International Journal of Pharmacy and Pharmaceutical Sciences

Print ISSN: 2656-0097 | Online ISSN: 0975-1491 Vol 12, Issue 11, 2020

Original Article

ANTIOXIDANT AND ANTI-PROLIFERATIVE EFFECTS OF AN ETHYL ACETATE FRACTION OF THE HYDRO-ETHANOLIC EXTRACT OF *SYNEDRELLA NODIFLORA* (L) GAERTN

PATRICK AMOATENG[1*], EMELIA OPPONG BEKOE[2*], SETH KWABENA AMPONSAH[3], REGINA APPIAH-OPONG[4], DORCAS OSEI-SAFO[5], BENOIT BANGA N'GUESSAN[1], M. V. N. L. CHAITANYA[6], RAMAN RAJESHKUMAR[7], S. P. DHANABAL[6]

[1]Department of Pharmacology and Toxicology, School of Pharmacy, College of Health Sciences, University of Ghana, [2]Department of Pharmacognosy and Herbal Medicine, School of Pharmacy, College of Health Sciences, University of Ghana, [3]Department of Medical Pharmacology, UG Medical School, College of Health Sciences, University of Ghana, [4]Department of Clinical Pathology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, [5]Department of Chemistry, School of Physical and Mathematical Sciences, College of Basic and Applied Sciences, University of Ghana, P. O. Box LG 56, Legon, Accra, Ghana, [6]Department of Pharmacognosy and Phytopharmacy, JSS College of Pharmacy (A constituent College of JSS University, Mysore), Rock lands, Ootacamund 643001, Tamilnadu, India, [7]Department of Pharmaceutical Biotechnology, JSS College of Pharmacy (A constituent College of JSS University, Mysore), Rock lands, Ootacamund 643001, Tamilnadu, India
Email: pamoateng@ug.edu.gh

Received: 03 Aug 2020, Revised and Accepted: 29 Sep 2020

ABSTRACT

Objective: *Synedrella nodiflora* is traditionally used in the treatment of several ailments. Pharmacologically, this plant has anticonvulsant, sedative, anti-nociceptive and anti-proliferative effects. This study further investigated *S. nodiflora* for its antioxidant and *in vitro* inhibition of cancerous cell lines.

Methods: Phytochemical assays, and the DPPH radical scavenging method were employed in preliminary screening for antioxidant activities of the crude hydro-ethanolic extract (SNE) and resulting fractions. The potent ethyl acetate fraction (EAF), was further investigated for total phenol and flavonoid contents, reducing power, lipid peroxidation potential, and cytotoxic effects on human breast cancer (MCF-7), leukemic (Jurkat), and normal liver (Chang's liver) cell lines.

Results: The extract contained phenols, flavonoids, tannins, glycosides, sterols, terpenoids, and alkaloids. It scavenged for DPPH with an IC_{50} of 114 µg/ml, whereas that of EAF was 8.9 µg/ml. EAF prevented peroxidation of egg lecithin at an IC_{50} of 24.01±0.08 µg/ml. These IC_{50s} are four and three times lower than the reference standards. EAF produced anti-proliferative effects against MCF-7, and Jurkat cell lines with IC_{50s} of 205.2 and 170.9 µg/ml, respectively. EAF had a high IC_{50} of 252.2 µg/ml against Chang's liver cells. At 0.1 mg/ml EAF had similar total flavonoid content to SNE, but a significantly higher total phenol content.

Conclusion: The ethyl acetate fraction of *S. nodiflora*, exhibited the most potent antioxidant activity. It inhibited the proliferation of breast and leukemic cancer cell lines, whiles having weak cytotoxic effect on normal liver cells. These can be explored for further drug development.

Keywords: *Synedrella nodiflora*, Antioxidant, Anti-proliferative, Ethyl acetate fraction, Cancer

DOI: http://dx.doi.org/10.22159/ijpps.2020v12i11.39301. Journal homepage: https://innovareacademics.in/journals/index.php/ijpps.

INTRODUCTION

Synedrella nodiflora (L.) Gaertn family Asteraceae is a common weed found growing along the banks of rivers, streams and roadsides [1]. This plant is an erect branched ephemeral herb that may grow to about 30-80 cm high. It originated from tropical America, but has currently spread all over the world. The leaves appear in opposite pairs, and are about 4-9 cm in length. The leaves are elliptic to ovate, with three prominent veins, and neatly toothed margins. The leave grows in small bunches of about 2-8 inflorescences at nodes and tips. Each inflorescence consists of several erect bracts 3-5 mm long surrounding 5-6 marginal ray florets and 10-20 central disc florets, each 3-4 mm long with a yellow petal [2]. In Ghana, the whole plant is boiled, and the aqueous extract drunk for the treatment of neurological conditions such as epilepsy, whiles the leaves are used for threatened abortion, hiccup, laxative and as feed for livestock [1]. The crude hydro-ethanolic extract of this whole plant has been found to possess anticonvulsant [3], sedative [4], antioxidant [5], anti-nociceptive properties [6, 7] and anti-proliferative effects on onion root and shoot apical meristem and wister rat bone marrow cells [8]. Hence, this study seeks to investigate further the antioxidant potential and anti-proliferative activities of various fractions of the hydro-ethanolic extract of this plant.

Oxidative stress exists when there is an imbalance between the production of free radicals and reactive metabolites, and their elimination by protective mechanisms involving antioxidant agents in the body [9]. Numerous findings have revealed that persistent oxidative stress can lead to chronic inflammation, which in turn mediates chronic diseases including cancer, diabetes, cardiovascular, neurological and pulmonary diseases [10]. Cancer is also currently one of the most severe health challenges worldwide [11], and reactive oxygen species in different phases of tumorigenesis has been strongly implicated [12, 13]. The use of antioxidants for cancer prevention, and therapy has been investigated, and several antioxidants such as curcumin, vanillin and tannic acid found in food products possess anticancer properties [14, 15]. Other antioxidants such as resveratrol, obtained from grapes and other food products, protect cells from oxidative damage, and programmed cell death [16, 17]. Quite a significant number of plants extracts with both antioxidant and anti-proliferative activities have been evaluated to have potential anti-cancer properties [18-21]. In view of this, *Synedrella nodiflora* which has already been shown to be effective in the treatment of a neurological condition such as epilepsy, and having both anti-oxidant and cell inhibitory activity was further investigated for its *in vitro* anti-oxidant and inhibitory effects on cancer cells.

