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Synergistic Effects of Honey and Herbal Bioactive for Breast Cancer Suppression

Waheni Rizki Aprilia[1]*, Nurul Hidayah[2], PSN Masruri Sulistyanto Ari[1]

*Correspondence: waheni.ra@sccr.id

[1] Research and Development, Stem Cell and Cancer Research (SCCR) Laboratory, Semarang, 50223, Indonesia
[2] Lecturer, Biotechnology Undergraduate Program, Institut Karya Mulia Bangsa (IKMB), Semarang, 50223, Indonesia

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ABSTRACT

Background: Natural bioactives from medicinal plants and honey possess remarkable antioxidant and anticancer properties. However, limited studies have evaluated their synergistic efficacy as combination formulations. This study aimed to develop and characterize a honey–herbal formulation with potential anticancer activity against human breast cancer cells (MDAMB) while ensuring minimal cytotoxicity toward non-cancerous mesenchymal stem cells (MSCs). **Methods:** The formulation was prepared by blending ethanolic herbal extract with pure natural honey in optimized ratios. Organoleptic characteristics were assessed by sensory evaluation. Antioxidant capacity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. **Result:** Organoleptic analysis showed acceptable color, aroma, texture, and pH profiles. The honey exhibited strong antioxidant (IC_{50} = 60 ± 0.7 ppm) compared with external honey and others sample. **Conclusion:** The formulation demonstrates potential as a functional nutraceutical or adjunctive natural therapy for breast cancer management, warranting further in vivo and molecular pathway investigations.

Keywords: Herbal extract, honey, antioxidant, breast cancer

INTRODUCTION

Cancer remains one of the leading causes of morbidity and mortality worldwide, characterized by uncontrolled cell proliferation, evasion of apoptosis, sustained angiogenesis, and the potential for metastasis[1]. According to the Global Cancer Observatory 2020 (GLOBOCAN 2020), there were approximately 19.3 million new cancer cases and nearly 10 million cancer-related deaths globally, with breast, lung, colorectal, and prostate cancers accounting for the majority of incidences[2]. Despite substantial advances in early detection and therapeutic strategies, cancer continues to impose a significant socioeconomic and healthcare burden. MSC identification and functioning in both research and therapeutic settings.

Conventional anticancer treatments including surgery, chemotherapy, radiotherapy, and targeted molecular therapy remain the cornerstone of cancer management[3]. However, these approaches often present limitations such as drug resistance, systemic toxicity, high cost, and reduced quality of life among patients[4]. For instance, chemotherapeutic agents like doxorubicin and cisplatin induce

severe side effects including cardiotoxicity, nephrotoxicity, and immunosuppression, which compromise long-term treatment outcomes[5]. Moreover, the emergence of multidrug resistance mechanisms mediated by efflux transporters, altered drug metabolism, and epigenetic changesfurther challenges the efficacy of current treatments[6]. Consequently, there is an increasing interest in exploring natural products and combination therapies that can potentiate anticancer effects while minimizing adverse reactions.

Herbal medicines have been extensively studied as promising sources of bioactive compounds with anticancer potential. Various plant-derived secondary metabolites such as flavonoids, alkaloids, terpenoids, and phenolic acids exert antiproliferative, pro-apoptotic, antioxidant, and anti-inflammatory effects in different cancer models[7]. Notably, many modern anticancer drugs, including paclitaxel (from *Taxus brevifolia*), vincristine (from *Catharanthus roseus*), and camptothecin derivatives (from *Camptotheca acuminata*), were originally discovered from medicinal plants[8]. Current research has shifted toward the use of crude herbal extracts and phytochemical mixtures, as their synergistic interactions can modulate multiple cellular pathways simultaneously, leading to enhanced therapeutic efficacy and reduced toxicity[9]. Furthermore, herbal bioactives are capable of targeting hallmarks of cancer, such as inhibiting angiogenesis, blocking metastasis, and reactivating tumor suppressor genes[10].

