocopherol (56.28 %), the known antioxidant at the same concentration used as standard. Results obtained in this study were lower than those obtained by (17). The extraction yield also increased significantly with increase in the polarities of the solvents as Ethanol > Methanol > Aqueous, which suggests that polyphenols of *N. mexicana* arepolar in nature. The water extract may either contain more non-phenolic compounds or possess phenolic compounds that contain a smaller number of active groups than other solvents used. However aqueous extracts of immature calamondin were shown to be more potent than both methanolic and ethanolic extracts (18).has also reported similarly ethanolic extracts have shown to have higher scavenging activity than water in case of *Nymphaea alba* **(19).**

3.1.2. Total phenols

Total phenols of *N. mexicana* varied significantly in different solvents (0.110 ± 0.05, 0.095 ± 0.05, 0.073 ± 0.05 GAE mg/100mg for methanol, ethanol, and water respectively (Fig 2). So the extractions with methanol resulted in the highest amount of total extractable compounds than in the remaining solvents which is in agreement with the results of (20) Ao et al., (2012) in *Ficusmicro carpa*. The results obtained were not in agreement to those with that of DPPH and it may be due to extraction of more potent polyphenols in ethanol which impart ethanolic extracts more antioxidant activity. Higher TPC in methanol despite of lower antioxidant activity of methanolic solvents can also be attributed to extraction of polyphenolic compounds of lower or no antioxidant activity as vitexin or isovitexin in methanol (21)(Inglett et al., 2011). Antioxidant activity of extracts is attributed to phenolic compounds present in the extract.

3.1.3. Reducing power

It has been reported that the reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (22). The data reveals that the reducing power of extract increased in a dose dependent manner showing an increase in the reducing power with increase in concentration of the extract. Reducing power of

sample were 56.87 %, 68.54 % & 75.93 % at a concentration of 50 µg/mL in ethanol, methanol and water respectively which was less in comparison to that of BHT (89.57 %) standard used at the same concentration (Fig. 3). Among different solvents used reducing power of extract increased with the polarity of the solvents (ethanol > methanol > aqueous) which was in agreement with results obtained in DPPH. The results were contrary to those found by Boulekbache et al (23) in egg plant extracts where methanolic extracts were more potent than ethanolic solvents. This suggests presence of highly polar polyphenolic compounds in *N. mexicana.* Higher reducing power of the ethanolic extract might be due to the greater hydrogen donating capability of the solvent and extraction of potent antioxidants by ethanol as compared to other solvents used in the analysis. In biological systems, hydroxyl radical is an extremely reactive free radical. It has been implicated as major active oxygen centered radical formed from the reaction of various hydroperoxides with transition metal ions which is capable of damaging almost every molecule found in living systems causing lipid perioation and biological damage.The hydro

3.1.4. Hydroxyl radical scavenging activity

In biological system, hydroxyl radical is an extremely reactive free radical. It has been implicated as major active oxygen centered radical formed from the reaction of various hydro peroxides with transition metal ions, which is capable of damaging almost every molecule found in living system causing lipid peroxidation and biological damage. The hydroxyl radical scavenging activity of *N. mexicana* in different solvents varied significantly and can be ranked as ethanol > methanol > aqueous.Madhusudhanan et al., (19) also reported higher activity in case of Ethanol extract than in aqueous. In vitro hydroxyl radicals (•OH) were generated by the mixture of Fe^{2+}-EDTA, H_2O_2 and ascorbic acid. The damages are evaluated by monitoring the degraded DNA fragments through the formation of MDA (malonyldaldehyde). The data showed that the extracts exhibited dose dependent inhibitory effects either by scavenging the radical or by chelating Fe^{2+} ion making them unavailable for Fenton's reaction. The ability of extracts to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and they seem to be good scavengers of active oxygen species, thus reducing the rate of reaction. The hydroxyl radicle scavenging capacity of *N. mexicana* in ethanol, methanol & aqueous varied significantly and were 78.54 %, 74.45 %, 57.95 % at a concentration of 50 µg/ml which is comparable to those of standard a-tocopherol (82.87 %) at the same concentration (Fig. 4).

