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PHYTOCHEMICAL SCREENING OF A POLYHERBAL EXTRACT AND ITS ANTICANCER POTENTIAL

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ABSTRACT

Individual ethnopharmacologic and phytochemical investigations of *Withania somnifera*, *Oroxylum indicum,* and *Calotropis gigentia* have revealed a wide range of active phytoconstituents with anticancer effects. The anticancer activity of a combination of these plant extracts as a polyherbal extract (PHE), however, remains unknown. The present study focused on the preliminary phytochemical and anticancer activities of this PHE. The *in vitro* anticancer potential was investigated using an MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) assay with MCF7 (breast carcinoma), HT29 (colon carcinoma), and HepG2 (hepatocyte carcinoma) cells. The mean GI_{50} (drug concentration inhibiting 50% cellular growth following 72 h drug exposure) of the PHE was 35.33 µg/mL. Total cellular growth inhibition required a drug concentration of 64 µg/ml for MCF7, 72 µg/ml for HT29, and 58 µg/ml for HepG2. The LC_{50} (drug concentration resulting in a 50% reduction at the end of the drug treatment compared with that at the beginning) was greater than 100 µg/ml for all three cell lines, confirming that this combination of extracts has potent synergistic anticancer activity.

KEY WORDS: Antioxidant activities, polyherbal extract (PHE), antitumor activity, EAC.

1.INTRODUCTION

Cancer is the excess proliferation of cell that cannot be completely abolished by chemotherapy because almost all the chemotherapeutic agents are toxic for tumor cells as well as for normal cells also. Cancer is a fatal disease rating the top three cause of death because of the lack of availability of effective drugs. Chemotherapeutic agents that are sold for the treatment of cancer are highly expensive, mutagenic, carcinogenic and teratogenic in nature. Therefore, researchers are giving efforts to find out the suitable anti-cancer drug of plant origin which ultimately prevent, slow/reverse cancer. India is the hub of many medicinal plants. Using this opportunity we carry out our research work to evaluate the anti-cancer potential of polyherbal extract of roots of *Withania somnifera*, stem bark of *Oroxylum indicum* and leaves of *Calotropis gigentia.*

The extensive literature search reveals that the plants and plant parts are the primary source of many anti-cancer constituents [e.g.- vincristine and vinblastine (*Catharanthus roseas*), etoposide and teniposide (*Podophylum* species), paclitaxel (*Taxus bravifolia*) (Shoeb M, 2006), Vinorelbine and Docetaxel (semisynthetic derivative from *Taxus buccata*) (Schmidt M and Bastians H, 2007), Irinotecan (Dholwani KK, 2008) and Topotecan (semisynthetic derivatives from *Camptotheca acuminata*) (Srivastava V, 2005). In the present market over 60% of anti-cancer drugs are from plant origin (Cragg GM, 1997) and shows effective anti-cancer potential on various experimental model.

W. somnifera Dunnl. (Ashwagandha, Family-Solanaceae), an erect evergreen shrub distributed through India (Unnikrishnan MC and Kuttan R,1990), was reported to have antiarthritic (Kurup PA,1956; Thakur RS, 1989 and Sethi PD, 1990), antipyretic, anti-inflammatory (Sethi PD, 1990; Budhiraja RD and Sudhir S, 1987), antihypertensive (Thakur RS , 1989) and anti-tumor activity whereas '*withania'* showed marked anti-tumor activity (Shohat B, 1972; Devi PU, 1996; Prakash J, 2002; Sbohat R, 1967; Devi PU, 1992; Jayaprakasam B, 2003; Kaur K, 2004). The main

mechanism of antitumor agents lies onto the induction of apoptosis and anti-proliferative strategies in tumor cells (Giridharan P, 2002; Yang KC, 2006; Lu KH, 2005; Hou DX, 2005).

Oroxylum indicum, small to medium sized tree found in China and India (Chen LJ, 2003), reported to show anti-inflammatory, antiallergic (Ikemoto S, 2000), antioxidant and anticancer activities due to active constituents, baicalein and chrysin (Kyo R, 1998; Takizawa H, 1998). Beyond its anti-inflammatory activity, few selected species have modulatory effect on the NF-B signaling pathway which has opened the gateway of most important targets of today's drug discovery for the treatment of autoimmune disorders as well as cancers (Bork PM, 1997; Baud V and Karin M, 2009; Sarkar FH, 2008; Sun SC and Ley SC, 2008; Aggarwal BB and Gehlot P, 2009).

