INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 26, No. 5, p. 153-160, 2025

RESEARCH PAPER OPEN ACCESS

Anticancer activity of *Mentha spicata* ethanolic extract against hepatic carcinoma cell lines

V. R. Rakhi, T. S. Dhanaraj, V. Ramamurthy*

P.G. & Research Department of Biochemistry, Maruthupandiyar College (Affiliated to Bharathidasan University), Thanjavur, Tiruchirappalli, Tamil Nadu, India

Key words: Anticancer, Cytotoxicity, *Mentha spicata*, HepG2 and SNU-423, MTT assay

http://dx.doi.org/10.12692/ijb/26.5.153-160 Article published on May 06, 2025

Abstract

Amongst liver cancers, the incidence of hepatocellular carcinoma (HCC) is increasing and has a high mortality rate. Chronic hepatitis B- or hepatitis C-induced cirrhosis is the leading risk factor for HCC. In this study, the anticancer potentials of plants was investigated against HepG2 and SNU-423 cells. Cytotoxicity of extracts was determined by MTT assay. The results showed that the ethanolic extract *Mentha spicata* possessed a moderate amount of anticancer activity and the IC_{50} value was recorded. The most potent anticancer activity was observed with the ethanolic extract of *Mentha spicata* with IC_{50} values of 55.75µg/ml and 48.50µg/ml on HepG2 and SNU-423 cellsc, respectively. Phytochemical analyses revealed the presence of large amount of phenols and flavonoids in the potent plant extracts which may be suggested to play an important role in their anticancer activities. These results suggest that *Mentha spicata* is a promising source of useful natural products and offers opportunities to develop novel anticancer drugs.

* **Corresponding Author:** V. Ramamurthy ✉ v.ramamoorthy07@gmail.com

Introduction

Cancer is currently one of the foremost health challenges and a leading cause of death worldwide. According to the World Health Organization (WHO), cancer was responsible for approximately 9.6 million deaths in 2018. Environment (physical carcinogen), certain viral infections (biological carcinogen) and diets (chemical carcinogen) are the main etiologies in the occurrence of cancer. Globally, cervical cancer is one of the most common cancers in women. Cervical cancer is caused by cofactors, including oral contraceptive use, smoking, multiparity and HIV infection. One of the major and considerable causes is the persistent infection of oncogenic human papilloma virus.

Cancer has been a constant battle globally with a lot of development in cures and preventative therapies. The disease is characterised by cells in the human body continually multiplying with the inability to controlled or stopped. Consequently, forming tumours of malignant cells with the potential to metastatic (Ochwang *et al.*, 2014). Currently treatments include chemotherapy, radiotherapy and chemically derived drugs are in use. Treatments such as chemotherapy can put patients under a lot of strain and further damage their health. Therefore, there is a focus on using alternative treatments and therapies against cancer.

For many years herbal medicines have been used and are still used in developing countries as the primary source of medical treatment. Plants have been used in medicine for their natural antiseptic properties. Thus, research has developed into investigating the potential properties and uses of terrestrial plants extracts for the preparation of potential nanomaterial based drugs for diseases including cancer (Rajeshwari Sivaraj *et al.*, 2014). Many plant species are already being used to treat or prevent development of cancer. Multiple researchers have identified species of plants that have demonstrated anticancer properties with a lot of focus on those that have been used in herbal medicine in developing countries.

Medicinal plants have been used for thousands of years in folk medicines in Asian and African populations and many plants are consumed for their health benefits in developed nations. According to the World Health Organisation (WHO) some nations still reply of plant-based treatment as their main source of medicine and developing nations are utilising the benefits of naturally sourced compounds for therapeutic purposes (Rajeswara Rao *et al.*, 2007). Compounds which have been identified and extracted from terrestrial plants for their anticancer properties include polyphenols, brassinosteroids and taxols.

