

Full Length Research Paper

Pharmacological effect of some fractions obtained from *Sapindus trifoliatus* acting as an antioxidant and against mammary cell proliferation

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To explore the naturally-occurring antioxidants having anticancer properties from plant origin since oxidants play a crucial role in developing various human diseases. The purpose of this research was to explore the antioxidant and anticancer properties of *Sapindus trifoliatus* (ST). The dried leaves of *S. trifoliatus* were ground into coarse powder and were exhaustively extracted with methanol; and the resulting crude methanolic extract (CME) was successively fractionated with petroleum ether, chloroform and ethyl acetate to obtain final extracts of petroleum ether (PEF), chloroform (CHF), ethyl acetate (EAF) and lastly the fractions of aqueous (AQF). Several assays were employed to determine the antioxidant activities, such as, 1,1-diphenyl-2-picrylhydrazine (DPPH) free radical scavenging assay, total antioxidant capacity assay, ferrous reducing antioxidant capacity, hydroxyl radical scavenging assay and lipid peroxidation inhibition assay. The *in vivo* anticancer activity of *S. trifoliatus* was deduced on Ehrlich's Ascites cell (EAC) induced Swiss albino mice. Majority of the extracts showed strong antioxidant activities related to the standard.

Key words: *Sapindus trifoliatus*, Sapindaceae, free radicals, polyphenolics, flavonoids, antioxidant activity, anticancer activity.

INTRODUCTION

Here, it is pertinent to mention the oxidative stress (OS) that results from an imbalance between formation and neutralization of pro-oxidants. Initiation of OS is due to free radicals which become stable through electron pairing with biological macromolecules. This causes protein and DNA damage progressively leading to lipid peroxidation. It has been observed that OS has been a major cause for pathogenesis of cancer, atherosclerosis, diabetes, ageing, inflammatory diseases and cardiovascular

diseases (Uttara et al., 2009; Halliwell and Gutteridge, 1984). The damage can become more widespread due to weakened cellular antioxidant defence systems. All biological systems have antioxidant defence mechanisms that protect against oxidative damages and repair enzymes to replace affected molecules. Dietary intake of antioxidant is important to stabilise the natural antioxidant defence mechanism.

Antioxidants are substances that prevent damage to cells

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caused by free radicals by supplying electron to these free radicals. Damage to the adjacent cells is thus prevented by stabilizing the molecules. They additionally change free radicals into waste by-products that eventually get eliminated from the body. Moreover, regular intake of fruits and vegetables is understood to lower the chance of many diseases, like cancer, vessel diseases and stroke caused by OS and such health benefits are primarily for the phytochemicals, such as poly-phenols, carotenoids and flavonoids (Buttar et al., 2005).

Sapindus trifolius belongs to the family Sapindaceae and is widely spread in India, Srilanka. The bark of the plant is astringent, sweet, refrigerant, carminative, diuretic, digestive, antihelmintic, febrifuge, respiratory diseases (Muruganandan et al., 2001), diarrhoea (Caceres et al., 1993) and antibacterial. The fruits and seeds are used to treat diabetes (Chakraborty et al., 1986), dysentery (Chopra et al., 1958), allergic disorders (Kim et al., 1958)], urethrorrhea and ringworm infection. The leaves have been extensively used to treat diabetes, constipation (Kim et al., 1998), leucorrhoea, stomachalgia, fever, gastropathy, strangury and dermopathy (Warrier et al., 1996) and to inhibit blood discharges in the faeces (Bhandary et al., 1995).

S. trifolius (ST) have been selected since the information of medicinal values on it is still lacking in the literature. Therefore, the research was carried out to enrich the information of the medicinal property of *S. trifolius* in terms of antioxidant and anticancer as well as its polyphenolic and flavonoid contents in the literature.

METHODOLOGY

Collection of plant materials

Leaves of ST were collected from Utkal University Campus, Bhubaneswar, Odisha, India in June 2012 and were identified by an expert taxonomist at Botany Department, Utkal University where a voucher specimen was deposited (Voucher specimen No:123-ST/BOT). Plant materials were then washed with fresh water to remove dirty materials and were shade dried for several days. They were sun dried sometimes. The dried materials were pulverised into coarse powder by grinding machine, and were stored at room temperature for future use.

Preparation of extract

The powdered plant materials of about 700 g were taken in an extraction bottle of 2.5 L capacity and they were soaked with methanol (1 L $\times$ 3 times) with timely stirring for 7 days. The extracts were filtered through cotton and filter paper. It was then concentrated with a rotary evaporator under reduced pressure at 45°C to afford 50 g crude extract. The extract was then fractionated successively to obtain petroleum ether fraction (PEF, 15.54 g), chloroform fraction (CHF, 11 g), ethyl acetate fraction (EAF, 8.68 g) and aqueous fraction (AQF, 14.50 g).