MATERIALS AND METHODS

Chemicals and reagents

RPMI 1640 (Roswell Park Memorial Institute medium), DMEM (Dulbecco's Modified Eagle's medium) and EMEM (Eagle's minimal essential medium) obtained from Sigma-Aldrich, (Illinois, USA) was

used for the various cell line culture. Curcumin (Sigma-Aldrich, Illinois, USA), 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical reagent and butylated hydroxytoluene (BHT) (Sigma-Aldrich Illinois, USA), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) dye (Sigma-Aldrich, Illinois, USA), while all solvents used including (e. g. methanol, chloroform, ethyl acetate, acetone, *n*-hexane, petroleum ether, glacial acetic acid) were of HPLC grade, and all other reagents were obtained from standard suppliers.

Plant collection and extraction

Samples of the plant were collected from the Botanical Gardens, University of Ghana, Accra, in August 2012. They were identified and authenticated at the Ghana Herbarium, Department of Plant and Environmental Science, University of Ghana, Accra where a voucher specimen (PA01/UGSOP/GH12) was kept. The whole plants were collected, air-dried for fourteen days and powdered. Three hundred (300 g) of pulverized plant material was cold-macerated with 70 % v/v of ethanol in water. The hydro-ethanolic extract was then evaporated using a rotary evaporator (Buchi Rotavapor® R-300, Flawil, Switzerland) under reduced pressure to remove ethanol. The aqueous portion was frozen at-20^{0}C, and lyophilized (Bench-top Freeze Dryer, Labfreez Instruments Co., Ltd, Beijing, China). The percentage yield of the dried sample (SNE) was calculated before it was kept in a desiccator.

Phytochemistry of crude extract

The crude extract was tested qualitatively for the presence of saponins, flavonoids, tannins, alkaloids, glycosides, steroids, terpenoids coumarins, and anthraquinones according to published methods in and Sofowora, 1993, and Evans, 2009 [22, 23].

Fractionation

The weight of 50 g of dried crude 70 % v/v hydro-ethanolic extract of *S. nodiflora* (SNE) was suspended in 500 ml of 70% ethanol (v/v) and successively partitioned with an equal volume of petroleum ether, n-hexane, dichloromethane, ethyl acetate, acetone, and glacial acetic acid to obtain a petroleum ether (PEF), n-hexane (NHF), dichloromethane (DCMF), ethyl acetate (EAF), acetone (ACF), glacial acetic acid (AAF), and the remaining aqueous (AQF) fractions respectively. Each fraction was concentrated at 40 °C, using a rotary evaporator, and lyophilised. Extract yields were recorded.

Total phenol assay

The total phenolic content of the crude extract (SNE) was assayed by the Folin Ciocalteu's method, as published by Amoateng et al., 2011 [5] with slight modifications. An aliquot (10 µl) of the extract (20 mg/ml) was placed into an Eppendorf tube, to which 0.79 ml of distilled water was added. Approximately 50 µl of Folin Ciocalteu's reagent was added, and mixed thoroughly by pipetting repeatedly. The mixture was then incubated at room temperature for 8 min. After incubation, 150 µl of Na2CO3 solution was added, mixed and incubated at room temperature for 2 h. Aliquots of 200 µl each mixture was then taken into wells 96-well-plates and the absorbance was read at 750 nm. Gallic acid (5 mg/ml) was used as a control and serial dilutions of both the gallic acid and extract were made with deionized water to obtain concentrations of 0.1, 0.3, 1, 3 mg/ml for the extract and 1, 0.2, 0.4, 06, 0.8, 1 mg/ml of gallic acid respectively. These measurements for both drug and control were performed in triplicates.

Total flavonoid assay

A volume of 50 µl of stock solution of the sample (20 mg/ml) was pipetted into a 96-well-plate, and 50 µl of 2 % aluminum chloride solution was added. The mixture was incubated at room temperature for 10 min. Absorbance was then taken after incubation, at a wavelength of 367 nm. Catechin was used as control. Two-fold serial dilutions were made for both the extract and the control to obtain initial concentrations of 0.1, 0.3, 1, 3 mg/ml for the extract and 0.1, 0.2, 0.4, 06, 0.8, 1 mg/ml for catechin respectively. Triplicate experiments were performed.

Preliminary antioxidant screening (DPPH Scavenging effect) of extract and fractions

The DPPH free radical scavenging effect was done as previously described by Amoateng *et al.*, 2011 [5] with slight modifications. The assay was carried out in a 96-well plate. To 200 µl of 100 mmol DPPH solution, 10 µl of each of the test samples or standard solution was added separately. The final concentration range of the extract and fractions was 1000 to 1.95µg/ml and ascorbic acid (AA) was 100 to 0.195 µg/ml. Methanol was used as the blank. The plates were incubated at 37 °C for 20 min and the absorbance was measured at a wavelength of 490 nm, using ELISA reader (Biotek® DR 2008C, Ring Bioscience, Beijing, China). The percent inhibition was calculated from the following formula:

$$\% \text{ DPPH Scavenging} = \left[\frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}}\right] \text{x } 100$$

IC_{50} values for the reference standard, and fractions were obtained from non-linear regression curves using the GraphPad Prism version 5.0 software (GraphPad Prism software Inc. San Diego CA, USA). IC_{50} value is the concentration of extract can scavenge the initial DPPH concentration by 50 %.

Phytochemistry of the ethyl acetate fraction (EAF)

EAF, which showed the highest DPPH radical scavenging activity was also tested qualitatively for the presence of flavonoids, tannins, saponins, alkaloids, steroids, glycosides, coumarins, terpenoids and anthraquinones by standard method [24]. The total phenol and flavonoid contents of EAF were also evaluated using the methods described above for the crude extract.