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is one of the most prevalent malignant diseases encountered in the world, killing up to 1 million people annually. Geographically, its prevalence varies greatly, with a very high incidence in sub Saharan Africa and south and northeast Asia, including Korea (WHO, 1993; Omata, 1987). Although many risk factors for HCC such as aflatoxin, persistent hepatitis C viral infection and alcoholic cirrhosis have been reported, hepatitis B viral infection has been known to be the most important etiologic factor.

Epidemiological and laboratory studies have confirmed a strong association between the hepatitis B virus (HBV) and HCC. Prospective studies have shown that in those infected with HBV, the risk of HCC was several hundred times higher than in uninfected individuals (Beasly, 1982).

Cell lines

HepG2

HepG2 cells were the first to exhibit the key characteristics of hepatocytes. This line was isolated in 1975 and described as hepatocellular carcinoma (or hepatoma, HCC). On the other hand, the earlier cell line SK-Hep1, created in 1971, although considered a model of HCC, does not possess critical markers of liver cells, including the expression of albumin and alpha and gamma-fibrinogen. A patent for the HepG2 cell line, “a human hepatoma-derived cell line” was filed in 1980 by researchers at the Wistar Institute.

Since then, HepG2 cells have been entered into the ATCC (American Type Culture Collection, Rockville, MD, USA) repository as a human cell line (HB 8065), "derived from the liver tissue of a 15-year-old white male with a well-differentiated hepatocellular carcinoma".

SNU-423

SNU (Seoul National University) cell lines have been established from Korean cancer patients since 1982. The cell line SNU-423 (Seoul National University-423) was established by J.G. Park and *et al.* It was derived from primary hepatocellular carcinoma taken from a Korean male. The cultured cells are multinucleated and HBV DNA was detected. The cell lines are generally used to examine the expression of HBV and IGF. The ERK, TGF-β, NF-κB and Akt/mTOR pathways are active in this cell line. SNU-423 has a doubling time of 72 hours.

Mentha spicata

Mentha spicata, also called *Mentha viridis*, is a medicinal plant of the Lamiaceae family characterized by its potency to synthesize and secret secondary metabolites, essentially essential oils. Different populations use the aerial parts of this plant for tea preparation, and this tisane has shown several effects, according to ethnopharmacological surveys carried out in different areas around the world.

M. spicata L. (spearmint) is a creeping rhizomatous, glabrous, and perennial herb with a strong aromatic odor, growing up to 30–100 cm tall with variably hairless to hairy stems and foliage, and a wide spreading fleshy underground rhizome (Kunwar *et al.*, 2017). The leaves are ovate to lancolate, 5–9 cm long and 1.5–3 cm broad, with a serrated margin. Spearmint produces flowers in slender spikes, each flower pink or white, and 2.5–3 mm long and broad.

The stem is square-shaped, a trademark of the mint family of herbs (Bayani *et al.*, 2017). *M. spicata* L is well adapted to climatic conditions in tropical and subtropical areas. It can be cultivated in wide range of soils and found in back gardens of homesteads (Kassahun *et al.*, 2014).

Mentha viridis is widely used in a variety of applications (Kee *et al.*, 2017). Since ancient times, Western and Eastern cultures have practiced *Mentha viridis* as a medicinal and aromatic plant against several diseases. Ethnobotanical investigations into *Mentha viridis* have suggested its potential medical applications in different disorders. It has beneficial effects on diabetes, digestive, skin, and respiratory disorders (El-hilaly *et al.*, 2003).

Apoptosis, or programmed cell death, is one of the most finely coordinated regulatory functions for maintenance of the homeostasis in the living organism. It involves the continuous checking of the cellular integrity and cascade-like events of self-destruction when the integrity of the organism is endangered. Morphological hallmarks of apoptosis are nuclear condensation, cell shrinkage, membrane blebbing and the formation of apoptotic bodies.