Chemicals

Catechin (CA), ferrous ammonium sulphate, butylated hydroxyl toluene (BHT), gallic acid (GA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), quercetin (QU), DMSO, EDTA, thiobarbituric acid (TBA), acetyl acetone and FeCl_3 , potassium ferricyanide, potassium acetate, phosphate buffer, ascorbic acid (AA), AlCl_3 , trichloro acetic acid (TCA), sodium phosphate, ammonium molybdate, and tannic acid were purchased from Sigma-Aldrich Chemical Co. (Bangalore); vanillin was procured from BDH; Folin-Ciocalteu's phenol reagent and sodium carbonate were also obtained from Sigma-Aldrich Chemical Co. (Bangalore).

Estimation of total phenolics

The extracts were taken for estimation of total phenolic contents by the modified Folin-Ciocalteu method (Wolfe et al., 2003). An aliquot of the extracts/standard was mixed with 2 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 2 ml (75 g/L) of sodium carbonate. The tubes were vortexed for 15 s, and allowed to stand for 20 min at 25°C for colour development. Absorbance was then measured at 760 nm UV-spectrophotometer (Shimadzu, USA). Samples of extracts standard were evaluated at a final concentration of 0.1 mg/ml. Total phenolic contents were expressed in terms of gallic acid equivalent (GAE; standard curve equation: $y = 0.011x + 0.066$, $R^2 = 0.997$), mg of GA/g of dry extract.

Total flavonoids estimation

From the leaves, total flavonoids were estimated using the following method (Ordenez et al., 2006). To 0.5 ml of samples/standard, 1.5 ml of methanol, 100 μl of 10% AlCl_3 , 100 μl of 1 M CH_3COOK solution and 2.8 ml of distilled water was added. After 1 h 30 min of incubation at room temperature (RT), the absorbance was recorded at 420 nm. The samples and the standard was evaluated at a final concentration of 0.1 mg/ml. Total flavonoid contents were expressed in terms of catechin equivalent (CAE; standard curve equation: $y = 0.003x + 0.022$, $R^2 = 0.996$), mg of CA/g of dry extract.

Estimation of total flavonols

Total flavonols in the leaf extracts were estimated using the following method (Kumaran and Karunakaran, 2007). To 2.0 ml of sample/standard, 2.0 ml of 2% AlCl_3 ethanol and 3.0 ml (50 g/L) sodium acetate solutions were added. The absorption at 450 nm was read after 2.5 h at 20°C. Extractives/standard were evaluated at a final concentration of 0.1 mg/ml. Total content of flavonols was expressed in terms of quercetin equivalent, QUE; standard curve equation:

$$y = 0.0255x + 0.0069, R^2 = 0.999 \text{ mg of QU/g of dry extract.}$$

Estimation of total proanthocyanidins

From the leaf extract, the content of proanthocyanidins was estimated based on the following method (Sun et al., 1998). From extracts, a volume of 0.5 ml of 0.1 mg/ml standard solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml hydrochloric acid; the mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm and samples; standard was evaluated at a final concentration of 0.1 mg/ml. Total content of proanthocyanidins was expressed in terms of catechin equivalent,

CAE; standard curve equation:

$$y = 0.567x - 0.024, R^2 = 0.982 \text{ mg of CA/g of dry extract.}$$

Determination of antioxidant activity

Estimation of total antioxidant capacity

Total antioxidant capacity (TAC) of samples and standard was determined by the following method (Prieto et al., 1999). Briefly, 0.3 ml of extracts in methanol (1 mg/ml) was added to 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The solutions formed were incubated at 95°C for 90 min. After cooling to room temperature, the absorbance was recorded at 695 nm. The results were evaluated through the standard curve of AA obtained by the same procedure. Increased absorbance of the reaction mixture indicated increase in total antioxidant capacity.

Determination of ferrous ions (Fe^{2+}) reducing antioxidant capacity

Different concentrations were taken for the ferrous reducing power of samples; standard was evaluated by the method of Oyaizu (1986). 0.25 ml samples in standard solution at different concentrations, 0.625 ml of potassium buffer (0.2 M) and 0.625 ml of 1% potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] solution were added into the test tubes. The mixture was incubated at 50°C for 20 min. After incubation, 0.625 ml of TCA (10%) was added to terminate the reaction and centrifuged at 3000 rpm for 10 min. The upper portion of the solution (1.8 ml) was mixed with 1.8 ml distilled water, and 0.36 ml FeCl_3 solution (0.1%) was added and the absorbance was measured at 700 nm against an appropriate blank solution. Ascorbic acid at various concentrations (10 to 100 $\mu\text{g}/\text{ml}$) was used as standard. Increased absorbance of the reaction mixture indicated increased reducing power.