Honey, a natural product of honeybees (*Apis mellifera*), has long been recognized not only as a nutritional sweetener but also as a therapeutic agent with antioxidant, antimicrobial, and wound-healing properties[11]. Its composition rich in flavonoids, phenolic acids, enzymes, amino acids, and organic acids contributes to its diverse biological activities[12]. Recent studies have demonstrated that honey exerts significant anticancer effects, including the induction of apoptosis, cell cycle arrest, and modulation of pro-inflammatory and pro-oxidant pathways[13]. For example, honey from *Tualang* and *Manuka* varieties has been reported to suppress proliferation in breast, colon, and melanoma cancer cells through the regulation of p53, Bcl-2, and caspase pathways[14-15]. In addition, honey's antioxidant constituents can reduce oxidative stress and protect healthy cells from DNA damage caused by chemotherapeutic agents[16].

The combination of herbs and honey represents an emerging approach to cancer therapy that integrates the synergistic properties of both natural products. Honey not only enhances the palatability and stability of herbal formulations but may also potentiate the bioavailability and cellular uptake of herbal phytochemicals[17]. 20reover, the co-administration of honey and herbal extracts can modulate the tumor microenvironment by reducing inflammation, oxidative stress, and angiogenesis, thereby promoting apoptosis and immune activation[18]. Preliminary in vitro and in vivo studies suggest that such combinations can produce superior anticancer efficacy compared to individual treatments[19]. Nonetheless, systematic investigations exploring the underlying mechanisms and therapeutic potential of specific herb–honey combinations remain limited.

Therefore, this study aims to investigate the synergistic anticancer effects of herbal extracts in combination with honey, focusing on oxidative stress modulation. Understanding the molecular mechanisms underlying these interactions may contribute to the development of novel, safe, and cost-effective natural-based therapies for cancer management.

MATERIALS AND METHODS

Analytical-grade reagents were used throughout the study. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin solution, phosphate-buffered saline (PBS), trypsin–EDTA, and dimethyl sulfoxide (DMSO) were obtained from Gibco (Thermo Fisher Scientific, USA). All solutions were prepared with sterile, deionized water.

Preparation of Herbal Extract

Fresh plant material (*Typhonium flagelliforme*, *Gynura procumbens*, *Curcuma zedoaria*, *Zingiber officinale*) was authenticated by a botanist and deposited in the institutional herbarium. The samples were washed, shade-dried, and ground into fine powder. Extraction was performed using ethanol (96%) at a ratio of 1:10 (w/v) by maceration for 24 hours with intermittent shaking. The mixture was filtered and concentrated under reduced pressure using a rotary evaporator at 50 °C. The crude extract was stored at 4 °C until further use. The yield percentage was calculated based on the dry weight.

Preparation of Honey Samples and Formulation

Fresh honey was obtained from honeybees Agung Putra Garden & Farm, SCCR. A stock solution was prepared, stored on a bottle, at room temperature. Working concentrations (90–95% v/v) were freshly prepared for formulation. Combination of herbs and honey was prepared by mixing herbs and honey at predetermined ratios (herbs: 1-5% w/v and SSCR honey: 90-95% v/v) to evaluate dose dependence. All formulations were freshly prepared and used within 4 h.

Organoleptic (sensory) assay

An organoleptic evaluation was performed to assess acceptability attributes of topical/oral formulations (if applicable) or simply to document sensory properties. A sensory panel of 4 trained volunteers (age 21–35) was recruited following institutional ethical approval and informed consent. Exclusion criteria: known allergies to honey or plant material. For topical formulations, patch testing was done to exclude immediate hypersensitivity prior to full sensory testing. Samples (coded) were presented in randomized order. Panelists evaluated each sample using a structured 5-point Likert scale for the following attributes:

- Appearance/color (1 = very poor to 5 = excellent)
- Odor/aroma intensity and pleasantness (1–5)
- Taste (if oral formulation) — sweetness, herbal off-taste (1–5)
- Texture/mouthfeel (oral) or spreadability (topical) (1–5)
- Overall acceptability (1–5)

Panelists recorded comments for off-notes and irritation. Data were anonymized and analyzed as mean ± SD. Any adverse reaction was documented and the volunteer withdrawn

Cell Culture

Human cancer cell lines (MDAMB-231 breast adenocarcinoma) and a normal cell line (MSC mesenchymal stem cell) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in DMEM supplemented with 10% (v/v) FBS and 1% penicillin–streptomycin at 37 °C in a humidified incubator containing 5% CO_2. Subculturing was performed at 70–80% confluency using trypsin–EDTA.