These changes are accompanied by biochemical features, including DNA fragmentation and the proteolytic cleavage of a variety of intracellular substrates. The present investigation was taken up for evaluating the antiproliferative potential possessed by the ethanolic leaves extract of *Avicennia marina* against different cancer cell lines.

Materials and methods

Collection and Identification of plant material

For the study, the whole plant of *Mentha spicata* belongs to Lamiaceae family was collected from Kerala, South India. The whole plant were identified taxonomically and authenticated according to various literatures, Flora of Madras Presidency and Wealth of India including other pertinent taxonomic literature.

Preparation of plant materials and extract

The leaves were carefully cleaned, shade dried and powdered. The powdered material was stored in a closed air-tight plastic container at low temperature.

The powdered plant material (50 g) was extracted with 300 mL of each solvent ethanol by maceration (3×24 h) at room temperature. The collected solvents were concentrated by rotary vacuum evaporator at 45°C and then dried using a freeze dryer. All extracts and acyclovir (extracted from commercial tablet) were dissolved in dimethyl sulphoxide (DMSO). The final concentration of DMSO was 0.1% v/v in cell culture environment.

Phytochemical analysis

The preliminary phytochemical evaluation of leaves was carried on extract prepared by successive extraction method in Soxhlet. The resultant extracts were evaporated to dryness under vacuum. These extract were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc. Chemical tests were identifying the phytochemicals as described (Trease and Evans, 1983; Harborne, 1973). Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Tumour cell lines

Cell lines of different tissue origin such as HepG2 and SNU-423 were used. Cells were cultured in MEM (Minimum Essential Media) supplemented with Sodium Bicarbonate, EDTA, FCS (Foetal Calf Serum) and incubated in humidified atmosphere of 5% CO_2 and 37°C. The culture medium was changed every two days. All cell lines used were of human origin in order to more closely mimic how plant extracts would affect human cancer cells. Cells were generally cultured in 10 mL of appropriate medium in 75 cm^2 tissue culture (T-75) flasks at 37˚C in a humidified atmosphere of 5% CO_2/ 95% air. Cells were passages weekly and medium replaced fortnightly.

MTT assay (Mossman, 1983)

Antiproliferative effects were measured *in vitro* by using MTT ([3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide]) assays. After treatment, the living cells were assayed by the addition of 20 µl of 5 mg/ml MTT solution. Finally, the reduced MTT was assayed at 545 nm wells with untreated cells were utilized as controls. Antiproliferative and cytotoxic effects were distinguished by cell number and the duration of treatment (72 h, 5000 cells/w, and 24 h, 25000 cells/w, respectively). Stock solutions of the tested materials were prepared with dimethyl sulfoxide (DMSO). The highest DMSO concentration (0.3%) of the medium did not have any significant effect on the cell proliferation. Extracts which demonstrated potent activity (growth inhibition > 50%) were selected for further in vitro testing (dose-response curve and cytotoxicity). To study the interactions between acridones and doxorubicin, a checkerboard method was applied. A series of 2-fold dilutions of the acridones was tested in combination with 2-fold dilutions of doxorubicin. The cell growth rate was determined with MTT staining drug interactions were evaluated according to the following system (fractional inhibitory index = FIX).

Synergism if FIX < 0.5
Additive effect if FIX = 0.51-1
Indifferent effect if 1 < FIX < 2
Antagonism if FIX > 2

Results and discussion

Phytochemical studies of *Mentha spicata* showed that it contains a number of phenolic and polyphenolic compounds, saponin, essential oil, polysaccharides and pectin. The main bioactive constituents found are bioflavonoid, amentoflavone with minute amount of cupressoflavone (Abinash *et al.*, 2012). All of the extracts from the *Mentha spicata* contained saponin, phenols, tannins, glycosides, terpenoids, flavonoids, alkaloids, and coumarins, according to a preliminary phytochemical examination. With the exception of the chloroform extract's lack of saponins, glycosides, and coumarins and the extract from ethyl acetate's absence of saponin. The results of the phytochemical analysis are displayed in Table 1. A higher degree of biological activity derives from the presence of a high concentration of phytochemicals in the plant.