DPPH free radical scavenging assay

Free radical scavenging activity was determined by DPPH radical scavenging assay (Choi et al., 2000). A solution of 0.1 mM DPPH in methanol was prepared and 2.5 ml of this solution was mixed with 1.5 ml of extractives in methanol at different concentrations. The mixture was mixed thoroughly and left in the dark at room temperature for 30 min. At 517 nm, the absorbance of the mixture was measured spectrophotometrically. BHT was used as reference standard. DPPH radical scavenging activity was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \{(A_0 - A_1)/A_0\} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the extractives/standard. Then percentage of inhibition was plotted against concentration, and from the graph, IC_{50} was calculated.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the extractives/standard was determined by the following method (Klein et al., 1981) with some changes. 0.5 ml of extractives/standard at various concentrations was taken in different test tubes. 1 ml of Fe-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 ml of 0.018% EDTA solution, 1 ml of 0.85% DMSO solution and 0.5 ml of 22% AA

were added into each of the test tubes. The test tubes were closed tightly and warmed for 15 min at 85°C into the water bath. After incubation, the test tubes were opened and 0.5 ml ice cold TCA (17.5%) was added to each of the test tubes immediately. 3 ml of reagent (7.5 g of ammonium acetate, 300 μl glacial acetic acid and 200 μl 2,4-pentanedione were mixed and adjusted to 100 ml) was added into all the test tubes and incubated at room temperature for 15 min. Absorbance was taken at 412 nm. Percentage hydroxyl radical scavenging activity was calculated by the following equation:

$$\text{Hydroxyl radical scavenging activity (\%)} = \{(A_0 - A_1)/A_0\} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the extractives/standard. Then percentage of inhibition was plotted against the concentration and IC_{50} was calculated.

Lipid peroxidation inhibition assay

The lipid peroxidation inhibition assay was determined according to the method described by Liu et al. (2000) with a slight modification. Excised rat liver was homogenized in buffer and then centrifuged to obtain liposome. 0.5 ml of supernatant, 100 μl of 10 mM FeSO_4 , 100 μl of 0.1 mM AA and 0.3 ml of extractives/standard at different concentrations were mixed to make the final volume of 1 ml. The reaction mixture was incubated at 37°C for 20 min. One millilitre of 28% TCA and 1.5 ml of 1% TBA was added immediately after heating. Reaction mixture was again heated for 15 min at 100°C and cooled at room temperature. After cooling, the absorbance was taken at 532 nm. Inhibition of lipid peroxidation was calculated by the following equation:

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

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard.

Then percentage of inhibition was plotted against concentration and IC_{50} was calculated from the graph.

Determination of anticancer activity

Cell growth inhibition

Cell growth inhibition was carried out by the method described by Sur and Ganguly (1994). Protocol used in this study for the use of mice as animal model for cancer research was approved by the Institutional Animal Ethical Committee, UDPS, Utkal University of CPCSEA (Government of India, Regn. No-990/c/CPCSEA/05).

In this research study, four groups of mice (6 in each group) were used on Day 1, 14×10^5 Ehrlich's Ascites cells (EAC)/mouse as inoculated into each group of mice. After 24 h of EAC inoculation treatment was started which continued for 5 days. Test compound (25 and 50 mg/kg) was given to Groups 1 and 2, respectively per day per mouse. In each case, the volume of the test solutions injected intraperitoneally (i.p.) was 0.1 ml/day per mouse. Group 3 received standard bleomycin (0.3 mg/kg, i.p) and were considered as positive control. Vehicle (normal saline) was given to Group 4 and was considered as negative or untreated control. On the 6th day after collection of tumour cells by repeated i.p. wash with 0.9% saline, the mice were sacrificed. Viable tumour cells per mouse of the treated group were compared with those of the control. The cell growth inhibition was calculated using the following formula:

$$\text{Cell growth inhibition (\%)} = (1 - \text{Tw} / \text{Cw}) \times 100$$

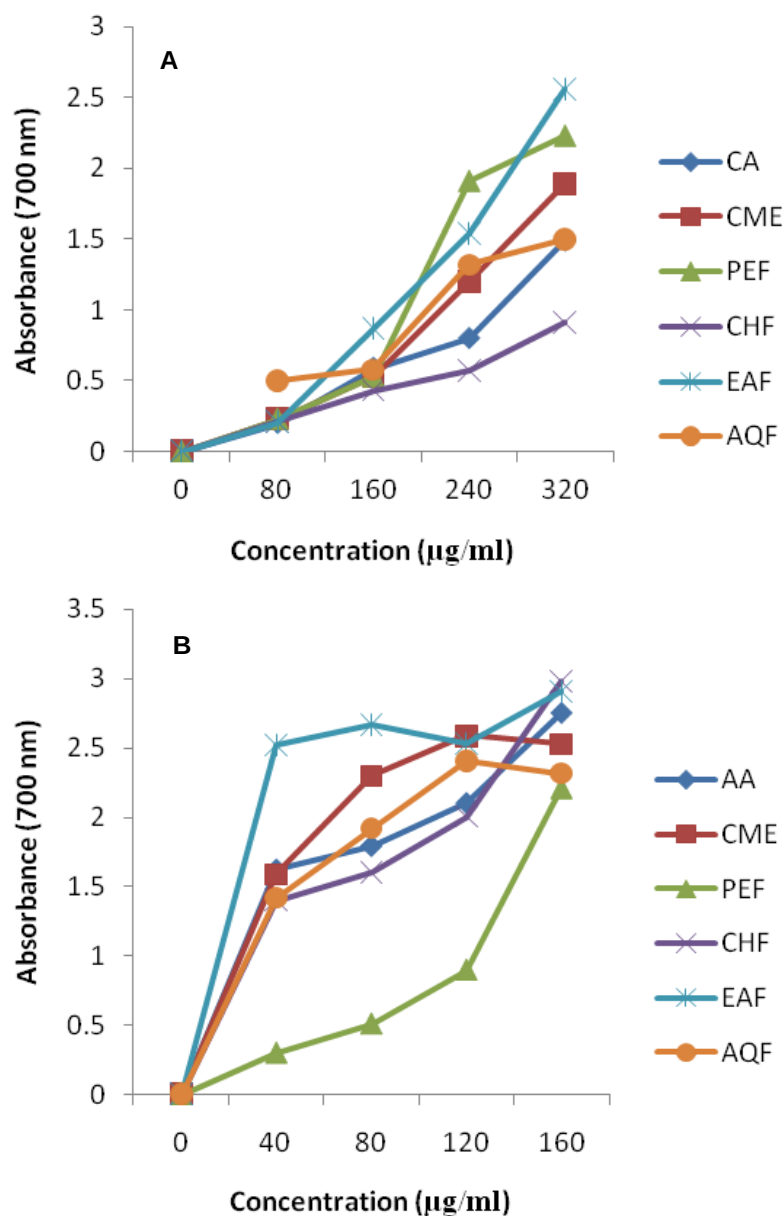


Figure 1. Determination of (A) total antioxidant capacity and (B) ferrous reducing antioxidant capacity of CME and its various fractions (PEF, CHF, EAF and AQF).

Data expressed as mean $\pm$ standard deviation (SD; $n=3$, $p < 0.05$) for all tested doses.

where Tw is the mean of the number of tumour cells of the treated group of mice and Cw is the mean of the number of tumour cells of the control group of mice.

Statistical analysis

Triplicate analyses were carried out and data were presented as mean $\pm$ standard deviation (SD). To evaluate significant relationships between experimental parameters by correlation and regression analyses, F- and t-tests ($p < 0.001$) were used.

RESULTS

TAC and ferrous reducing antioxidant capacity estimation

The TAC of CME and its four fractions of leaves of ST are as shown in Figure 1A. CME of leaves of ST showed higher antioxidant activity as compared to the reference standard CA at all the concentrations. The absorbance of