Calotropis gigantea commonly known as milkweed or swallowwort is one of the latex bearing plants (Family-Ascleapiadeceae) (Singh U, 1996; Rastogi RP and Mehrotra BN, 1991) having activity as analgesic (Kirtikar KR and Basu BD, 1995) and also in anxiety and pain (Boericke W, 1999; Sharma V, 2001), epilepsy (Jain SK, 1989) and mental disorder (Upadhyaya AS, 1994). Some of the constituent like flavonoids, terpinoids that possess antitumour, antioxidant and related biological activities (Ferguson PJ, 2004; Hudson EA, 2000), have been isolated from the different parts of the plant.

Though, individually, these three plants contain large group of phyto constituent and have proven anticancer activities from the previous literature. The preliminary Phytochemical and evaluation of anti-cancer activity of the combined plant extract of *W. somnifera*, *O. indicum* and *C. gigantica* as PHE using three human cell lines like breast cancer (MCF 7), colon cancer (HT 29) and liver cancer (Hep G2) has not been reported yet. The aim of the present study was also to investigate chemical constituents and anticancer activity of the plants in combination.

2.MATERIALS AND METHODS

Chemicals*:* Fetal bovine serum and trypsin were obtained from Gibco's (USA) and Gibco's (UK), respectively. Amphotericin-B was collected from Dr.V.Venkateshwarlu, University College of Pharmaceutical Sciences, Kakatiya University (Warangal, A.P., India). Beyond this, a list of highly pure chemicals like RPMI-1640 media, Penicillin-G, Streptomycin, Phosphate buffered saline (PBS), Ethylenediamine tetra-acetic acid (EDTA), Trypan blue, SDS lysis buffer, MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) were supplied by Himedia (Mumbai, India). All the rest chemicals were of high reagent grade and pure.

Collection of plant materials: The roots of *W. somnifera*, stem bark of *O. indicum* and leaves of *C. gigantea* were collected from Chennai, India in the month of February 2009 and authenticated by Botanical Survey of India, Chennai, Tamil Nadu, India (Ref No. BSI/LS/215).

Preparation of PHE: All the plant materials were collected, washed with water and dried under shade at about 30-35ºC for several days, then pulverized to fine powder using a laboratory scale mill. The individual powder was extracted with methanol and water using a soxhlet apparatus in the ratio of 1:6 [powder (in g): solvent (in mL)]. The extract obtained were vacuum dried at 40ºC in a rotary evaporator (Buchi, Switzerland). *W. somnifera, O. Indicum* and *C. gigantea* yielded 20, 8 and 4 gm/kg, respectively. The samples were stored in a vacuum desiccator at room temperature until further use. The three extracts were mixed together and suspended in 5%w/v Carboxy methyl cellulose for pharmacological studies.

Preliminary phytochemical study (C.K kokate, 2007): The extract was subjected to different phytochemical test like carbohydrates, glycosides, alkaloids, phytosterol, fixed oils, mucilages, saponins, proteins, tannins and flavonoids to identify the presence of phytoconstituents in the extract.

***In vitro* anti-tumor activity**

Cytotoxicity Assay (Scudiero DA, 1988): The cytotoxicity of the PHE was tested against MCF7 (breast carcinoma), HT29 (colon carcionama) and HepG2 (hepatocellular carcinoma) tumor cell lines. Cells are cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum, 100U/mL Penicillin G sodium, 50µg/mL streptomycin and 2µg/mL Amphotericin B at 37^0C with 5% CO_2. Cells were kept at a concentration of 10^5 cells/well in 96 well microtitre plates. After 24 h, the cells were treated with different concentration of extract (6.125-100 µg/mL) which was dissolved in 1%

DMSO. The control groups were received 1% DMSO only. At the end of 72 h, 20µL of MTT was added in each well and incubated for 2 h in CO_2 incubator. After incubation, 80 µL of lysis buffer (15% SLS in 1:1 DMF and water) was added and kept in rotary shaker for 8 h. The growth of tumor cell was determined by the ability of living cell to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a purple formazan product . The MTT-frozen product was dissolved in DMSO and estimated by measuring the absorbance at 562 nm in an ELISA plate reader.

