Original Article

Therapeutic Effect of Hydro-Ethanolic Extract of *Pothos scandens* L on key Carbohydrate Metabolizing Enzymes and Xenobiotic Marker Enzymes in DMBA Induced Experimental Mammary Carcinoma

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ABSTRACT

Objective: In the present study, phytochemical evaluation and the therapeutic effect of 50 % hydro-ethanolic extract of aerial parts of *Pothos scandens* (HEEPS) on glycolytic enzymes, TCA cycle enzymes, gluconeogenic enzymes and xenobiotic marker enzymes in the liver and breast tissues of 7, 12 -Dimethylbenz [a] anthracene (DMBA) induced breast cancer bearing rats were carried out. **Materials and Methods:** Five groups of (each group six rats) female albino Spargue-Dawley rats were taken. Group I (control), Group II (DMBA induced, 20 mg/ kg b.wt p.o), Group III (DMBA + Cyclophoshamide, 10mg/kg b.wt p.o), Group IV (DMBA + 200 mg/kg b.wt p.o of HEEPS) and Group V (DMBA + 400 mg/kg b.wt p.o of HEEPS). The alteration in the level of carbohydrate metabolizing enzymes such as Hexokinase, Phosphogluco isomerase, Glucose-6-phosphatase, Fructose-1,6-bis phosphatase, Succinate dehydrogenase and Malate dehydrogenase together with the level of xenobiotic markers like Cytochrome P450 reductase, Cytochrome C oxidase and Glutathione -S- transferase were analyzed in all the groups. GC-MS analysis of the plant extract was also carried out. **Results:** Increased level of glycolytic enzymes, decreased level of TCA cycle enzymes, gluconeogenic enzymes and altered level of xenobiotic marker enzymes observed in Group II was brought to near normal in treatment groups in a dose dependent manner suggesting potential anticancer activity of HEEPS. This was supported by the GC-MS analysis of the plant extract which revealed the presence of many compounds with anticancer activity. **Conclusion:** The study revealed that HEEPS is effective in restoring the activity of mitochondria in DMBA induced breast cancer.

Key words: *Pothos scandens* L, GC-MS, 7, 12 -Dimethylbenz [a] anthracene (DMBA), Mammary Carcinoma, Carbohydrate Metabolizing Enzymes, Xenobiotic Markers.

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INTRODUCTION

Cancer can be described as a group of more than 100 different diseases which are characterized by uncontrolled cellular growth, local tissue invasion and distant metastasis.[1] Cancer claims over 6 million lives every year and it continues to represent the largest cause of mortality in the world.[2] Breast cancer is the most common form of cancer and the leading cause of cancer mortality among women worldwide.[3,4] The number of new breast cancer cases in India is about 100,000 per year.[5] It is a major clinical problem that possesses significant social and economic challenges to the health care system.[6] Breast cancer is a collection of breast diseases that have diverse genomic variations histopathologies and clinical outcomes.[7]

Mitochondrial dysfunction, rapid growth and cellular proliferation are hallmarks of tumor cells. In order to support continuous cell growth and proliferation in a hypoxic

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and acidic conditions generated during progressive tumor cell growth, cancer cells alter their metabolism by shifting the burden of energy metabolism from mitochondrial oxidative phosphorylation to glycolysis.[8] Molecular defects in mitochondrial or nuclear genes that code for proteins that are involved in mitochondria biogenesis; respiratory maintenance of membrane potential; chain assembly and function and energy metabolic or signal transducing pathways can be the cause of mitochondrial dysfunction.[9] Despite various therapeutic strategies including chemotherapy to treat cancer, drug resistance and high systemic toxicity limit the successful outcomes in most cases. Moreover cancer treatment is usually accompanied by diverse side effects to different body organs.[10] Hence there is a global trend to go back to natural resources (medicinal plants) which are more therapeutically effective, culturally acceptable and economically affordable.[11] Phytochemicals with anticancer potential which interfere with carcinogenesis makes them interesting tools in cancer research.[12]