3.1.5. Lipid peroxidation (Microsomal).

In food deterioration and in oxidative modification of biological molecules, lipid peroxidation plays an important role. Capacity to inhibit lipid peroxidation by any substance is often used to assess its antioxidant activity. Lipid peroxidation (auto-catalytic, free radical mediated destructive process), where wide variety of products are formed on decomposition including ketones, fatty acids, low molecular weight hydrocarbons, alkenols and alkanals, in particular malonaldehyde (MDA) when polyunsaturated fatty acids in bio-membranes undergo degradation to form lipid hydro peroxides (24). The reduction of MDA production would indicate lipid peroxidation inhibition. Fe^{2+}/Ascorbate/H_2O_2 model system were used to initiate lipid peroxidation in rat liver microsomes. MDA forms a

pink chromogen with TBA which gives maximum absorbance at 535 nm. Initiation of lipid peroxidation by ferric nitrate, ascorbic acid and H_2O_2 takes place either through ferryl–perferryl complex or through hydroxyl radical (•OH) in the Fenton reaction. *N. mexicana* extract inhibited MDA formation, and thus lipid peroxidation in liver microsomes in concentration dependent manner (Fig. 5). The extract produced 78.98 % LPO inhibition at 50 µg/ml concentration. BHT at the same concentration produced 90 % LPO inhibition. The beneficial effect of *N. mexicana* on lipid peroxidation may be attributed to its high polyphenolic content.

3.1.6. IC50 and Correlation analysis

In order to compare the antioxidant potential of extracts IC_{50} values have been used and are calculated using linear regression model (25). IC_{50} refers to the half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. Thus higher the IC_{50} value of a sample lower is the antioxidant activity. IC_{50} value of samples for DPPH radicle scavenging activity was lowest for ethanol (IC_{50} = 47.79 µg/mL) in comparison to methanol (48.65 µg/mL) and water (49.55 µg/mL). Similarly lower values of IC_{50} were seen for reducing power, OH• radicle scavenging activity (Table 3). This further confirms the superiority of pure ethanol as an extraction solvent in comparison to other solvents used in this study. Similar results were seen in *Limnophila aromatica* by Do et al., (26). A high level of correlation was seen between antioxidant activity and total phenol content. However Khled-khoudja et al., (27) reported water extracts more potent than ethanolic extracts for three selected Algerian lamiaceae varieties.

Phenolics are main antioxidant compounds that impart antioxidant activity to a sample extract (25). In this study the antioxidant activity of extracts is due to phenolics present in it as is indicated by high correlation coefficients between TPC and antioxidant tests carried. Similar results were found by (26).

3.2. Chromatographic profiling using GC-MS

Among different solvents used GC-MS was performed on methanolic extracts only because highest TPC was seen in methanol. Various compounds were identified through GC-MS which have already been reported to have strong antioxidant activity. The compounds identified are shown in Table 1 & GC-MS chromatogram (Fig. 7) and table 2 shows the health benefits of the compounds that contribute major portion in methanolic extract. Relative percentage of polyphenolic compounds present can be deduced from the chromatogram however the quantity of compounds in the extract is a function of solvent used e.g. The area percentage under the peak designating 9-Hexadecenoic acid was approximately 21 % in methanol however the solubility of 9-Hexadecenoic acid is 11.8 mg/L and 31.9 g/L in water and ethanol respectively. This suggests that the actual antioxidant and antiproliferative activity of the sample can be greater than the crude extracts used in the study. Various other components present in the *N. mexicana* were Octadecanoic acid, 2,3-didexyribonolactone, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, Vitamin E, Nonanoic acid, n-Decanoic acid, γ-tocopherol, Squalene, 3,7,11,15-tetramethyl-2 hexadecen-1-ol. These compounds have prominent anticancerous and antioxidant activity (28-31). Health benefits of such compounds are given in table 3.