Table 1. Qualitative phytochemical screening on extracts of *Mentha spicata*

Name of test	Test applied / Reagent used	Ethanol	Water	Chloroform	Hexane	Acetone	Ethyl acetate
Alkaloids	Mayer's	+++	++	++	++	+++	++
	Wagner's	+++	++	++	++	+++	++
	Hagner's	+++	++	++	+++	+++	++
	Dragndorff's test	++	++	++	++	++	+
Flavonoids	HCl and magnesium turnings	+++	++	+	++	+	++
Carbohydrate	Molisch's test	+	+	+	+	+	+
Tannins & phenols	10% Lead acetate	+++	+	++	++	++	++
	$Fecl_3$	+++	+	++	++	++	++
Test for steroids	Salkowski's test	++	++	++	++	++	++
	Libermann-Burchard's test	++	++	++	++	++	++
Gums & mucilages	Alcoholic precipitation	-	-	-	-	-	-
Fixed oil & fats	Spot test	+	-	+	+	-	-
Saponins	Foam test	+	+	+	+	+	+
Phytosterols	LB test	+	+	+	+	+	+
Volatile oils	Hydro distillation method	+	+	+	+	+	+
Protein & free amino acids	Biuret test	++	++	++	++	++	++
	Ninhydrin test	+++	++	++	++	++	++
	Xanthoprotein test	+++	++	++	++	++	++

This plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized (Sofowora, 1993). In the present study, it was clearly understood that the ethanolic extracted maximum amount of the different type of metabolites present in the *Mentha spicata*. Boominathan and Ramamurthy (2009) reported that the phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins.

For instance, the presence of flavonoids suggest that the plant have been reported to exert multiple biological effects including, anti-allergic, anti-inflammatory, anti- microbial antioxidant, anti-cancer activity (Kunle and Egharevba, 2009). It also suggests that the plant might have diuretic properties (Jayvir *et al.*, 2002). The presence of tannins shows that the plant is astringent as documented and suggests that it might have antiviral and anti-bacterial activities and can relief in wound healing and burns (Haslem, 1989). Saponins and glycoside are also very important classes of secondary metabolites as some are cardio-active and used in treatment of heart conditions (Oloyode, 2005). Some researchers have also investigated that some saponins have anti-cancer and immune modulatory properties (Evans and Trease, 2002). Volatile oils are used in the industries for various purposes, both as a pharmaceutical/ cosmetic raw material for production of emollients and active ingredient for the respiratory tract infections. Anticancer activity of *Mentha spicata* was studied in different mammalian cell line.

Anticancer activity of ethanolic extract of *Mentha spicata* as well as standard was determined through MTT cytotoxicity assay. In the preliminary study, the ethanolic extract showed the good yielding capacity of phytocompounds activity. In this regards, the present investigation the ethanolic extract of *M. spicata* was studied in HepG2 and SNU-423 cell lines and its result labelled in the Table 2 and also made with standard drug tamoxifen.

The minimum cell viability (19.5%) and maximum cell inhibition (80.5%) were noted in 200 µg/ml concentration of *M. spicata*. The IC_{50} value (54.50µg/ml and 43.75µg/ml) was calculated for

anticancer activity of ethanolic extract of *M. spicata* against HepG2 and SNU-423 cell lines. The tamoxifen used as a standard for this study. In the standard, the minimum cell viability (17.5%) and maximum cell inhibition (82.5%) were observed in higher concentration. The percentage of cell inhibition was noted in the different concentrations of ethanolic extract of HepG2 and SNU-423 ranges from 20 to 200 µg/ml.