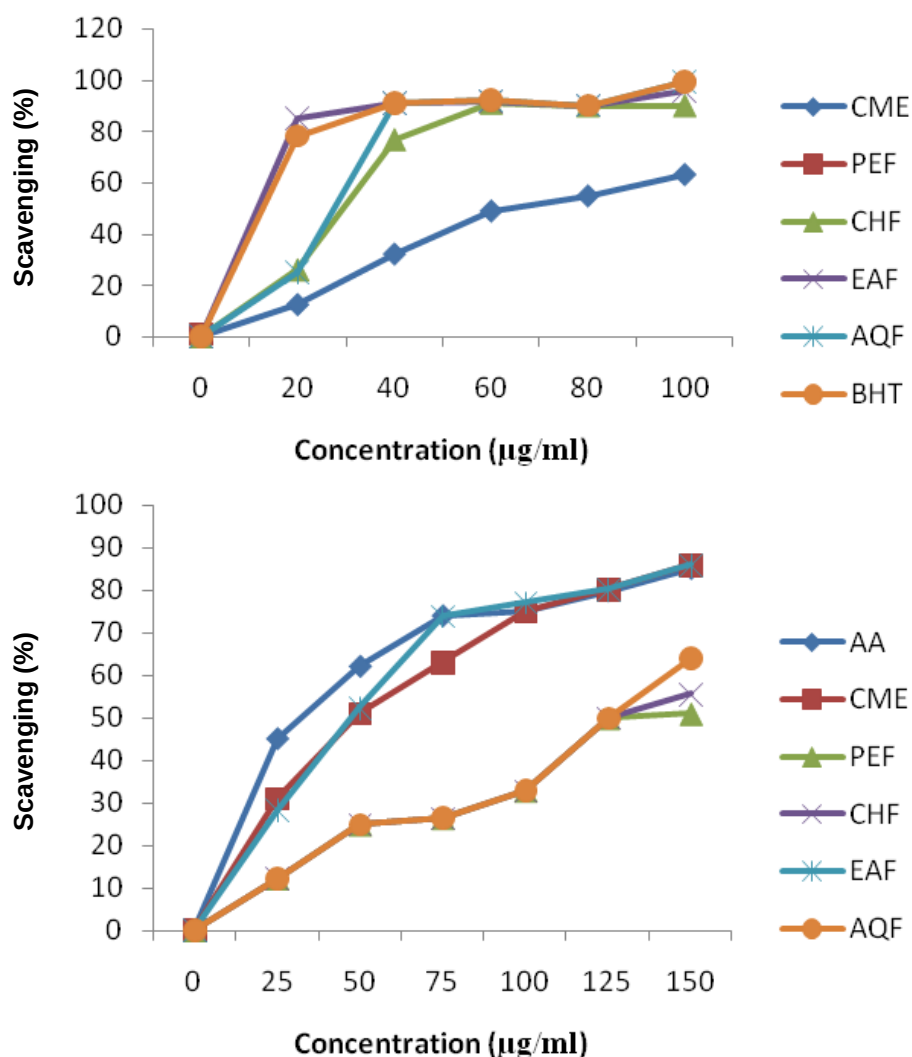


Figure 2. Determination of (A) DPPH radical scavenging activity and (B) OH radical scavenging activity of CME and its various fractions. Data expressed as mean $\pm$ standard deviation (SD; $n=3$, $p < 0.05$) for all tested dosages.

CME, PEF, CHF, EAF, AQF, and standard CA were 1.90, 2.21, 0.96, 2.65, 1.49 and 1.35, respectively at 324 µg/ml. The TAC of EAF was significantly higher ($p < 0.01$) than standard CA. The extracts were found to increase the total antioxidant activity with the increasing concentration of the extracts.

The ferrous reducing antioxidant capacity of CME and its four fractions are as shown in Figure 1B. At 160 µg/ml, the absorbance of CME, PEF, CHF, EAF, AQF, and standard AA were 2.75, 2.53, 2.21, 2.98, 2.91 and 2.32, respectively. A higher absorbance indicates a higher reducing power, hence CME, EAF and AQF showed higher reducing activity than standard AA. The ferrous reducing capacity of EAF was significantly higher ($p < 0.01$) than standard AA. CHF and PEF had mild to moderate iron

reducing capacity. It was observed that with increasing concentration of the extracts reducing activity also increased.

DPPH radical scavenging activity

Figure 2A showed free radical scavenging activity of the CME and its four fractions. At a concentration of 25 µg/ml, the scavenging activity of the CME, EAF and AQF were 95.93, 95.67 and 93.41%, respectively, while at the same concentration, the activity of BHT was 88.53%. Thus, CME, EAF and AQF exhibited significant free radical scavenging activity (Figure 2A). The scavenging activity of the PEF and CHF was in moderate level when

compared with BHT (Figure 2A). The IC_{50} of CME, PEF, CHF, EAF and AQF were 9.92, 63.2, 23.5, 95.93 and 10.0 $\mu\text{g/ml}$, respectively. The IC_{50} of BHT was 9.83 $\mu\text{g/ml}$, which was almost double than the IC_{50} of EAF (4.55 $\mu\text{g/ml}$). The inhibitory activity of different extractives and BHT were in the following order: EAF > BHT > CME > AQF > CHF > PEF. Our results revealed that the EAF had higher scavenging activity than that of other extractives as compared to BHT and CME and AQF had similar activity with BHT.