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3.3 Antiproliferative effects of Nymphaea mexicana on colo-205 human cancer cell lines

Human colo-205 cancer cells were used for the study of anti-proliferative effect of extract in comparison to 5-florouracil. 5-florouracil is used to treat several types of cancer including colon, rectum, and head and neck cancers. It is also used for other types of cancer, and the skin cream is used for other conditions as well 5-florouracil belongs to the class of chemotherapy drugs known as anti-metabolites. It interferes with cells making DNA and RNA, which stops the growth of cancer cells. Fig. 8 shows the aqueous extract of the *Nymphaea mexicana* significantly reduced the growth rate of colo-205 cancer cells at an initial concentration of 20 mg/mL after 24 hr treatment. Dose-dependent inhibition against the proliferation of the human cervical epithelial adenocarcinoma HeLa cell line in case of dulse and kelp extracts has also been reported (32). Methanol extracts of various kelps including *L. japonica* and the red algae *P. tenera* and *Gelidiumamansii* exhibited dose-dependent inhibition of the growth of human gastric (AGS) and HT-29 colon cancer cells (33). Further studies on *N. mexicana* are needed to identify the antioxidant and anti-proliferative molecules for better understanding of the bioactivity of these underutilized aquatic plants for production of functional foods

4. Conclusions

The use of traditional medicine is widespread and plants still represent a large source of natural antioxidants that might serve as leads for the development of novel drugs. The results of this work have revealed that *N. mexicana* has got excellent antioxidant and antiproliferative properties. This suggests that *N. mexicana* can be used as a functional food or it can be incorporated in other foods for preventing oxidative damage. Further, the GC-MS analysis of the extract verified presence of various compounds that were responsible for strong flavor and anti-oxidant activity of the *N. mexicana*. The potential of this extractas an antioxidant, particularly hydroxyl radical scavenging using model DNA and antiproliferative activity using human cancer cell lines may open ways on future in vivo studies on prevention and management of cancer. These properties will help regulating carcinogenesis at the initiation as well as progressive stages. Due to its suitable flavor and high antioxidative activity, *N. mexicana* should be considered as an additive for development of functional foods and protection from oxidative damage.

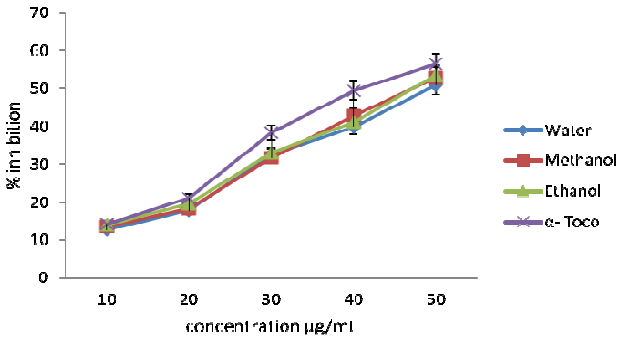

Fig. 1: DPPH radical scavenging activity of *Nymphaea mexicana* extracts in different solvents at different concentrations.

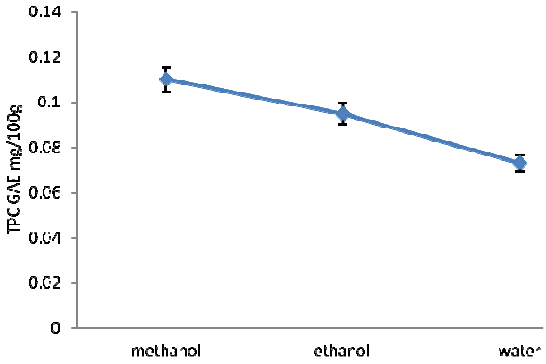

Fig. 2: Total Phenolic content of *Nymphaea mexicana* extracts in different solvents at different concentrations.

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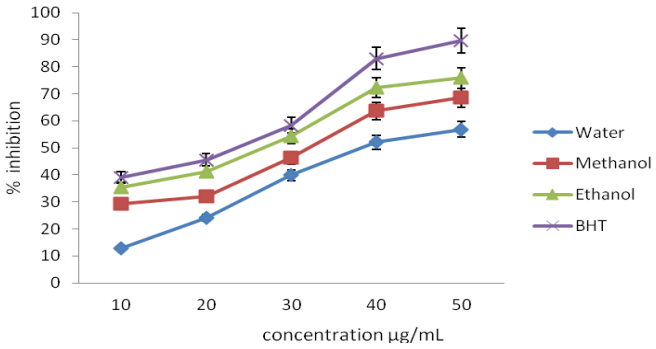

Fig.3: Reducing power of *Nymphaea mexicana* extracts in different solvents at different concentrations.