Reducing power assay of the ethyl acetate fraction (EAF)

The reducing capacity of EAF was determined using the method as previously described by Amarowicz *et al.*, 2005 [25]. A mixture of 200 µl of extract solution (5 mg/ml) with 200 µl of 1 % potassium ferricyanide was prepared and incubated at 50 °C for 20 min. After incubation, a 200 µl of 10 % trichloroacetic acid solution was added. After further incubation, the mixture was centrifuged at 3000 revolutions per minute for 10 min. An aliquot of 200 µl of the supernatant was taken and mixed with 200 µl of distilled water and 40 µl of ferric chloride solution. The mixture was incubated for 30 min, after which absorbance was read at 700 nm. BHT was used as the standard. Serial dilution of the extract (l1.14-833.33 µg/ml) and standard (0.0011-0.833 µg/ml) were made and triplicated.

Lipid peroxidation (LPO) assay of the ethyl acetate fraction (EAF)

To prepare egg lecithin, three eggs were obtained from ZamZam store, 10A Bombay Castle, Ooty-1, India. The egg yolks were separated from the albumin, and mixed with the aid of a glass rod. A volume of 100 ml acetone was added, mixed and allowed to stand for about 5 min. A yellowish supernatant which formed was discarded. This was done successively until the entire yellowish colour of the egg yolk mixture was bleached and the supernatant was colourless. The creamy mixture was poured into a glass dish and dried in an oven at 37 °C. The dried white powdered precipitate (egg lecithin) was collected and stored in a refrigerator until use. Test samples of 100 µl aliquots of different concentrations (l1.14-833.33 µg/ml), and rutin (0.0011-0.833 µg/ml) were added to 1 ml of egg lecithin in phosphate buffer, while control was without test sample. Lipid peroxidation was induced by adding 10 µl $FeCl_3$ (400 mmol) and 10 µl L-ascorbic acid (200 mmol). After incubation for 1 h at 37 °C, the reaction was stopped by adding 1 ml of 15 % trichloroacetic (TCA) and 1 ml 0.375% thiobarbituric acid (TBA) in 20% acetic acid and the reaction mixture was boiled for 15 min then cooled and centrifuged. The absorbance of the supernatant was measured at a wavelength of 532 nm.

Cell culture

All the cell lines were cultured as previously described by Ham *et al.,* 2012 [26]. The Jurkat cell lines were cultured in RPMI 1640, the MCF-7 in a DMEM medium and the Chang liver cell lines in EMEM medium. All culture media were supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin L-glutamine (PSG) (Sigma-Aldrich, Illinois, USA). The cells were maintained in a humidified incubator with 5 % CO_2 at 37 °C. The cells were harvested and plated either for cytotoxicity tests or subcultures when they have reached about 80 % confluence. The DPPH reagent and BHT were dissolved in methanol to a concentration of 1 mg/ml

and MTT dye was dissolved in phosphate-buffered saline (PBS) to the same concentration.

***In vitro* antiproliferative activity of ethyl acetate fraction**

Cytotoxic (MTT) assay

The tetrazolium-based colorimetric assay (MTT) was employed to determine the effect of the ethyl acetate fraction of *S. nodiflora* (EAF) on the cell growth and viability of cancerous and normal cell lines as described previously described by Apprey *et al.*, 2015 [27]. The anti-proliferative effects of this fraction on leukemia (Jurkat), breast cancer (MCF-7) and normal liver (Chang's liver) cell lines were. The cells were plated at 1.0×10^3 cells per well in 96-well plates and incubated for 24 h. The cells were then incubated with EAF (62.5, 125, 250, 500 and 1000 µg/ml) or curcumin (1.15, 2.3, 4.6, 9.2, and 18.4 µg/ml,) for 72 h. Curcumin was dissolved in DMSO. After incubation, 20 µl of MTT solution (2.5 mg/ml) was then added to each well and the plates were further incubated for 4 h. Insoluble purple formazan crystals formed were dissolved by adding 150 µl of acidified isopropanol and incubating the plates overnight. Absorbance was taken at a wavelength of 570 nm using a microplate reader. The percentage of cell viability was calculated from the formula

$$\% \text{ Cell viability} = \left[\frac{A0 - A1}{A0 - A}\right] \times 100$$

Where A0 = Mean absorbance of control wells; A1 = Mean absorbance in test wells and A =Mean absorbance of blank wells.

The concentration of the ethyl acetate fraction causing 50 % inhibition of the cancer cell growth was considered as IC_{50}. This was obtained from a plot of percentage cell viability versus concentration of sample tested.

Statistical analysis

The IC_{50} (concentration responsible for 50 % of the maximal effect) and inhibitory effects of drugs were analyzed by using an iterative computer least squares method, GraphPad Prism for Windows version 5.0 (GraphPad Software, San Diego, CA, USA) with the following nonlinear regression (four-parameter logistic equation).

$$Y = \frac{a + (a - b)}{1 + 10^{((Log\ ED_{50} - X) \times Hill\ Slope))}}$$

Where X is the logarithm of concentration. Y is the response and starts at ***a*** and goes to ***b*** with a sigmoid shape.

The fitted midpoints (IC_{50}s) of the curves were compared statistically using the *F* test [28, 29]. $P<0.05$ was considered statistically significant in all analyses (one or two-way ANOVA followed by an appropriate *posthoc* test). The graphs were plotted using Sigma Plot for Windows Version 11.0 (Systat Software Inc., Germany).

RESULTS

Phytochemical analysis

The 70% hydro-ethanolic extract of *S. nodiflora* (SNE) showed the presence of the following compounds: flavonoids, tannins, saponins, alkaloids, cardiac glycosides, coumarins, triterpenes, sterols, anthraquinones and phenols. Similar constituents were also observed in the ethyl acetate fraction (EAF).