Pothos scandens (*P. scandens*), a medicinal aroid, is a climbing shrub with aerial roots growing on trees and rocks like ivy which belongs to the family Araceae. After being fried in oil, the bruised root of the plant is applied to promote healing of abscesses. An infusion of the leaves of this plant is used by the Indian people as a bath for curing convulsions and epilepsy. Apart from that, the stem is also widely used to treat asthma. It has been also reported that the whole plant is used against various health problems and disorders such as diarrhea[13], small pox[14], muscle catches, sprains[15] and bone fracture.[16] Recently, the ethanolic extract of *P. scandens* has been reported to be effective in wound healing[17] and the plant has also been reported to have significant antioxidant and antipyretic activity.[18] The ethanolic extracts of aerial part of *P. scandens* showed the presence of phytochemicals such as alkaloids, glycosides, flavonoids and phenolic compounds and has been shown to inhibit mast cell derived immediate-type allergic reactions and mast cell degranulation.[19] The methanolic extract of leaves of *P. scandens* has been reported to have cytotoxic as well as thrombolytic potential.[20] From the literature survey it was found that the phytochemical analysis of leaf extract of *P. scandens* showed the presence of alkaloid, catachin, coumarin, flavonoid, phenolics, saponins, sugar, glycoside, xanthoprotein[21] and phytosterols.[18] 1,2- Benzenedicarboxylic acid, diisooctylester; n-Hexadecanoic acid, 9,12,15-Octadecatrienoic acid, (ZZZ)-; Octadecanoic acid; Phytol and 9, 12 Octadecadienoic acid (ZZ) were detected as major compounds in the GC-MS analysis of the ethanolic extract of *P. scandens* leaf.[22]

As per the ethnobotanical data collected during the field surveys made on several visits between 2004 and 2006 to three Akha communities in Chiang Rai in northern Thailand, it was found that the whole aerial parts of *P. scandens* were used by the traditional healers to treat cancer.[23] However, on thorough literature survey, it was found that there were no studies related to the anticancer properties of this plant. Hence, the present study aims to investigate the therapeutic properties of *P. scandens* for its potential anticancer activity by evaluating their effect on carbohydrate metabolizing enzymes and biotransformation enzymes in DMBA induced experimental mammary carcinoma.

MATERIALS AND METHODS

Chemicals and reagents

7, 12 -Dimethylbenz [a] anthracene (DMBA) was obtained from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals and solvents used were of analytical grade.

Plant material

P. scandens were collected from in and around Palai, Kottayam, Kerala. They were identified and certified by the Taxonomist, Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India (Plant identification no.-BSI/SRC/5/23/2013-14/Tech/685).

Extraction Procedure

The aerial parts of *P. scandens* were shade dried and ground to coarse powder. The coarse powder was extracted using 50% ethanol using soxhlet apparatus for 48 hours. The extracts were filtered and condensed to dryness using a rotary evaporator and stored at -20^{0}C for further studies.

Animals

Female albino Spargue-Dawley rats (150-200 g) used in the present study was procured from the animal breeding station, Mannuthy, Kerala, India. They were housed in polypropylene cages (38 × 23 × 10cm) with not more than six animals per cage and maintained under standard environmental conditions (14h dark /10h light cycles; temp 25±2°C; 35-60% humidity, air ventilation) and were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water *ad libitum*. The animals were acclimatized to the environment for two weeks prior to experimental use. Animals were fasted over night before the experimental schedule, but have free access for water *ad libitum*. Ethical clearance for the handling of experimental animals was obtained from the Institutional Animal Ethics Committee

(IAEC) constituted for the purpose and care of laboratory animals as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (IAEC No. MLEA-CPCSEA/09-2014/02).