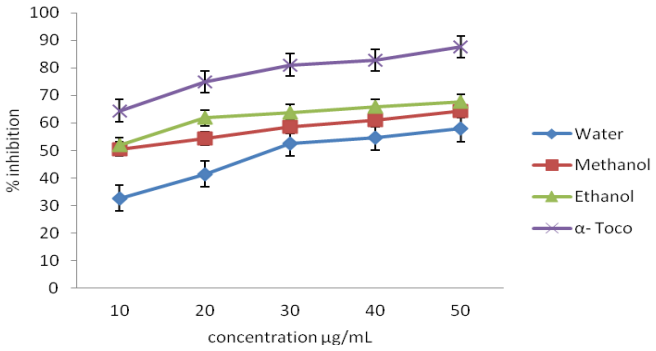

Fig. 4: Hydroxyl radical scavenging activity of *Nymphaea Mexicana* extracts in different solvents at different concentrations.

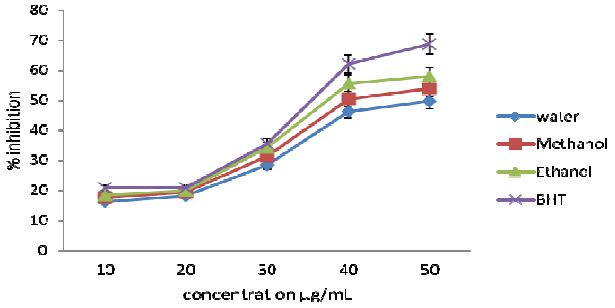

Fig.5: Effect of *Nymphaea Mexicana* and known antioxidant BHT on microsomal lipid peroxidation

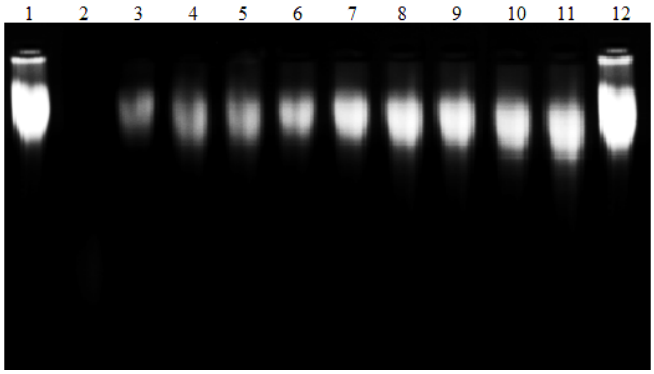

Fig 6: (lanes1-12) Protective effect of *Nymphaea mexicana* extracts in different solvents on oxidative damage to calf thymus DNA at varied concentrations LANE **1**: Native calf thymus DNA. LANE **2**: DNA + 15 mM ferric nitrate + 50 mM ascorbic acid 15 mM H_2O_2 LANE **3**: DNA + 15 mM ferric nitrate + 50 mM ascorbic acid + 15 mM H_2O_2 + 30 µg/mL of *Nymphaea mexicana* aqueous extract. LANE **4**: DNA + 15 mM ferric nitrate + 50 mMascorbic acid + 15 mM H_2O_2 + 40 µg/mL of *Nymphaea mexicana* aqueous extract. LANE **5**: DNA + 15 mM ferric nitrate + 50 mM ascorbic acid + 15 mM H_2O_2 + 50 µg/mL of *Nymphaea mexicana* aqueous extract. LANE **6**: DNA + 15mM ferric nitrate + 50 mM ascorbic acid + 15mM H2O2+ 30 µg/mL of *Nymphaea mexicana* methanolic extract. LANE **7**: DNA + 15mM ferric nitrate + 50 mM ascorbic acid + 15mM H2O2+ 40 µg/mL of *Nymphaea mexicana* methanolic extract. LANE **8**: DNA + 15mM ferric nitrate + 50 mM ascorbic acid + 15mM H_2O_2+ 50 µg/mL of *Nymphaea mexicana* methanolic extract. LANE **9**: DNA + 15mM ferric nitrate + 50 mM ascorbic acid + 15mM H_2O_2+ 30 µg/mL of *Nymphaea mexicana* ethanolic extract. LANE **10**: DNA + 15mM ferric nitrate + 50 mM ascorbic acid + 15mM H_2O_2 + 40 µg/mL of *Nymphaea mexicana* ethanolic extract. LANE **11**: DNA + 15mM ferric nitrate + 50 mM ascorbic acid + 15mM H_2O_2+ 500 µg/mL of *Nymphaea mexicana* ethanolic extract. LANE **12**: DNA + 15mM ferric nitrate + 50 mM ascorbic acid + 15mM H_2O_2+ 50 µg/mL of *Nymphaea mexicana* ethanolic extract.

