INTRODUCTION

Cancer is one of the leading causes of death worldwide. According to the WHO (2004), 12.5% of the population dies due to cancer. The disease is characterized by the uncontrolled and abnormal growth of cells in the human body, forming tumors of malignant cells with the potential to be metastatic [1,2]. Major causes of cancer may be physical inactivity, heredity, unbalanced diet, and various environmental factors [3].

Currently, chemotherapy, radiotherapy, and immunotherapy treatments and surgery cause several toxic effects on non-targeted cells/tissues. This arouses the need of using alternative treatments and therapies against cancer [1,4]. Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with the present chemotherapeutic agents [5]. Over the past decades, herbal medicines have made an impact on both global health, and they have been well accepted worldwide [6].

Medicinal plants play a critical role in the healthcare system of a majority of the world’s population. Among several medicinal plants worldwide, including India, only a few medicinal plants have attracted the interest of scientists to investigate the remedy for the prevention and treatment of cancer [5]. Natural bioactive compounds such as phenol and flavonoids occurring in the medicinal plants protect the biological systems against harmful effect. They have been studied for their antitumor, proapoptotic, and antiangiogenic effects [7,8].

In history, plant secondary metabolite derives anticancer constituents such as vincristine, vinblastine, camptothecin, podophyllotoxin, flavipiridol, and silvestrol have been used worldwide [9]. Medicinal plants possess good immunomodulatory and antioxidant properties, leading to anticancer activities. The antioxidant phytochemicals protect the cells from oxidative damage. Further, it will be of great significance to develop new drugs from these medicinal plants. Taking into consideration the above facts, an attempt has been made to evaluate the in vitro anticancer activity particularly against totally unexplored ovarian cancer cell lines i.e. SiHa cervix and ovcar-5 ovary cancer cell lines and phytochemical study of the seeds of Annona squamosa more over LCMS analysis will be the part of study.

METHOD

Chemicals and drugs

The following drugs and chemicals were used: Ethanol, Roswell Park Memorial Institute (RPMI) media, trypsin-EDTA (Sigma Chemical Co.), fetal bovine serum, penicillin, streptomycin, phosphate-buffered saline, sulforhodamine b, and dimethyl sulfoxide (DMSO). All chemicals used were of analytical grade.

Plant material

The seeds of AS were obtained from Botanical Garden, Dr. H. S. Gour University, Sagar, M.P, specimen No. BOT/H/12/14/20.

Extract preparation

The seeds of AS were washed with running water to remove impurities. It was dried to avoid the growth of microorganisms and subjected to hydroalcoholic extraction with the help of a Soxhlet apparatus. The hydroalcoholic extract was filtered, concentrated under reduced pressure, and lyophilized for drying. The dried extract was kept in the airtight container and stored at 4°C till further studies. The air-dried seeds (150 g) were powdered and then extracted with 1 L of 50% ethanol using Soxhlet apparatus at 68°C [10]. Percentage yield was calculated (Table 1).

Phytochemical tests

Phytochemical analysis was performed to detect various compounds such as tannins, flavonoids, alkaloids, and steroids [11,12].
Table 1: Percentage yield of hydroalcoholic extract of the plant

| Solvent   | Plant | Part used | Dry weight (g) | Yield (g) | Time (h) | Temperature (°C) | % yield |
|-----------|-------|-----------|----------------|-----------|----------|------------------|--------|
| 50% ethanol | AS    | Seeds     | 150            | 25.25     | 96       | 68               | 16.83  |

AS: Annona squamosa

Table 2: LCMS analysis of AS seed extract

| Retention time range (min.) | Mass-to-charge (m/z) |
|-----------------------------|----------------------|
| 2.3–2.5                     | 181.10               |
| 6.3–6.6                     | 313                  |
| 7.0–7.3                     | 327                  |
| 8.2–8.7                     | 652                  |
| 9.9–10.3                    | 848                  |
| 12.9–13.2                   | 837.30               |
| 15.0–15.4                   | 549.10               |
| 16.1–16.5                   | 269.10               |
| 18.3–18.7                   | 635.40               |
| 18.9–19.2                   | 656.40               |
| 19.9–20.1                   | 654.40               |
| 11.7–12.1                   | 832.40               |
| 21.7–22.2                   | 638.40               |
| 24.4–24.6                   | 638.40               |
| 25.2–25.7                   | 638.40               |

LCMS: Liquid chromatography and mass spectroscopy; AS: Annona squamosa

Table 3: Anticancer activity of hydroalcoholic extract of AS

| Type of cell line | Concentration of Extract (µg/ml) | % inhibition |
|-------------------|----------------------------------|--------------|
| MCF 7             | 100                              | 53.34        |
| SiHa              | 100                              | 67.15        |
| HT                | 100                              | 67.66        |
| Ovcar             | 100                              | 69.72        |
| HepG2             | 100                              | 63.82        |

AS: Annona squamosa, MCF 7: Breast cancer cell line, SiHa: Cervix cancer cell line, HT: Colon cancer cell line, Ovcar: Ovary cancer cell line, HepG2: Liver cancer cell line

Fig. 1: Anticancer activity of hydroalcoholic extract of Annona squamosa

Fig. 2: Liquid chromatography and mass spectroscopy chromatogram of hydroalcoholic extract of Annona Squamosa
RESULTS AND DISCUSSIONS

The percentage yield of the extract of seeds of AS was 16.83 (Table 1). It showed the presence of flavonoids, tannins, alkaloids, and other phytochemicals. The LC/MS analysis revealed the presence of 15 different molecular weight compounds at different retention time (Table 2 and Fig.2). The mass spectrum of extract by LC/MS showed various peaks of different peak of 15 compounds of which molecular ion peak at $m/z$ 316 which resembles the molecular weight of the Isorhamnetin. However, isorhamnetin is methylated metabolite of quercetin which may acting as flavonoid in hydroalcoholic extract of extract of seeds of AS. The extract showed an average in vitro anticancer activity at a concentration of 100 µg/ml against all cancer cell lines. The best activity was observed against Ovar cell line (69.72) and was also significant against HT and SiHa cell lines (Table 3). The anticancer activity of the plant may be due to the presence of phytochemicals, such as flavonoids and flavonoids, which were investigated to determine chemoprevention activity against cancer [18]. Phenols, flavonoids, quercetin, genistein, and baicalein obtained from plant extracts are also effective against tumor [19]. Similarly, alkaloids such as schischkinnin and montamine have been isolated from the seeds of Centaurea schischkinii and Centaurea montana showed anticancer property [20]. Further, the active compounds of the plant can be isolated and their individual activity can be analyzed for several pharmacological activities.

CONCLUSION

On the basis of phytochemical and anticancer activity, it has been found that the Annona Squamosa seeds indicates potential against ovarian cancer cell lines. Phytochemical analysis indicates the presence of flavonoids and its derivatives are present in the Annona Squamosa seeds and can be concluded that anticancer potential may be due to presence of isorhamnetin.

AUTHOR CONTRIBUTIONS

Sarvesh Paliwal planned, design and supervised the research and manuscript. Shuchi Dave Mehta involved the design of the research, interpretation of data, analysis of the result and to the writing of the manuscript.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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