Experimental Design

The animals were randomly divided into five groups of six animals each. Group I served as control rats. Group II rats were induced with a single dose of DMBA (20 mg/kg b. wt, dissolved in 0.75 ml sunflower oil and 0.25 ml physiological saline) by oral gavage at the first day of the experimental period. Group III received Cyclophoshamide (CP) (10mg/kg p.o), Group IV received 200 mg/kg b.wt and Group V received 400 mg/kg b.wt of HEEPS orally for 60 days after 90 days of DMBA induction. At the end of the experimental period, all the animals were anesthetized with diethyl ether, and they were sacrificed by decapitation. Animals were starved overnight before sacrifice. The breast and liver tissues were excised and rinsed 2 to 3 times in ice cold normal saline followed by 0.15 M Tris- HCl (pH 7.4), blotted dried and known weight of breast and liver were homogenized in 0.25 M Tris-HCl buffer (pH 7.5). The homogenate was subjected to differential centrifugation at 4°C. The cell organelle such as mitochondria, microsomes and cytosolic fractions were isolated. Total homogenate and subcellular fractions were used for the assay of the following parameters in breast and liver samples.

Biochemical Analysis

The following biochemical parameters in the liver and breast tissue were analysed. Hexokinase (HK), Branstrup *et al.,*[24]; Phosphogluco isomerase (PGI), Horrocks *et al.,*[25]; Glucose-6-phosphatase (G-6-Pase), King [26]; Fructose-1, 6-bis phosphatase (F-1,6-BPase), Gancedo and Gancedo [27]; Succinate dehydrogenase (SDH), Slater and Bonner [28]; Malate dehydrogenase (MDH), Mehler *et al.,*[29]; Cytochrome P450 reductase (POR), Masters *et al.,*[30]; Cytochrome C oxidase (COX), Wharton and Tzagoloff [31] and Glutathione -S- transferase (GST) Habig *et al.,*.[32]

Gas Chromatography - Mass Spectrometry (GC-MS) analysis of hydro-ethanolic extract of *P. scandens* (HEEPS)

GC- MS analysis of HEEPS was performed with Shimadzu GC- MS (model QP2010) operating in EI mode at 70 eV with a Restek -5MS column (30m × 0.25mm × 0.25µm). Helium gas was used as carrier gas at a constant flow rate 1ml/ min and an injection volume was 1µL. The oven temperature programs was initially kept at 150^{0}C and hold for 1 min, and then with a rise of 10^{0}C/ minute upto 250^{0}C and hold for 3 min, and again with a rise of 10^{0}C/ min upto 280^{0}C and hold for 5 min. Injector temperature 260^{0}C; ion source temperature 250^{0}C, quadrupole temperature 150^{0}C, interface temperature 250^{0}C, full scan mode, scan range 40-600 m/z and solvent delay of 4 min was employed.

The interpretation of the spectrum from GC- MS analysis was done using the database of National Institute of Standard and Technology (NIST). By comparing the mass spectrum of the unknown component with the known component in the NIST library; the name, structure and molecular weight of the components of the test materials were ascertained.

Statistical analysis

The results are expressed as mean ± standard deviation (SD).Differences between groups were assessed by one-way analysis of variance (ANOVA) using the Statistical Package of Social Sciences (SPSS, Version 16.0 for windows). The group means were compared by Duncan's Multiple Range Test (DMRT). Values were considered statistically significant when $p < 0.05$.

RESULTS

Effect of HEEPS on carbohydrate metabolizing enzymes

The effect of HEEPS on carbohydrate metabolizing enzymes in the liver and mammary gland tissue of mammary carcinoma bearing rats is given in Table 1. The result indicated that the activity of glycolytic enzymes namely HK and PGI in the liver and mammary tissues of rats bearing mammary carcinoma (Group II) was significantly increased by 81.82% and 84.13% in liver and by 60% and 67.74% in mammary tissue respectively when compared with control rats (Group I). However, it was seen that the activity reverted to near normal when treated with plant extract (Group IV and Group V). The activity shown by the treatment group was almost near to those treated with standard drug (Group III).