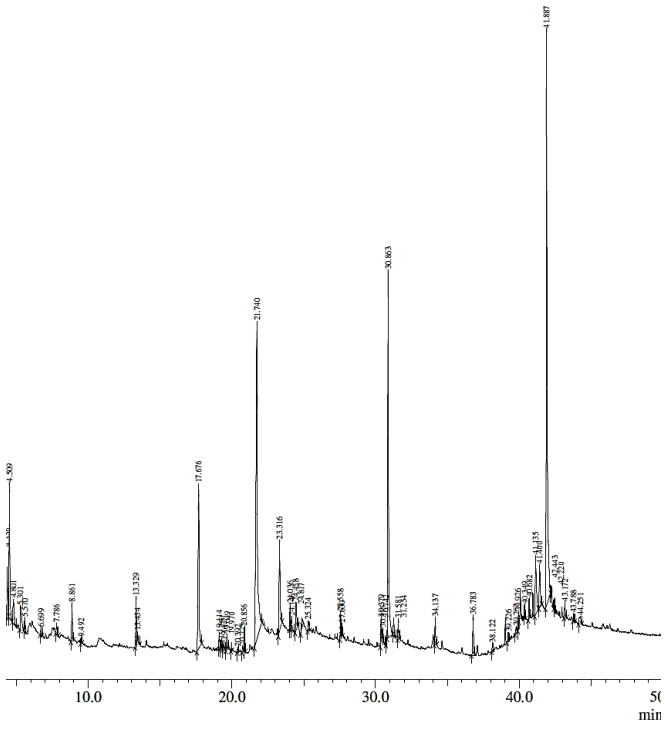

Fig. 7: GC-MS Chromatograph of methanolic extract of *Nymphaea mexicana*.

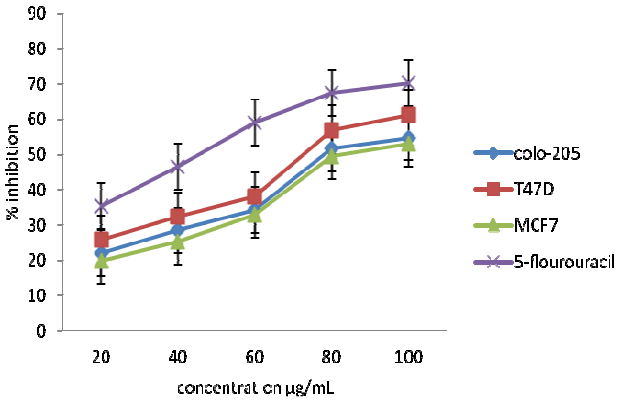

Fig. 8: Antiproliferative activity of aqueous *Nymphaeamexicana* extract at different concentrations