3.RESULTS

Preliminary phytochemical studies: Preliminary phytochemical screening of combined plant extract of all plants was performed using standard chemical tests. Table 1 shows that alkaloid, glycoside, tannin, protein, saponin, sterol and flavonoid were presented in the PHE.

***In vitro* antitumor activity**

Cytotoxicity assay: In the present study, the cytotoxic activity of PHE using three human cancer cell lines was evaluated by MTT assay (Table 2). When the cells were treated for 72 hrs with various concentration of aqueous extract (5–100 µg/ml), the relative cell survival progressively decreased in a dose dependant manner. The GI50, TGI, LC50 of the extract was found to be 29, 40, 37 µg/mL, 64, 72, 58 µg/mL, greater than 100 µg/mL in three cell lines MCF7, HT29 and HepG2, respectively.

DISCUSSION

Developing novel antitumor agents having a well-defined mechanism of action is still an emerging field of oncology where researchers in both basic and clinical sciences are facing great challenges. In this direction, plants are being actively explored as a source of new molecules that can curtail cancer growth (Dredge K, 2003; Lee EJ, 2003). Supportive to this, many plant extracts containing antioxidant principles have been reported to possess antitumor activity (Ruby AJ, 1995). Hence, plants containing flavonoids, alkaloids, glycosides etc. are being screened constantly for antitumor activity that proposed for choosing these plants for the present study.

The *in vitro* cytotoxicity assay was carried out to determine possible cytotoxic effect of PHE on different human cancer cell lines. Flavonoids are generally regarded to have antitumor activity and inhibit the growth of leukemia cell to some extent. Flavonoids included in almost all plants we usually are consuming may therefore be considered as tumor preventing compounds, but the present result suggested that the mechanism of PHE action on tumor cells should be elucidate in detailed manner. MTT assay and *in vitro* cytotoxicity assay result using three cell lines clearly explain the reductive potential of the experimental extract though the overall clinical accuracy of MTT assay has been reported to be 83.3% (Suto A , 1989). Observations from the experimental studies on various parameters confirm the primary focus of our study, the potential tumoricidal role of PHE. Further studies are essential to elucidate the mechanism of action of the PHE.

4.CONCLUSION

The component of the poly herbal extract (PHE), root of *Withnia somnifera,* stem bark of *Oroxylum indicum* and leaves of *Calotropis gigentia* were reported individually to possess anticancer activity with different mechanism of action on different animal models and cell lines. The results of the present study revealed an excellent synergistic anti-cancer effect of these plant parts in combination as PHE. The mechanism of action of the PHE may be due to effect of PHE on signal transduction in cell proliferation and angiogenesis or by selectively increasing cytotoxicity through apoptosis without producing toxicity. Hence further investigation are in progress to establish it's exact cellular level mechanism of action.

Table 1: Phytochemical studies of *Annona squmosa*, *O. indicum* and *C. gigantea* extract

PHE		PHE		PHE	
Test for alkaloid	+	Test for carbohydrate	-	Test for glycoside	+
Test for phenolic compound	-	Test for tannin	+	Test for protein	+
Test for saponin	+	Test for gum and mucilage	-	Test for sterol	+
Test for oil and fat	-	Test for flavonoid	+		

Notes:+present;-absent

Table 2: Effect of PHE on human cancer cell lines

Test compound	GI_{50}(µg/ml)			TGI (µg/ml)			LC_{50}(µg/ml)			MEAN GI_{50}
	MCF7	HT29	HepG2	MCF7	HT29	HepG2	MCF7	HT29	HepG2	
PHE	29	40	37	64	72	58	>100	>100	>100	35.33

Notes: n=3; results are expressed as average of three determinations in three replicate; **GI_{50}** : Drug concentration inhibiting 50% cellular growth following 72 h drug exposure; **TGI**: Total cellular growth inhibition; **LC_{50}** : Concentration of drug resulting in a 50% reduction at the end of the drug treatment as compared to that at the beginning.

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