The activity of TCA cycle enzymes like SDH and MDH were significantly lowered by 75.22% and 76.09% in liver and by 71.85% and 72.18% in mammary tissue respectively in Group II rats compared to the Group I rats. It was observed from the result that the treatment groups (Group III, Group IV and Group V) showed near normal activity of these enzymes.

Gluconeogenic enzymes like G-6-Pase and F-1, 6-BPase showed a decrease in activity by 52.86% and 51.60% in liver and by 68.72% and 48.39% in mammary tissue respectively in Group II rats when compared with control rats whereas the near normal activity was shown

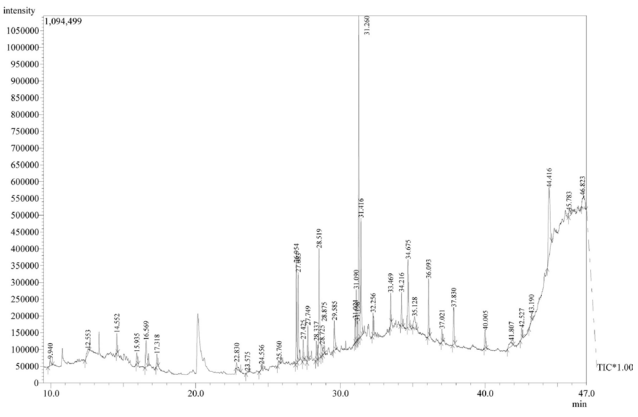

Figure 1: GC-MS chromatogram of 50% ethanolic extracts of *Pothos scandens* L

by Group III which was treated with standard drug and in Group IV and Group V rats treated with plant extract.

Effect of HEEPS on xenobiotic marker enzymes

Table 2 shows the effect of HEEPS on various xenobiotic marker enzymes in the liver and mammary gland tissue of mammary carcinoma bearing rats. When the rats bearing mammary carcinoma (Group II) showed a decrease in the activity of POR, COX and GST by 56.11%, 77.68% and 58.11% in liver and 69.14%, 66.06% and 51.31% in mammary tissue respectively, the activity reached near normal in the groups treated with standard drug (Group III) and extract of *P. scandens* (Group IV and Group V).

GC-MS analysis

The GC-MS analysis of the HEEPS is given in Figure 1. Thirty nine compounds were revealed by the GC-MS analysis. The identified compounds with their retention time, molecular formula and molecular weight are given in Table 3. The results showed the presence of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Synonym: Phytol) 19.30%, Stigmast-5-en-3-ol, (3 beta) (Synonym: β Sitosterol) 9.96%, Hexadecanoic acid, methyl ester 8.66%, Octadecanoic acid, methyl ester 7.21%, Hexahydrofarnesyl acetone 5.10%, Dotriacontane 4.27%, Hexatriacontane 3.13%, 9-Octadecenoic acid (Z)-, methyl ester 2.97%, α Curcumene 2.66%, Cedrol 2.40%, Tetrapentacontane 2.37%, Ethyl heptadecanoate 2.20%, 5-methyl-5-(4,8,12-trimethyltridecyl)oxolan-2-one 2.06%, Nopol 1.76%, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester 1.30% and Eicosane 1.29% as major compounds.

DISCUSSION

DMBA efficiently induces breast cancer in female rats. Oral delivery of DMBA to rats has been used intensively for carcinogenic studies since 1960 as a model mimicking the dietary intake of carcinogens in humans.[33] It has been shown that DMBA-induced mammary carcinoma leads to disruption of redox balance of tissues which cause undesirable consequences to the mitochondrial membrane suggesting that biochemical and physiological alterations may result from oxidative damage.[34,35]