Table 1: Compositional analysis of methanolic extract of*N.mexicanea*by GC-MS

Retention Time	Area Percentage	Name
4.356	1.49	2-acetyl-2-hydroxy-.gamma.-butyrolactone
4.509	4.25	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
4.801	1.45	Stigmasta-5,22-dien-3-ol $$ stigmasta-5,22e-dien-3b-ol
5.301	1.39	(S)-(+)-2',3'-dideoxyribonolactone
5.570	0.41	Acetic acid, anhydride
6.699	0.24	Nonanoic acid
7.786	0.40	2-Methoxy-4-vinylphenol
8.861	1.22	N-Decanoic acid
9.492	0.14	Heptadecane
13.434	0.23	3',5'-dimethoxyacetophenone
17.676	7.24	Tetradecanoic acid
19.114	0.47	2,6,10-trimethyl,14-ethylene-14-pentadecne
19.250	0.27	2-Pentadecanone, 6,10,14-trimethyl-
19.427	0.41	2-Decene, 3-methyl-, (Z)-
19.649	0.57	Pentadecanoic acid
19.970	0.14	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
20.392	0.15	2-heptadecanone
20.725	0.15	Tetradecanal
20.856	0.55	Hexadecanoic acid, methyl ester
21.740	20.98	N-Hexadecanoic acid
23.316	3.95	9-Hexadecenoic acid
24.036	0.65	9,12-Octadecadienoic acid, methyl ester
24.146	0.51	9,12-Octadecadienoyl chloride, (Z,Z)-
24.458	1.26	Phytol
24.817	0.17	9,12-Octadecadienoic acid, methyl ester
25.324	0.43	Octadecanoic acid
27.558	0.55	Hexadecanoic acid, 1-((((2-aminoethoxy)hydroxyphosphinyl)o
27.630	0.18	9-Hexadecenoic acid, methyl ester, (Z)-
30.379	0.62	9,12-Octadecadienoyl chloride, (Z,Z)-
30.515	0.20	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-
30.742	0.10	Heneicosane
30.863	12.94	13-Docosenoic acid, methyl ester, (Z)-
31.254	0.63	Heneicosanoic acid, methyl ester
31.581	0.51	1,2-benzenedicarboxylic acid
34.137	1.00	Cis-15-tetracosensaeure, methylester
36.783	1.74	Squalene
38.122	0.26	Tetratriacontane
39.226	0.35	Pentatriacontane
39.767	0.22	Gamma.-tocopherol
39.926	0.33	Cholesta-4,6-dien-3-ol, (3.beta.)-
40.349	0.30	Vitamin E
40.682	0.47	1-hentetracontanol
41.135	2.82	Ergost-5-en-3-ol, (3.beta.)-
41.400	1.89	Stigmasta-5,22-dien-3-ol
41.887	22.51	Gamma.-sitosterol
42.220	0.34	1-hentetracontanol
42.443	0.28	Stigmast-5-en-3-ol, oleate
43.172	0.55	Stigmast-4-en-3-one
43.788	0.23	2,6,10-trimethyl,14-ethylene-14-pentadecne

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Table 2: Phyto-components identified in *Nymphaeamexicania* by GC-MS.

R. Time	Area %	Name of the compound	Activity
4.509	4.25	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	Antimicrobial, anti-inflammatory
20.856	0.55	Hexadecanoic acid	Antioxidant
5.301	1.39	2,3-didexyribonolactone	Antimicrobial
25.324	0.43	Octadecanoic acid	Antioxidant
40.349	30	Vitamin E	Antioxidant,antileukemic, antitumor,anticancer.
6.699	0.24	Nonanoic acid	Anti-bacterial, anti-oxidant
8.861	1.22	n-Decanoic acid	Anti-oxidant, anti-bacterial
39.767	0.22	gamma- tocopherol	Anti-oxidant
36.783	1.74	Squalene	Anti-Oxidant
19.970	0.14	3,7,11,15-tetramethyl-2 hexadecen-1-ol	Anti-oxidant and hepatroprotective
24.458	1.26	Phytol	Anti-oxidant

Table 3: IC 50 values of *Nymphaea mexicana* in different solvents

	IC50 values (µg/mL)			Correlation coefficient		
	ethanol	methanol	water	ethanol	methanol	water
DPPH	47.79a	48.65b	49.56c	0.9730**	0.9636**	0.9614**
Reducing power	26.95a	33.10b	41.04c	0.9776**	0.9303**	0.9954**
OH radicle scavenging activity	0.56a	7.78b	27.46c	0.9346**	0.9998**	0.9968**
Lipid peroxidation	37.55a	42.30b	46.73c	0.9051**	0.9148**	0.9267**
Anticancerous activity	-	-	-	-	-	0.997**

Values followed by different superscript letter in a row are significantly different (p≤0.05).

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