Fractionation of 70% hydro-ethanolic extract of *S. nodiflora* (SNE)

The successive fractionations of the 70 % hydro-ethanolic extract of *S. nodiflora* resulted in the extract yields of: dichloromethane (DCMF) (0.32 %), glacial acetic acid (AAF) (0.65 %), n-hexane (NHF) (0.68 %), ethyl acetate (EAF) (2.00 %), petroleum ether (PEF) (2.79 %), acetone (ACF) (14.74 %), and aqueous (AQF) (78.82 %). The highest amount of constituents remained in the aqueous fraction.

DPPH scavenging effect of the extract and fractions of 70 % hydro-ethanolic extract of *S. nodiflora* (SNE)

DPPH analysis showed that SNE exhibited a concentration-dependent free radical scavenging activity (fig. 1), with an IC_{50} of 114.3±0.01 µg/ml in the DPPH scavenging assay (table 1). This was significantly lower than ascorbic acid (AA). Table 1 shows that the fractions with significant DPPH scavenging effects (with regards to the IC_{50} values) were EAF and DCMF. EAF was four times lower in activity than ascorbic acid.

Table 1: IC_{50} (µg/ml) values of DPPH free radical scavenging assay of crude extract and fractions of *S. nodiflora*

IC_{50} (µg/ml)±SEM								
SNE	**AqF**	**AAF**	**ACF**	**EAF**	**DCMF**	**NHF**	**PEF**	**AA**
114.3±0.01	118.0±0.03	26.7±0.29	42.0±0.01	8.9±0.02	23.5±0.03	904.0±0.20	351.4±0.06	2.1±0.02

n=3, data given is mean±SEM

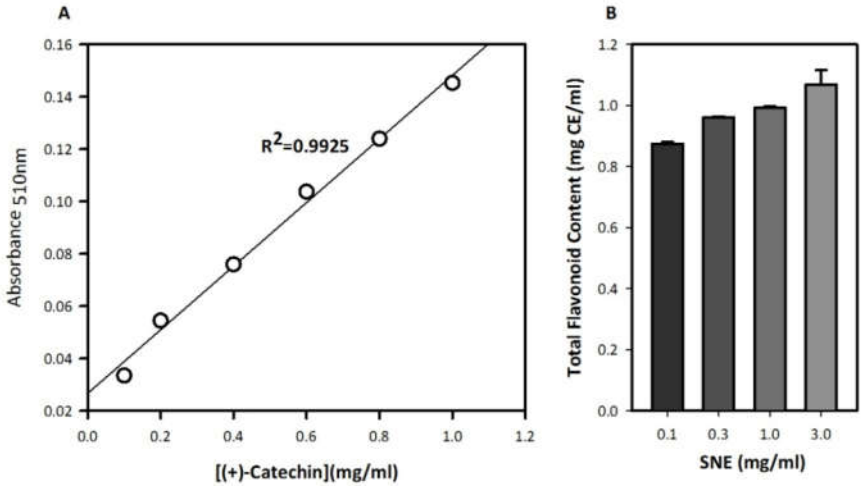

Fig. 1: (A) Calibration curve of (+)-catechin (0.1-1.0 mg/ml) and (B) total flavonoids present in various concentrations of SNE (0.1-3 mg/ml) expressed as catechin equivalents. Each point in A and columns in B represent the mean±SEM (n=3)

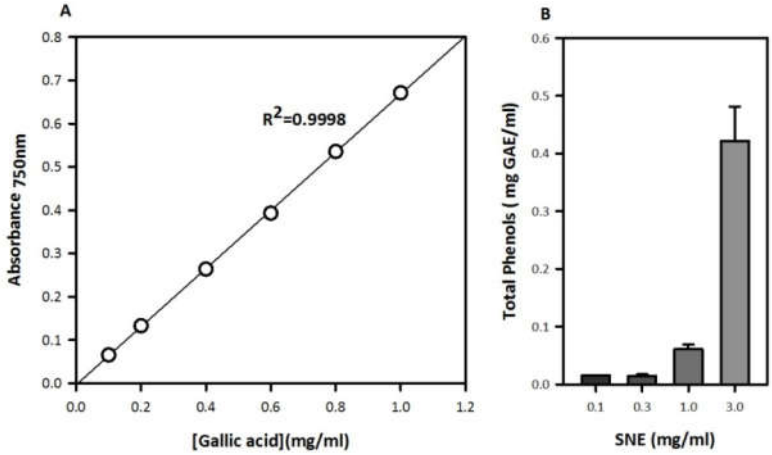

Fig. 2: (A) Calibration curve of gallic acid (0.1-1.0 mg/ml) and (B) total phenolic substances present in various concentrations of SNE (0.1-3 mg/ml) expressed as gallic acid equivalent. Each point in A and columns in B represent the mean±SEM (n=3)

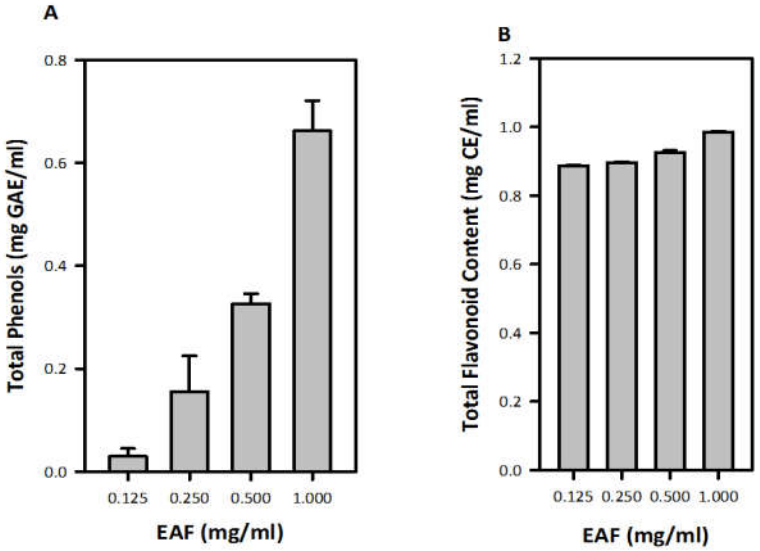

Fig. 3: (A) Total phenolic content of EAF (0.125-1 mg/ml) expressed as gallic acid equivalent and (B) total flavonoids content of EAF (0.125-1 mg/ml) expressed as catechin equivalents. Each point in A and B represent the mean±SEM (n=3)