Hydroxyl radical scavenging activity

At a concentration of 150 $\mu\text{g/ml}$, the scavenging activity of CME and its four fractions PEF, CHF, EAF and AQF reached 88.29, 51.03, 57.85, 87.15 and 63.95%, respectively; while at the same concentration, the activity of AA was 84.25% (Figure 2B). The IC_{50} of CME, PEF, CHF, EAF, AQF and AA were 50.25, 145.29, 133.45, 43.33, 124.65 and 32.15 $\mu\text{g/ml}$, respectively. The result demonstrates that CME and EAF significantly scavenged hydroxyl radicals when compared with standard AA.

Lipid peroxidation inhibition assay

The lipid peroxides scavenging activity of CME of leaves of ST was investigated and compared with standard CA. At a concentration of 150 $\mu\text{g/ml}$, the scavenging activity of CME and its fractions, PEF, CHF, EAF and AQF were 79.25, 56.35, 54.98, 81.65 and 66.12%, respectively; whereas the activity of CA was 80.54% (Figure 3A). The EAF exhibited higher activity than other extractives, even though higher than standard CA. The IC_{50} of CME, PEF, CHF, EAF and AQF were 72.52, 135.34, 135.25, 70.15 and 100.25 $\mu\text{g/ml}$, respectively; on the other hand, the IC_{50} of CA was 58.25 (Figure 3B). Significant correlations ($p < 0.001$) were observed between percentage of lipid peroxidation inhibition and hydroxyl radical scavenging (Figure 3).

Total polyphenol contents

Tumor cell growth inhibition

Since EAF showed the strongest antioxidant activity in all antioxidant tests, it was chosen for *in vivo* tumor cell growth inhibition at various doses (25 and 50.0 mg/kg, i.p). Maximum cell growth inhibition (67.35%, $p < 0.01$) was found after treatment with EAF at dose 50.0 mg/kg (i.p) on day six of tumor inoculation. On the other hand, standard bleomycin at dose 0.3 mg/kg i.p inhibited the tumor cell growth by 83.81% (Table 2). This result implies that the EAF had moderate anticancer activity, and the

plant might therefore be considered as an effective source of active chemopreventive agents.

DISCUSSION

Total antioxidant capacity

Total antioxidant potentials of leaves of *S. trifoliatum* extracts were estimated from their ability to reduce the reduction of Mo (VI) to Mo (V) and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. The antioxidant potential of the extractives was in the range of 0.95 ± 0.003 to 2.65 ± 0.004 μm green phosphate/Mo (V) (Figure 1A). All the fractions showed a good total antioxidant activity, which was concentration-dependent. The antioxidant potential of EAF was significantly higher ($p < 0.05$) than standard antioxidant which may be due to its chemical composition and phenolic content.

Ferrous reducing antioxidant capacity

Reducing power is also widely used in evaluating antioxidant activity of plant polyphenols and reductones that show antioxidant action by breaking the free radical chains by donating a hydrogen atom. The presence of reductants in the antioxidant sample causes the reduction of the Fe^{3+} /ferricyanide complex to the Fe^{2+} /ferrous form. The reducing power of the sample can be observed by measuring the formation of Perl's Prussian blue at 700 nm. The reducing ability of the extractives was in the range of 1.32 ± 0.004 to 2.96 ± 0.002 μm Fe (II)/g. The EAF exhibited strong reducing power and was higher than other fractions, even significantly higher ($p < 0.05$) than AA as shown in Figure 1B.

DPPH radical scavenging activity

DPPH radicals antioxidant activity is thought to be due to their hydrogen donating potential (Klein et al., 1981). In order to prevent the deleterious role of free radical in different diseases including cancer, radical scavenging activities are necessary. Relatively short time is required for this analysis which makes this method popular among the researchers. DPPH radical scavenging activity of all the fractions from *S. trifoliatum* increased with increase in fraction concentration (Figure 2A). The IC_{50} of EAF was significantly higher ($p < 0.01$) than that of other fractions and BHT with the order of EAF > BHT > CME > AQF > CHF > PEF. It has been found that mainly phenolics and flavonoids have a vital role in reducing the DPPH radicals by their hydrogen donating ability (Nariya et al., 2013). The results obtained in this study reveal that all the fractions from *S. trifoliatum* leaves are free radical scavengers