Non malignant cells depend primarily on mitochondrial oxidative phosphorylation (OXPHOS) than aerobic glycolysis for its energy requirement. However, Otto Warburg suggested that in contrast to non malignant cells, malignant cells depend on glycolysis rather than OXPHOS for ATP generation which is considered by some as the seventh hallmark of cancer. This could be due to the damage of mitochondrial respiration in tumor cells.[8,36] In the present study, increased activity of cytosolic glycolytic enzymes in DMBA induced mammary carcinoma (Group II) suggest that DMBA induced carcinoma had lead to an alteration in carbohydrate metabolism. An increase in the rate of glycolysis confers the tumor cells with advantages like synthesis of ATP independent of oxygen; faster synthesis of ATP as compared to OXPHOS, generation of fewer reactive oxygen species and support cell proliferation by the provision of glycolytic intermediates many of which are intermediates of biosynthetic pathways.[37,38] The level of hexokinase plays an important role in determining the glycolytic activity of cancer cells.[39] An increase in activity of hexokinase in the Group II may be due to the consumption of large amount of glucose by cancer cells. Elevated level of PGI was reported in sarcoma and cancers in the breast.[40] The elevated level of PGI observed in Group II in the present study may be due to increase in aerobic glycolysis as a result of metabolic alteration. The activity of the glycolytic enzymes reached near normal activity in groups treated plant extract in a dose dependent manner suggesting curative effect of this plant.

A decrease in the activity of TCA cycle enzymes were observed in Group II animals in the current study. The decreased activity of mitochondrial enzymes may be due to the damage to the mitochondria caused by oxidative stress which could have altered the mitochondrial membrane, morphology leading to its dysfunction[41] and may be due to decrease in mitochondrial fluidity, alteration in membrane ionic permeability including proton permeability which uncouples OXPHOS from electron transport chain.[42] Animals treated with extract of *P. scandens* showed significant restorative activity of mitochondria in mammary carcinoma bearing rats.

In the present study, carcinoma bearing rats showed a decrease in the activity of G-6-Pase and F-1, 6-BPase.

Decreased activity of gluconeogenic enzymes viz G-6-Pase and F-1, 6-BPase might lead to an increase in the concentration of their substrates glucose-6-phosphate and fructose-1, 6-bisphosphate. The resultant accumulated glucose-6-phosphate might be utilized for the pentose phosphate pathway to produce ribose-5-phosphate for the rapid synthesis of nucleotide in cancer bearing cells. On the other hand fructose-1, 6-bisphosphate acts as an allosteric activator of pyruvate kinase.[43] The activities of these enzymes were returned to near normal when treated with plant extract suggesting a protective role of plant.

POR is a flavoenzyme ubiquitously expressed in all cells including malignant cells and catalyzes the electron transfer required for the P450 enzyme activity.[44] It has been confirmed from studies that POR overexpression substantially increases the potency of prodrug mediated tumor cell killing.[45-48] In the present study, a decrease in the activity of POR in both liver and breast tissues has been observed in cancer induced rats (Group II) which was brought to near normal in groups treated with plant extract. This suggests the capability of plant extract in augmenting the expression of POR.

COX is a protein present in the inner membrane of mitochondria and is responsible for the conversion of

Table 1: Effect of HEEPS on carbohydrate metabolizing enzymes of liver and breast tissue against DMBA induced tumor