Total phenolic content and total flavonoid content of 70 % hydro-ethanolic extract of *S. nodiflora* (SNE) and the ethyl acetate fraction (EAF)

The total flavonoid and phenol contents of SNE at a concentration of 1 mg/ml was 0.9 mg/CE/ml and 0.03 mg/GAE/ml respectively (fig. 1B and 2B). EAF, which was the fraction having shown the most potent ability to scavenge for DPPH, was further assessed for its total phenolic and flavonoid content. The fraction EAF showed a concentration-dependent increase in both total phenol (fig. 3A) and total flavonoid contents (fig. 3B). EAF at an approximate concentration of 0.1 mg/ml, had a total phenol content of 0.07 mg/GAE/ml (fig. 3A). The flavonoid content of the same concentration of EAF was 0.9 mg/CE/ml.

Reducing power and lipid peroxidation effects of EAF

Both BHT (the reference antioxidant) and the EAF exhibited a concentration-dependent reduction of $Fe^{3+}$to Fe^{2+}, resulting in a concentration-dependent increase in absorbance (fig. 4). BHT was found to be about 1000 fold more potent than EAF (table 2). Also, Both rutin and EAF exhibited concentration-dependent inhibition of lipid peroxidation, as seen in fig. 5, however as seen with the IC_{50} values, EAF is about 3-fold less potent than rutin (table 2).

Table 2: IC_{50} values (µg ml) of EAF and reference antioxidant drugs in reducing power and lipid peroxidation assays

	Reducing power	Lipid peroxidation
EAF	151.1±0.04	24.01±0.08
BHT	0.15±0.02	-
Rutin	-	8.38±0.04

n=3, data given is mean±SEM

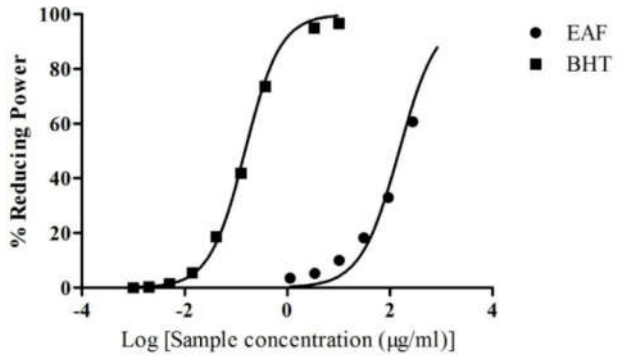

Fig. 4: Reducing power effects of EAF (1.14-833.33 µg/ml) and BHT (0.001-10 µg/ml). Each point represents the mean±SEM (n = 3)

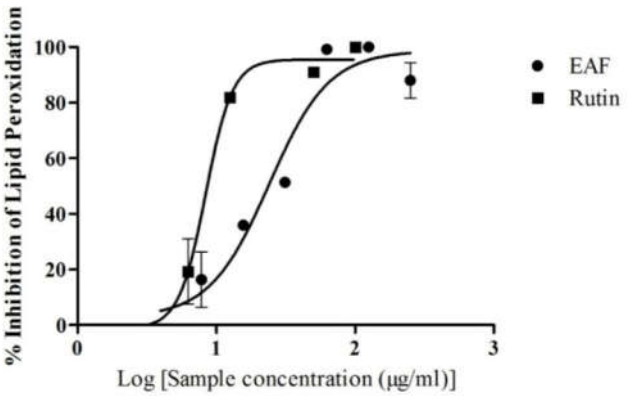

Fig. 5: Effects of the inhibition of lipid peroxidation by EAF (3.906-1000 µg/ml) and rutin (3.125-100 µg/ml). Each data point represents the mean±SEM (n = 5)

Cytotoxicity of ethyl acetate fraction (EAF) on cell lines

Both curcumin (the reference compound) and EAF exhibited concentration-dependent inhibition of the cell growth of Chang's liver (fig. 6), Jurkat (fig. 7), and MCF-7 (fig. 8) cell lines. The rank order of potency, as defined by the IC_{50} (table 3), showed that EAF was about 114-fold less cytotoxic to normal human (Chang's liver) cells than curcumin. The EAF also showed 187-fold less cytotoxicity than curcumin on the Jurkat cells and 57-fold less than curcumin on the MCF-7 cells (table 3).

Table 3: IC_{50} (µg/ml) values of EAF and curcumin on MCF-7, Chang liver and Jurkat cell lines

	MCF-7	Chang's liver	Jurkat
EAF	205.2±0.14	252.2±0.14	170.9±0.02
Curcumin	3.56±0.06	2.20±0.03	0.91±0.05

n=3, data given is mean±SEM

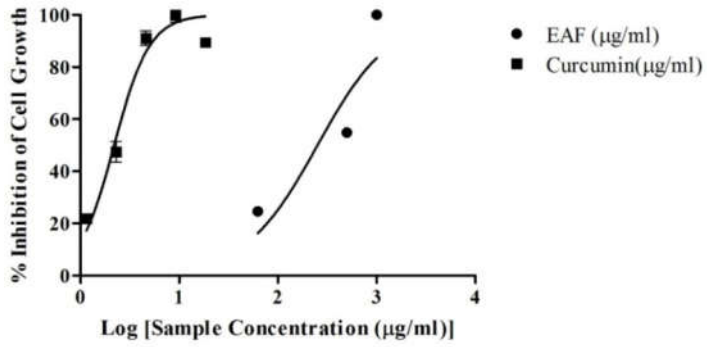

Fig. 6*:* Effects of EAF (62.5-1000 µg/ml), and curcumin (1.15-18.4 µg/ml) on the cell growth of chang liver cells. Each data point represents the mean±SEM (n = 3)