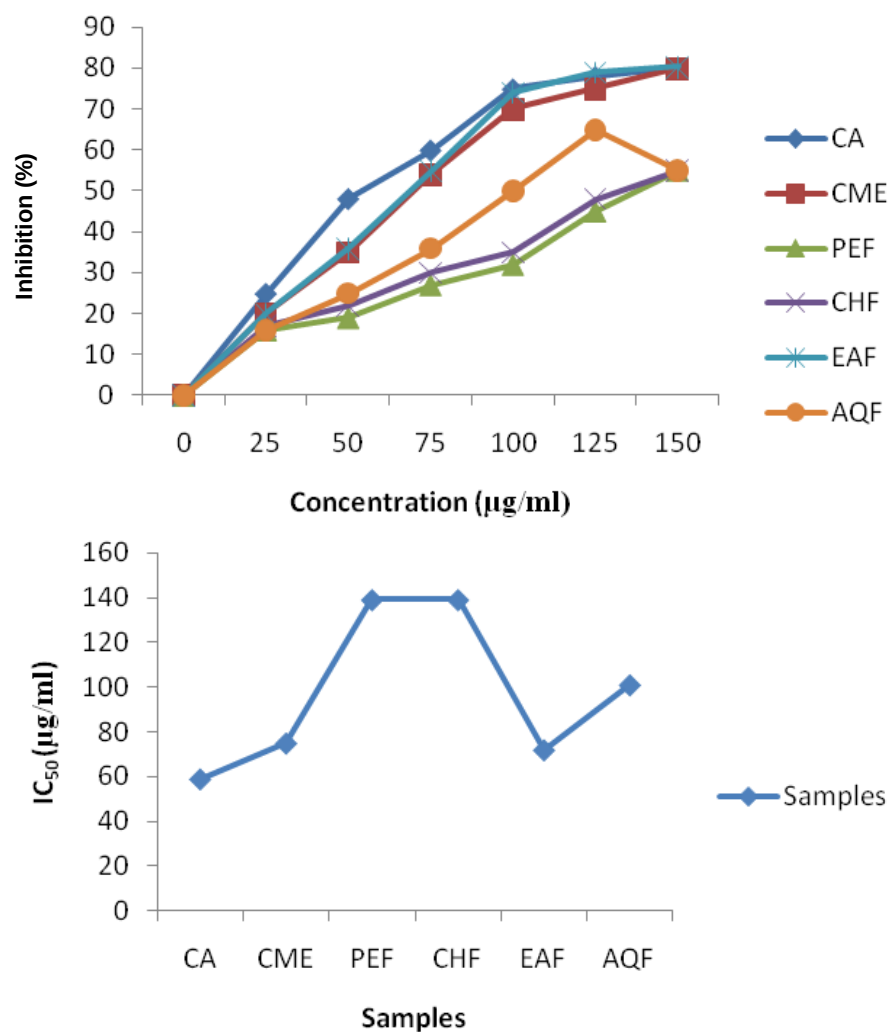


Figure 3. Determination of (A) lipid peroxidation inhibition of CME and its various fractions and (B) IC₅₀ of CME and its various fractions obtained from lipid peroxidation inhibition assay. Data expressed as mean $\pm$ standard deviation (SD; n=3, p<0.001).

and are able to react with the DPPH radical, which might be responsible for the antioxidant potential.

Hydroxyl radical scavenging activity

Free radicals mutagenic capacity is due to the direct interactions of hydroxyl radicals with DNA and therefore playing an important role in cancer formation (Rahman, 2007). Hydroxyl radicals can be generated by biochemical reaction. The results reveal that EAF of the leaves of *S. trifoliatum* had appreciable hydroxyl radical scavenging activity when compared with standard antioxidant BHT (Figure 2B) and could be used as anticancer agent by inhibiting the interaction of hydroxyl radical with DNA. Inhibition of lipid peroxidation may result due to

quenching of the hydroxyl radicals by the extract.

Lipid peroxidation inhibition assay

The reactive oxygen species (ROS) induced membrane damage by peroxidising lipid moiety, especially the polyunsaturated fatty acids with a chain reaction known as lipid peroxidation (Mylonas and Kouretas, 1999). Lipid peroxidation has been reported to be elevated in the cancer (Cai et al., 2012). In this study, lipid peroxidation of mouse liver homogenates was induced by ferric ion plus ascorbic acid. The CME of the leaves of *S. trifoliatum* and its four fractions, especially EAF had appreciable lipid peroxidation inhibition activity (Figure 3). The ST

Table 1. Total polyphenols contents in the CME and its four fractions: PEF, CHF, EAF and AQF.