Parameter	Group I (Control)	Group II (DMBA)	Group III (DMBA+CP)	Group IV (DMBA + HEEPS 200 mg/kgbw)	Group V (DMBA + HEEPS 400 mg/kgbw)
LIVER					
HK	0.011 ± 0.002	0.020 ± 0.003*	0.011 ± 0.001*	0.014 ± 0.002*	0.013 ± 0.001*
PGI	0.063 ± 0.010	0.116 ± 0.022*	0.062 ± 0.005*	0.076 ± 0.011*	0.070 ± 0.011*
SDH	1.703 ± 0.18	0.422 ± 0.08*	1.325 ± 0.21*	0.672 ± 0.05*	1.040 ± 0.07*
MDH	1.748 ± 0.13	0.418 ± 0.03*	1.263 ± 0.32*	0.623 ± 0.07*	1.098 ± 0.05*
G-6-Pase	0.367 ± 0.06	0.173 ± 0.04*	0.344 ± 0.03*	0.289 ± 0.04*	0.314 ± 0.01*
F-1, 6-BPase	0.281 ± 0.05	0.136 ± 0.03*	0.264 ± 0.02*	0.200 ± 0.02*	0.243 ± 0.02*
BREAST					
HK	0.045 ± 0.006	0.072 ± 0.012*	0.047 ± 0.004*	0.051 ± 0.004*	0.049 ± 0.003*
PGI	0.040 ± 0.018	0.124 ± 0.020*	0.041 ± 0.017*	0.085 ± 0.017*	0.052 ± 0.020*
SDH	1.197 ± 0.18	0.337 ± 0.03*	1.042 ± 0.17*	0.602 ± 0.05*	0.933 ± 0.08*
MDH	6.568 ± 0.96	1.827 ± 0.51*	5.938 ± 0.89*	3.642 ± 0.70*	5.068 ± 0.43*
G-6-Pase	0.227 ± 0.01	0.071 ± 0.04*	0.194 ± 0.01*	0.147 ± 0.03*	0.176 ± 0.02*
F-1, 6-BPase	0.746 ± 0.07	0.385 ± 0.10*	0.688 ± 0.08*	0.586 ± 0.04*	0.639 ± 0.04*

Values are expressed as mean ± SD for six rats in each group.*p<0.05. Group I vs Group II, Group II vs Group III, Group IV and Group V. Units: HK- µ moles of glucose 6 phosphate formed/min/mg protein; PGI - µ moles of fructose formed/min/mg protein; SDH - µmoles of succinate oxidized /min/mg protein; MDH - µmoles of NADH oxidized/min/mg protein; G-6-Pase and F-1, 6- BPase - µ moles of phosphorous liberated/min/mg protein

Table 2: Effect of HEEPS on xenobiotic marker enzymes of liver and breast tissue against DMBA induced tumor

Parameter	Group I (Control)	Group II (DMBA)	Group III (DMBA+CP)	Group IV (DMBA + HEEPS 200 mg/kgbw)	Group V (DMBA + HEEPS 400 mg/kgbw)
LIVER					
POR	20.038 ± 0.33	8.795 ± 0.58*	15.780 ± 0.28*	11.612 ± 0.31*	13.098 ± 0.52*
COX	223.143 ± 1.18	49.810 ± 5.39*	160.650 ± 1.63*	135.670 ± 6.15*	151.075 ± 7.01*
GST	52.800 ± 1.29	22.118 ± 1.71*	43.463 ± 1.24*	36.803 ± 1.15*	39.868 ± 1.94*
BREAST					
POR	11.680 ± 1.54	3.605 ± 0.89*	9.587 ± 1.48*	6.750 ± 1.59*	7.817 ± 1.07*
COX	475.923 ± 8.57	161.510 ± 8.81*	412.637 ± 6.48*	292.653 ± 6.37*	372.192 ± 7.27*
GST	43.742 ± 5.27	21.300 ± 5.86*	37.872 ± 6.18*	31.813 ± 2.32*	35.908 ± 4.19*

Values are expressed as mean ± SD for six rats in each group.*p<0.05. Group I vs Group II, Group II vs Group III, Group IV and Group V. Units: POR - µ moles of Cytochrome reduced/min/mg protein; COX - µ moles of Cytochrome oxidised/min/mg protein; GST - µ moles of CDNB conjugation formed/min/mg protein