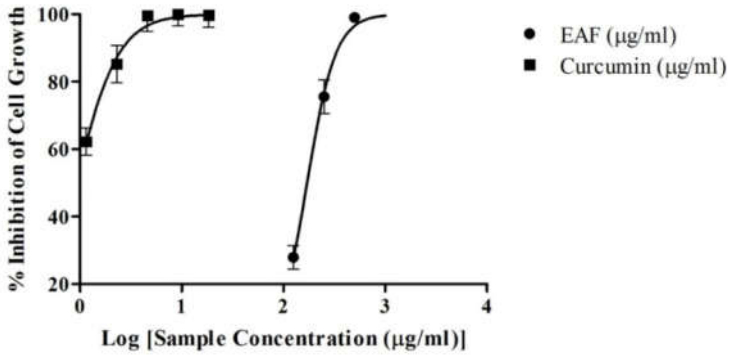

Fig. 7: Effects of EAF (62.5-1000 µg/ml), and curcumin (1.15-18.4 µg/ml) on the cell growth of Jurkat cells. Each data point represents the mean±SEM (n = 3)

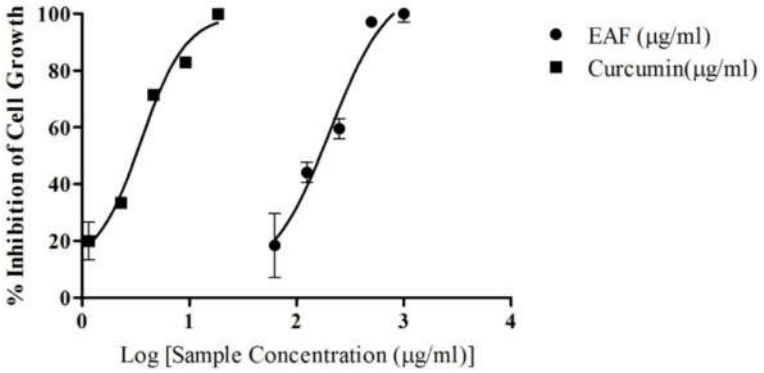

Fig. 8: Effects of EAF (62.5-1000 µg/ml), and curcumin (1.15-18.4 µg/ml) on the cell growth of MCF-7 cells. Each data point represents the mean±SEM (n = 3)

DISCUSSION

This study demonstrates the free radical scavenging, as well as the anti-lipid peroxidative effects of the hydro-ethanolic extract, and its solvent-extracted fractions *in vitro*. This results further supports the basic antioxidant activities of *S. nodiflora* which has already been published [5]. Antioxidants protect the body against various reactive oxygen species [30], which otherwise are linked to the etiology of several diseases. The phytochemistry of *S. nodiflora* revealed the presence of flavonoids, tannins, saponins, alkaloids, cardiac glycosides, coumarins, triterpenes, sterols, anthraquinones and phenols. This confirms earlier findings on the phytoconstituents present in similar extracts of the plant [31]. The phytochemicals of first-time identification for this extract are the cardiac glycosides and coumarins. Many of the secondary metabolites present in this plant has been found to possess antioxidant properties [32]. Many alkaloids, and polyphenols such as tannins and flavonoids, are known to be potent free radical scavengers [33-36]. Polyphenols are electron-rich compounds and can go into electron-donation reactions with oxidizing agents to form stable species [37], and thus inhibit or delay the oxidation of different biomolecules [25, 38]. Phenolic antioxidants are potent free radical terminators and this is thought to be due to the ability to donate hydrogen to free radicals, and their presence in *S. nodiflora* is a good marker of potential antioxidant activity [39, 40].

The DPPH test is valid for the primary characterization of the scavenging potential of compounds [41]. *S. nodifolia* extract's ability to scavenge for DPPH concentration-dependently confirms earlier reports [5]. Fractionation showed an increase in this activity for the acetone, glacial acetic acid, dichloromethane and ethyl acetate fractions. These fractions most likely retained polar phenolic compounds that may possess antioxidant activities [42, 43]. However, the most potent fraction was the ethyl acetate fraction (EAF) with IC_{50} of 8.9±0.02 µg/ml. This is about 4 times lower in activity than ascorbic acid. This suggests the need for further optimization, to isolate the antioxidant component from this fraction. Hence is was selected for further assessment for reducing power, lipid peroxidation and inhibition of the cancerous cells. At the concentration of 0.1 mg/ml, the total phenolic content per mg GAE/ml of the EAF was about ten times higher than that of the extract SNE, but the total flavonoid content was similar. This suggests that the antioxidant activity may likely be due to a non-flavonoid component of the extract. This implies that EAF, and other fractions may need to be optimized to determine the most potent fractions with regards to biological activities also.

Reducing power reflects the ability of an agent to convert iron (Fe) from the ferric (Fe^{3+}) to the Fe^{2+}state. In principle, an agent having this reduction potential will react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric-ferrous complex [44]. Physiologically, iron in the ferric state reacts with peroxides to form free radicals that initiates further oxidation of physiological molecules. Lipid peroxidation has been implicated in several diseases, conditions and ageing; atherosclerosis, cataract, rheumatoid arthritis, and neurodegenerative disorders [45]. Antioxidants that inhibit lipid peroxidation may thus have a potential role in the prevention of such diseases. Four main mechanisms associated with antioxidant defense against lipid peroxidation include: prevention of the formation of active oxidizing agents, scavenging, quenching and removal of active oxidants, repair of damage and excretion of products of toxic oxidation, and adaptive responses [45]. EAF inhibited the peroxidation of egg lecithin in a concentration-dependent order which may be attributed to the presence of antioxidant phytoconstituents.

EAF produced anti-proliferative activity against breast cancer (MCF-7), and leukemia (Jurkat) cell lines. The MCF-7 cell lines are known

to possess progesterone and estrogen receptors and provides a proliferative response to estrogen [46-48]. Jurkat cells are immortalized line of T-lymphocyte cells which are used to determine the mechanism of the susceptibility of leukemic cancers to drugs [49]. Thus the ability of EAF to inhibit these cell lines suggests that it may have a potential use in breast and leukemic cancers. Several findings for example, have proven that phytoconstituents such as flavonoids have significant anticancer properties [50].