Polyphenol	CME	PEF	CHF	EAF	AQF
Phenolics*	301.63 ± 4.36 ¹	17.56 ± 1.41	118.13 ± 1.61	526.22 ± 1.55	266.88 ± 2.78
Flavonols**	119.38 ± 1.24	185.48 ± 1.19	149.01 ± 2.78	220.38 ± 1.26	132.54 ± 1.77
Flavonoids***	219.88 ± 15.17	152.13 ± 6.34	128.21 ± 9.38	612.75 ± 5.37	185.71 ± 11.35

Each value is the average of 3 analyses ± standard deviation (SD). *, ** and *** expressed in terms of GAE, QUE and CAE, respectively.

Table 2. Effect of EAF on EAC cell growth inhibition in mice (*in vivo*).

Group	Dose (mg/kg/day; i.p)	No. of EAC cells in mouse×10 ⁷ on day 6 after tumour cell inoculation	Cell growth inhibition (%)
Control (EAC cell bearing mice)	-	5.12 ± 0.23	-
Bleomycin	0.3 mg/kg	0.73 ± 0.03**	85.92
EAF	25 mg/kg	2.2 ± 0.23*	50.45
	50 mg/kg	1.13 ± 0.12**	65.14

n=6, results were shown as mean ± standard error of mean (SEM), *p < 0.05, **p < 0.01.

extracts can prevent the cell abnormalities caused by cancer through breaking down of chain reactions responsible for lipid peroxidation. Inhibition of lipid peroxidation by the EAF fraction was significantly correlated with hydroxyl radical scavenging (Figure 3). This result reveals that the extractives differentially inhibit lipid peroxidation by virtue of their varying degrees of free radical quenching potential. Thus, ST is a good source for antioxidant thereby can be used as anticancer agent.

Effect of antioxidant on EAC-induced tumor cells

Free radicals are neutralized by free radicals, which are molecules with incomplete electron shells that make them more chemically reactive than those with complete electron shells. In humans, the most common form of free radicals is oxygen. When an oxygen molecule (O₂) becomes electrically charged or radicalized, it tries to steal electrons from other molecules, causing damage to the DNA and other molecules. Over time, such damage may become irreversible and lead to diseases including cancer. Antioxidants are often described as “mopping up” free radicals, meaning they neutralize the electrical charge and prevent the free radical from taking electrons from other molecules, thereby prevent cancer. In this study, the anticancer activity of EAF at low dose (below 25 mg/kg) on EAC-induced cancer in mice was not observed (data not shown). The EAF showed anti-cancer activity only at higher concentrations (25 and 50 mg/kg) which are comparable with bleomycin (Table 2). Thus, EAF might be a good source for isolating anticancer

agent.

Total phenolic, flavonoids, flavonols and proanthocyanidin contents

A number of polyphenols are identified as potential anti-tumor agents that could be used to combat biologically aggressive cancers, including metastasizing cancers, through the targeting of specific kinases (Lamoral-Theys et al., 2010). Secondary metabolites like flavonoids have good antioxidant potential which have also been shown to possess antimutagenic and antimalignant effect (Lamoral-Theys et al., 2010). Furthermore, flavonoids have a chemopreventive role in cancer through their effect on signal transduction in cell proliferation and angiogenesis (Ramos, 2007). Dietary flavonols and proanthocyanidins in particular offer significant cardiovascular health benefits (Ramos, 2007). Results obtained in the present study revealed that the level of these phenolic compounds in leaves of ST were significant (Table 1). Our findings strongly suggest that the phenolics are important components of ST pharmacological effects like anticancer activity and antioxidant activity could be due to the presence of these metabolites.

Conclusion

The present study shows that as compared to the other fractions, the ethyl acetate fraction of *S. trifoliatum* possessed

the highest phenolic content and also exhibited strong antioxidant with moderate anticancer activities. Thus, it can be concluded that the *S. trifoliatum* extract can be used as natural antioxidant as well as an anticancer agent. Further analysis and characterization of the phenolic compounds may help in discovering the chemical moieties responsible for the antioxidant and anticancer activities from the ethyl acetate fraction of *S. trifoliatum*.

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ABBREVIATIONS

AA, Ascorbic acid; **AQF**, aqueous fraction; **CAE**, catechin equivalent; **CHF**, chloroform fraction; **CME**, crude methanolic extract; **DPPH**, 1,1-diphenyl-2-picrylhydrazine; **EAC**, Ehrlich's Ascites cells; **EAF**, ethyl acetate fraction; **GA**, gallic acid; **GAE**, gallic acid equivalent; **OS**, oxidative stress; **PEF**, petroleum ether fraction; **QE**, quercetin equivalent; **ROS**, reactive oxygen species; **RT**, room temperature; **ST**, *Sapindus trifoliatum*; **TAC**, total antioxidant capacity; **TCA**, trichloro acetic acid.

Conflict of Interest

The authors declare that there is no conflict of interest.

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