Table 3: Phytochemical compounds identified through the GC-MS study of HEEPS

Sl. No	Retention Time (min)	Compound	Molecular Formula	Molecular Weight
1	9.940	3,7- Dimethylnonane	$C_{11}H_{24}$	156
2	12.553	Silane, trimethyl (1-methyl dodecyl oxy)-	$C_{16}H_{36}OSi$	272
3	14.552	1,3-Di isopropoxy-1,3-dimethyl-1,3-disilacyclobutane	$C_{10}H_{24}O_2Si_2$	232
4	15.935	Sym-tetra (isopropyl) disiloxane	$C_{12}H_{30}OSi_2$	246
5	16.569	α Curcumene	$C_{15}H_{22}$	202
6	17.318	α Bergamotene	$C_{15}H_{24}$	204
7	22.830	Butyldimethyl (2-styryl[1,3]dithian-2-yl) silane	$C_{18}H_{28}S_2Si_2$	336
8	23.575	6-Methyl-6-(5-methylfuran-2-yl) heptan-2-one	$C_{13}H_{20}O_2$	208
9	24.556	1,1,3,3-Tetramethylcyclopentane	C_9H_{18}	126
10	25.760	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	$C_{10}H_{16}O_2$	168
11	26.954	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Synonym: Phytol)	$C_{20}H_{40}O$	296
12	27.083	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	268
13	27.425	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Synonym: Phytol)	$C_{20}H_{40}O$	296
14	27.749	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Synonym: Phytol)	$C_{20}H_{40}O$	296
15	28.337	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	268
16	28.519	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
17	28.725	2-Octenoic acid, 4-isopropylidene-7-methyl-6-methylene-, methyl ester	$C_{14}H_{22}O_2$	222
18	28.875	1-Hexadecen-3-ol, 3,7,11,15-tetramethyl- (Synonym: Isophytol)	$C_{20}H_{40}O$	296
19	29.585	Ethyl heptadecanoate	$C_{19}H_{38}O_2$	298
20	31.021	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294
21	31.090	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296
22	31.158	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296
23	31.260	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Synonym: Phytol)	$C_{20}H_{40}O$	296
24	31.416	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298
25	32.256	Ethyl octadecanoate	$C_{20}H_{40}O_2$	312
26	33.469	Eicosane	$C_{20}H_{42}$	282
27	34.216	5-methyl-5-(4,8,12-trimethyltridecyl)oxolan-2-one	$C_{21}H_{40}O_2$	324
28	34.675	Dotriacontane	$C_{32}H_{66}$	450
29	35.128	Nopol	$C_{11}H_{18}O$	166
30	36.093	Hexatriacontane	$C_{36}H_{74}$	506
31	37.021	Mono (2-ethylhexyl)phthalate	$C_{16}H_{22}O_4$	278
32	37.830	Tetrapentacontane	$C_{54}H_{110}$	758
33	40.005	Tetrapentacontane	$C_{54}H_{110}$	758
34	41.807	Methyl steviol	$C_{21}H_{32}O_3$	332
35	42.527	1-Bromotriacontane	$C_{30}H_{61}Br$	501
36	43.190	Docosa-2,6,10,14,18-pentaen-22-al, 2,6,10,15,18-pentamethyl-, all trans	$C_{27}H_{44}O$	384
37	44.416	Stigmast-5-en-3-ol, (3 beta) (Synonym: β Sitosterol)	$C_{29}H_{50}O$	414
38	45.783	5-Nitrobenzofuran-2-one	$C_8H_5NO_4$	179
39	46.823	Cedrol	$C_{15}H_{26}O$	222

molecular oxygen to water in the terminal complex of mitochondrial respiratory chain.[49] In the present study reduction in the activity of the enzyme was found in DMBA induced breast cancer. Defective complex IV has been observed in various studies conducted in many human tumors and mouse xenografts.[50] The activity of the enzyme was significantly increased in the treatment groups indicating the capability of *P. scandens* extract to repair the mitochondrial membrane damage. Similar results were observed in the studies conducted using tangeretin in breast cancer bearing rats.[51]

GST is a major group of phase II enzymes for which glutathione is the substrate. The expression of GST is controlled by multiple transcription factors and is highly inducible by dietary components.[52] The enzyme is involved in the detoxification of variety of xenobiotics and also plays an important role in carcinogen detoxification.[53,54] In the present study, the level of GST was decreased in carcinoma bearing rats depicting the deleterious effect of carcinogen. *P. scandens* extract treated groups showed near normal activity showing curative effect of the plant extract. Chemopreventive compounds act as blocking agents that affect the initiation phase of cancer by detoxification of carcinogen through increasing the activity of GST.[55] Our results are in accordance with the above findings as we have observed a significant decline in the activity of GST in DMBA induced cancer bearing animals (Group II) and a significant increase in the activity of the enzyme in treatment groups.