Anticancer properties of many natural compounds isolated from different plant extracts have been reported. Research is being carried out throughout the world to find a lead compound which can block the development of cancer in humans. Nature has always been a great contributor towards this goal. Plant-derived natural products such as flavonoids, terpenoids and steroids have received considerable attention due to their diverse pharmacological properties, which include cytotoxic and chemopreventive effects (Abdullaev, 2001). They were the first agents to advance into clinical use for the treatment of cancer (Cragg and Newman, 2005).

Table 2. Anticancer activity of hepatic cancer cells treated with ethanolic extracts of *Mentha spicata*

Concentrations (µg/ ml)	HepG2		SNU-423	
	% of cell viability	% of cytotoxicity	% of cell viability	% of cytotoxicity
Control	100	0	100	0
7.8	68	32	79	21
15.6	53.1	46.9	72.1	27.9
31.2	49.5	50.5	63.8	36.2
62.5	40.8	59.2	57.9	42.1
125	33.9	66.1	45.2	54.8
250	28.2	71.8	35.5	64.5
500	20.4	79.6	26.4	73.6
1000	9.5	90.5	5.5	94.5
Vehicle control (DMSO)	1.8	98.2	3.8	96.2

Withania somnifera as a potential source of new molecules that can curtail cancer growth were studied by Dredge *et al.* (2003). *A. marina* has also been shown to inhibit the growth of human cancer cell lines comparable to that produced by tamoxifen. The peels extract produced antiproliferative activity on HepG2 and SNU-423 tumor cell lines. Jayaprakasam (2003) reported that the inhibitory concentrations obtained was 25.1±0.91 against colon cell line HCT-116, but in this study plant extracts from different cancer cell treatments of *M. spicata* cultivated in fly ash containing soil had shown more than 85% inhibition against human cell lines. Furthermore this study has reported growth inhibitory importance in *M. spicata* against various cancer cell lines, i.e., HepG2 and SNU-423 tumor cell lines. Hence, this study has revealed remarkable anticancer potential in the leaves extract of *M. spicata*.

Conclusion

Nowadays plants are extensively used for the research purpose and it possesses more than one chemical entity so it has been widely used for the research investigations. Anticancer properties of many natural compounds isolated from different plant extracts have been reported. Research is being carried out throughout the world to find a lead compound which can block the development of cancer in humans. Nature has always been a great contributor towards this goal. Furthermore this study has to prove the cytotoxic effects of ethanolic leaves extract of *M. spicata* may be conducted in clinical trials on patients suffering from cancer disease. To the best of our knowledge, the present study concluded that the *M. spicata* have an anticancer activity against HepG2 and SNU-423cell line. From this study, it is clear that *M. spicata* extract have significant anti-cancer activity in cell line. The anti-cancer activity is probably due to the presence of phytocompounds.

References

Abdullaev FI. 2001. Plant derived agents against cancer. In: Gupta SK, editor. Pharmacology and therapeutics in the new millennium. Narosa Publishing House: New Delhi, India, p. 345–354.

Abinash C, Bharati, Sahu AN. 2012. Ethnobotany, phytochemistry and pharmacology of *Biophytum sensitivum* DC. Pharmacogn Rev **6**(11), 68–73.

Bayani M, Ahmadi-hamedani M, Jebelli Javan A. 2017. Study of hypoglycemic, hypocholesterolemic and antioxidant activities of Iranian *Mentha spicata* leaves aqueous extract in diabetic rats. Iranian Journal of Pharmaceutical Research **16**(8), 75–82.

Beasley RP. 1982. Hepatitis B virus as the etiologic agent in hepatocellular carcinoma: Epidemiologic considerations. Hepatology **2**, 21S–27S.

Boominathan M, Ramamurthy V. 2009. Antimicrobial activity of *Heliotropium indicum* and *Coldenia procumbens*. Journal of Ecobiology **24**(1), 11–15.

Cragg GM, Newman DJ. 2005. Plants as source of anticancer agents. Journal of Ethnopharmacology **100**, 72–79.