In this study, the extract had the highest cytotoxic effect on the breast cancer cell line. A recent report showed that the aqueous extract from *S. nodiflora* was able to inhibit the proliferation of onion root and shoot apical meristem and Wister rat bone marrow cells [8]. EAF had a very high IC_{50} (252.2±0.14 µg ml), hence it is less inhibitory to the human hepatocyte-derived cell line (Chang Liver cell) in comparison to curcumin [15]. This suggests that EAF has some selective killing potential on cancerous cells, whiles leaving the normal cells, and this may warrant further investigation.

CONCLUSION

In summary, the ethyl acetate fraction from the hydro-ethanolic extract of the whole plant of *Synedrella nodiflora* possesses potent antioxidant activities and anti-proliferative activities against MCF-7, Jurkat, and Chang liver cells. These results may warrant further investigation for potential anti-cancer drug development.

ACKNOWLEDGEMENT

The authors wish to show their appreciation to the technical staff of the Departments of Pharmacognosy and Phytopharmacy, and Pharmaceutical Biotechnology, JSS College of Pharmacy, Ootacamund, TN, India and Clinical Pathology. Appreciations also go to the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, for providing the technical support in the laboratory work.

FUNDING

Department of Science and Technology, FICC, Government of India funded the *in vitro* antioxidant assay under the CV Raman Postdoctoral fellowship awarded to Dr Patrick Amoateng. The *in vitro* cytotoxic effects of the ethyl acetate fraction was supported by Noguchi Memorial Institute for Medical Research and School of Pharmacy, University of Ghana, Legon.

AUTHORS CONTRIBUTIONS

PA, EOB, RAO, and SPD participated sufficiently in the intellectual content, conception and design of this work. PA, RAO, MVLNC and RR performed the experiment, analysis and interpretation. PA and EOB drafted the initial manuscript and RAO, BBN and DOS made inputs and corrections. All authors read and made corrections to the finalised manuscript before submission.

CONFLICT OF INTERESTS

None

REFERENCES

1. Mshana NR, Abbiw DK, Addae Mensah I, Adjanohoun E, Ahyi MRA, Enow Orock EG, *et al.* Traditional medicine and pharmacopoeia. Contribution to the revision of ethnobotanical and floristic studies in Ghana. Scientific, Technical and Research Commission of the Organization of African Unity: Scientific, Technical and Research Commission (OAU); 2000.
2. Invasive Species Compendium. Available from: https://www.cabi.org/isc/datasheet/52325 [Last accessed 26 Nov 2019]
3. Amoateng P, Woode E, Kombian SB. Anticonvulsant and related neuropharmacological effects of the whole plant extract of *Synedrella nodiflora* (L.) Gaertn (Asteraceae). J Pharm Bioallied Sci 2012;4:140-8.
4. Woode E, Amoateng P, Abotsi WMK. Ethopharmacological analysis of the effects of the whole plant extract of synedrella nodiflora (L.) gaertn (Asteraceae) in murine models. Pharm Sin 2011;2:54-67.
5. Amoateng P, Assumeng Koffuor G, Sarpong K, Oteng Agyapong K. Free radical scavenging and anti-lipid peroxidative effects of a hydro-ethanolic extract of the whole plant of synedrella nodiflora (L.) gaertn (Asteraceae). Free Rad Antiox 2011;1:70-8.
6. Woode E, Amoateng P, Ansah C, Duwiejua M. Anti-nociceptive effects of an ethanolic extract of the whole plant of synedrella nodiflora (L.) gaertn in mice: involvement of adenosinergic mechanisms. JPT 2009;4:17-29.
7. Amoateng P, Adjei S, Osei Safo D, Ameyaw EO, Ahedor B, N'Guessan BB, *et al*. A hydro-ethanolic extract of Synedrella nodiflora (L.) Gaertn ameliorates hyperalgesia and allodynia in vincristine-induced neuropathic pain in rats. JPCPP 2015;26:383-94.
8. Sanjib R. Antiproliferative activity of allelochemicals present in aqueous extract of synedrella nodiflora (L.) gaertn. In apical meristems and wistar rat bone marrow cells. IOSR J Pharm 2013;3:1-10.
9. Durackova Z. Some current insights into oxidative stress. Physiol Res 2010;59:459-69.
10. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free Radical Biol Med 2010;49:1603-16.
11. Ma X, Yu H. Global burden of cancer. Yale J Biol Med 2006;79:85-94.
12. Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. Toxicol Pathol 2010;38:96-109.
13. Sosa V, Moline T, Somoza R, Paciucci R, Kondoh H, ME L. Oxidative stress and cancer: an overview. Ageing Res Rev 2013;12:376-90.
14. Johnson IT, Williamson G, Musk SR. Anticarcinogenic factors in plant foods: a new class of nutrients? Nutr Res Rev 1994;7:175-204.
15. Sa G, Das T. Anti-cancer effects of curcumin: the cycle of life and death. Cell Div 2008;3:14.
16. Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, *et al.* Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science (New York, NY) 1997;275:218-20.
17. Clement MV, Hirpara JL, Chawdhury SH, Pervaiz S. Chemopreventive agent resveratrol, a natural product derived from grapes, triggers CD95 signaling-dependent apoptosis in human tumor cells. Blood 1998;92:996-1002.
18. Gul Mir Z, Bhakshu LM, Ahmad F, Kondapi AK, Qureshi IA, Ghazi IA. Evaluation of Abelmoschus moschatus extracts for antioxidant, free radical scavenging, antimicrobial and antiproliferative activities using *in vitro* assays. BMC Complem Altern Med 2011;11:64.
19. Gul MZ, Ahmad F, Kondapi AK, Qureshi IA, IA G. Antioxidant and antiproliferative activities of Abrus precatorius leaf extracts-an *in vitro* study. BMC Complem Altern Med 2013;13:53.
20. Khanapur M, Avadhanula RK, Setty OH. *In vitro* antioxidant, antiproliferative, and phytochemical study in different extracts of nyctanthes arbortristis flowers. BioMed Res Int 2014;2014:1-0.
21. Jimenez Estrada M, Velazquez Contreras C, Garibay Escobar A, Sierras-Canchola D, Lapizco Vazquez R, Ortiz Sandoval C, *et al*. *In vitro* antioxidant and antiproliferative activities of plants of the ethnopharmacopeia from northwest of Mexico. BMC Complem Altern Med 2013;13:12.
22. Evans WC. Trease and evans pharmacognosy. 16th edn. London: Saunders Publishers; 2009.
23. Sofowora A. Medicinal plants and traditional medicinal in Africa. 2 edn. Ibadan, Nigeria: Spectrum Books Ltd; 1993.
24. Evans WC. Trease and Evans' Pharmacognosy. 15 edn. Edinburgh: Saunders Ltd; 2002.
25. Amarowicz R, Troszynska A, Shahidi F. Antioxidant activity of almond seed extract and its fractions. J Food Lipids 2005;12:344-58.
26. Ham YM, Yoon WJ, Park SY, Jung YH, Kim D, Jeon YJ, et al. Investigation of the component of Lycopodium serratum extract that inhibits proliferation and mediates apoptosis of human HL-60 leukemia cells. Food Chem Toxicol 2012;50:2629-34.
27. Apprey C, Larbie C, Arthur FKN, Appiah Opong R, Annan RA, Tuffour I. Anti-proliferative effect of Isoflavones Isolated from