The HEEPS contains rich phytochemicals which was identified by GC-MS analysis. In the present study in terms of percentage phytol, β-sitosterol, hexadecanoic acid methyl ester, octadecanoic acid methyl esters were predominant in the extract. Phytol has been reported to have anticancer[56], antimicrobial[57], diuretic and anti-inflammatory activity.[58] β-sitosterol has been reported to impart protection to cells against damage by reactive oxygen species by increasing the activities of antioxidant enzymes.[59] Also it was pointed out in the study of Awad *et al.,*[60] that β-sitosterol can alleviate cancer development by reducing production of carcinogens and its treatment has reduced the breast cancer cell growth.[61] Hexadecanoic acid methyl ester was reported to have anticancer and antioxidant properties and octadecanoic acid has been reported to have anticancer, antioxidant antibacterial, antifungal, hypocholesterolemic, antihistamic and anti-coronary properties.[62] Apart from these, remaining compounds mentioned in Table 3 detected by GC-MS has been reported to have significant application in drug industry.

CONCLUSION

Damage to the mitochondrial membrane and increased rate of glycolytic enzymes during cancer is an important field of research in breast cancer studies. From the present study, it was evident that significant damage was caused to mitochondria in breast cancer induced animals which resulted in an increase in the glycolytic enzymes and a decrease in the level of TCA cycle, gluconeogenic enzymes. Also it was found that the levels of marker enzymes such as POR, COX and GST were also altered in breast cancer induced animals. All the altered enzyme activities were brought to near normal activity in plant extract treated groups which suggest the potential therapeutic effect of the plant. The GC-MS analysis of the plant also well supported the fact that the plant contained important compound which could be employed for the treatment of breast cancer. To the best of our knowledge, this is the first publication about the therapeutic activity of *P. scandens* extract against breast cancer. However, compounds responsible for its anticancer property and its mode of action need further study.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ABBREVIATION USED

HEEPS: 50 % hydro-ethanolic extract of *Pothos scandens*; **DMBA:** 7, 12 -Dimethylbenz [a] anthracene; **CP:** Cyclophoshamide; **HK:** Hexokinase; **PGI:** Phosphogluco isomerase; **SDH:** Succinate dehydrogenase; **MDH:** Malate dehydrogenase; **G-6-Pase:** Glucose-6-phosphatase; **F-1,6-BPase:** Fructose-1,6-bisphosphatase; **POR:** Cytochrome P450 reductase; **COX:** Cytochrome C oxidase; **GST:** Glutathione -S- transferase; **NIST:** National Institute of Standard and Technology; **SPSS:** Statistical Package of Social Sciences; **OXPHOS:** Oxidative phosphorylation.

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SUMMARY

- The whole aerial parts of *Pothos scandens* were not evaluated for its anticancer properties though it was used by traditional healers to treat cancer.
- The present study investigated the therapeutic effect of 50% hydro-ethanolic extract of aerial parts of *Pothos scandens* (HEEPS) on key carbohydrate metabolizing enzymes (glycolytic [Hexokinase, Phosphogluco isomerase], gluconeogenic [Glucose-6-phosphatase, Fructose-1,6-bis phosphatase]) and TCA cycle [Succinate dehydrogenase, Malate dehydrogenase] and xenobiotic marker (Cytochrome P450 reductase, Cytochrome C oxidase and Glutathione -S- transferase) enzymes in DMBA induced experimental mammary carcinoma.
- HEEPS showed significant anticancer activity which was revealed by its ability to restore the enzymes levels which were altered in cancer induced groups to near normal in HEEPS treated groups. The phytochemical analysis of HEEPS also showed the presence of compounds with proved anticancer activity.

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