Dredge K, Dalgleish AG, Marriott JB. 2003. Angiogenesis inhibitors in cancer therapy. Current Opinion in Investigational Drugs **4**, 667–674.

El-Hilaly J, Hmammouchi M, Lyoussi B. 2003. Ethnobotanical studies and economic evaluation of medicinal plants in Taounate province (Northern Morocco). Journal of Ethnopharmacology **86**(2–3), 149–158.

Evans WC, Trease G. 2002. Pharmacognosy. 15th ed. W.B. Saunders, London, pp. 183–184, 191–393.

Harborne JB. 1973. Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman and Hall, London, pp. 33–41.

Haslem E. 1989. Plant polyphenols: Vegetable tannins revisited – chemistry and pharmacology of natural products. Cambridge University Press, Cambridge, 169.

Jayaprakasam B, Zhang Y, Seeram NP, Nair MG. 2003. Growth inhibition of human tumor cell lines by withanolides from *Withania somnifera* leaves. Life Sci **74**, 125–132.

Jayvir A, Minoo P, Gauri B, Ripal K. 2002. Nature Heals: A glossary of selected indigenous medicinal plant of India. 2nd ed. SRIST Innovations, India, 22.

Kassahun BM, Egata DF, Lulseged T, Yosef WB, Tadesse S. 2014. Variability in agronomic and chemical characteristics of spearmint (*Mentha spicata* L.) genotypes in Ethiopia. International Journal of Advanced Biological and Biomedical Research **2**(10), 2704–2711.

Kee LA, Shori AB, Baba AS. 2017. Bioactivity and health effects of *Mentha spicata*. Integrative Food, Nutrition and Metabolism **5**(1), 1–2.

Kunle OF, Egharevba HO. 2009. Preliminary studies on *Vernonia ambigua*: Phytochemistry and antimicrobial screening of whole plant. Ethnobotanical Leaflets **13**, 1216–1221.

Kunwar G, Pande C, Tewari G. 2017. Essential oil composition of the aerial parts of *Mentha spicata* L. Journal of Essential Oil-Bearing Plants **13**(3), 353–356.

Mossman T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods **65**, 55–63.

Ochwangi DO, Kimwele CN, Oduma JA, Gathumbi PK, Mbaria JM, Kiama SG. 2014. Medicinal plants used in treatment and management of cancer in Kakamega County, Kenya. Journal of Ethnopharmacology **151**(3), 1040–1055.

Oloyode OI. 2005. Chemical profile of unripe pulp of *Carica papaya*. Pakistan Journal of Nutrition **4**(6), 379–381.

Omata M. 1987. Current perspectives on hepatocellular carcinoma in Oriental and African countries compared to developed western countries. Dig Dis **5**, 97–115.

Rajeshwari Sivaraj, Rahman PKSM, Rajiv P, Abdul Salam H, Venckatesh R. 2014. Biogenic copper oxide nanoparticles synthesis using *Tabernaemontana divaricata* leaf extract and its antibacterial activity against urinary tract pathogen. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy **133**(10), 178–181.

Rajeswara Rao BR, Singh K, Sastry KP, Singh CP, Kothari SK, Rajput DK, Bhattacharya AK. 2007. Cultivation technology for economically important medicinal plants. In: Janardhan Reddy K, Bahadur B, Bhadraiah B, Rao MLN (eds). Advan. in medicinal plants, Universities Press India Pvt Ltd, Hyderabad, 112–122.

Sofowora EA. 1993. Medicinal plants and traditional medicine in Africa. John Wiley and Sons Ltd, Nigeria, pp. 1–3.

Trease GE, Evans WC. 1983. Textbook of Pharmacognosy. 12th ed. Balliere Tindall, London, pp. 57–59.

World Health Organization (WHO). 1993. Causes of deaths by sex and age. In: World Health Statistics Annual. WHO, Geneva.