soybean and soymilk powder on lymphoma (DG 75) and leukemia (CEM) cell lines. Br J Pharm Res 2015;7:206-16.

28. Motulsky HJ, Christopoulos A. Fitting model to biological data using linear and nonlinear regression. A practical guide to curve fitting. San Diego CA: GraphPad Software Inc; 2003.
29. Miller JR. GraphPad Version 4.0. step-by-step examples. GraphPad Software Inc, San Diego CA. In; 2003.
30. Fitrotunnisa Q, Arsianti A, Tejaputri NA, Qorina F. Antioxidative activity and phytochemistry profile of hibiscus sabdariffa herb extracts. Int J Appl Pharm 2019;11:29-32.
31. Martin Rathi J, Gopalakrishnan S. Insecticidal activity of aerial parts of syndrella nodiflora gaertn (Compositae) on spodoptera litura (Fab). J Cent Eur Agric 2006;7:289-96.
32. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 2004;74:2157-84.
33. Dekkers JC, van Doornen LJP, Kemper HCG. The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage. Sports Med 1996;21:213-38.
34. Clarkson PM. Antioxidants and physical performance. Crit Rev Food Sci Nutr 1995;35:131-41.
35. Ismail A, Marjan ZM, Foong CW. Total antioxidant activity and phenolic content in selected vegetables. Food Chem 2004;87:581-6.
36. Skandrani I, Boubaker J, Bouhlel I, Limem I, Ghedira K, Chekir Ghedira L. Leaf and root extracts of Moricandia arvensis protect against DNA damage in human lymphoblast cell K562 and enhance antioxidant activity. Environ Toxicol Pharmacol 2010;30:61-7.
37. Kang H, Mansel RE, Jiang WG. Genetic manipulation of stromal cell-derived factor-1 attests the pivotal role of the autocrine SDF-1-CXCR4 pathway in the aggressiveness of breast cancer cells. Int J Oncol 2005;26:1429-34.
38. Seidel V, Verholle M, Malard Y, Tillequin F, Fruchart JC, Duriez P, *et al*. Phenylpropanoids from ballota nigra L. inhibit *in vitro* LDL peroxidation. Phytother Res 2000;14:93-8.
39. Soobrattee MA, Neergheen VS, Luximon Ramma A, Aruoma OI, Bahorun T. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. Mutat Res Fund Mol M 2005;579:200-13.
40. Wijaya S, Nee T, Jin KT, Hon LK, San LH, Wiart C. Antibacterial and antioxidant activities of synedrella nodiflora (L.) gaertn. (Asteraceae). J Complement Integrt Med 2011;8:13.
41. Krings U, Berger RG. Antioxidant activity of some roasted foods. Food Chem 2001;72:223-9.
42. Cho J, Kim HM, Ryu JH, Jeong SY, Lee YS, Jin C. Neuroprotective and antioxidant effects of the ethyl acetate fraction prepared from Tussilago farfara L. Biol Pharm Bull 2005;28:455-60.
43. Sowndhararajan K, Kang SC. Protective effect of ethyl acetate fraction of Acacia ferruginea DC. against ethanol-induced gastric ulcer in rats. J Ethnopharmacol 2013;148:175-81.
44. Arulpriya P, Lalitha P, Hemalatha S. *In vitro* antioxidant testing of the extracts of Samanea saman (Jacq.) merr. Chem Sin 2010;1:73-9.
45. Niki E, Yoshida Y, Saito Y, Noguchi N. Lipid peroxidation: mechanisms, inhibition, and biological effects. Biochem Bioph Res Co 2005;338:668-76.
46. Holliday DL, Speirs V. Choosing the right cell line for breast cancer research. Breast Cancer Res 2011;13:215.
47. Soule HD, Vazguez J, Long A, Albert S, Brennan M. A human cell line from a pleural effusion derived from breast carcinoma. J Natl Cancer Inst 1973;51:1409-16.
48. Levenson AS, Jordan VC. MCF-7: the first hormone-responsive breast cancer cell line. Cancer Res 1997;57:3071-8.
49. Schneider U, Schwenk HU, Bornkamm G. Characterization of EBV-genome negative "null" and "T" cell lines derived from children with acute lymphoblastic leukemia and leukemic transformed non-hodgkin lymphoma. IJC 1977;9:621-6.
50. Lotha R, Sivasubramanian A. Flavonoids nutraceuticals in prevention and treatment of cancer: a review. AJPCR 2018;11:42-7.