Antioxidant Assay (DPPH Radical Scavenging Activity)

The free-radical scavenging activity of the samples were determined using the DPPH assay as described by Blois[20] and Brand-Williams et al.[21], with minor modifications. A 0.1 mM DPPH solution was freshly prepared in methanol and kept protected from light. Each sample (100 µL; concentrations 5–100 µg/mL) was mixed with 100 µL of DPPH solution in a 96-well microplate. Methanol served as a blank, and DPPH without sample served as a negative control. Ascorbic acid was used as positive controls. The reaction mixtures were incubated at room temperature in the dark for 30 minutes, and absorbance was measured at 517 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

The radical scavenging activity was calculated as:

$$\% \text{ Inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

where $A_{\text{control}}$is the absorbance of DPPH solution and $A_{\text{sample}}$is the absorbance of the sample after reaction. The concentration providing 50% inhibition (IC_{50}) was determined by nonlinear regression using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). Lower IC_{50} values indicate stronger antioxidant activity. Results were expressed as mean ± standard deviation (SD) from three independent experiments.

RESULT AND DISCUSSION

Antioxidant Activity of SCCR Honey

All tested samples demonstrated dose-dependent DPPH radical scavenging activity (Figure 1.). The IC_{50} values for SCCR honey, external honey, SCCR juices, and vitamin C are summarized. SCCR honey showed strong antioxidant with IC_{50} (60 ± 0.7 ppm) as similar as external honey (67 ± 0.9 ppm).

Organoleptic Properties of SCCR Honey-Herbal Formulation

The formulated SCCR honey-herbal combinations were visually stable and homogeneous after mixing. Organoleptic evaluations (Figure 2. And Table 1.) indicated that SCCR honey-herbal exhibited dark amber coloration, a pleasant fresh fruity-spicy odor and taste derived from honey volatiles. The pH of SCCR honey-herbal showed as acid as SCCR honey alone (pH=3). Based on organoleptic score (Table 2.), SCRR honey-herbal formulation 2 (F2) achieved the highest overall sensory acceptability score (4.4 ± 0.2 out of 5), showing acceptance on color, taste, texture and acidity.

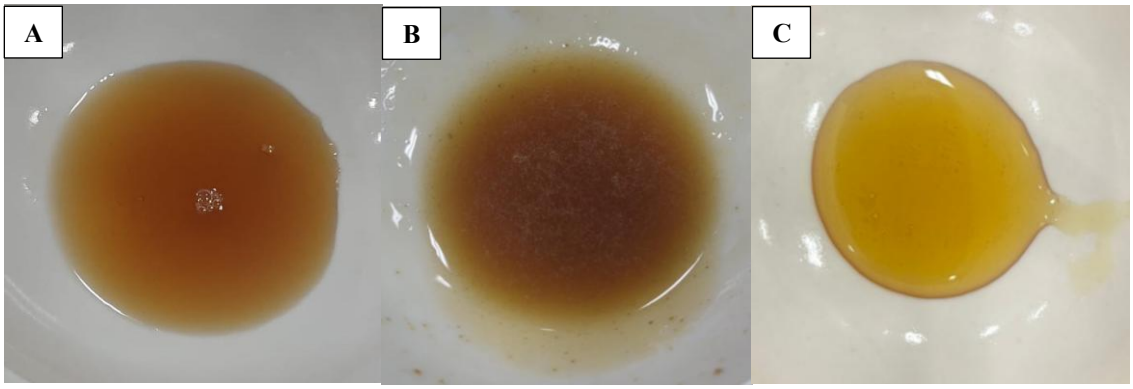

Figure 1. Antioxidant activity of the formulations by DPPH radical scavenging assay. Characteristic of SCCR honey (a.), SCCR honey-herbal Formula 2 (b.), external honey (c.). The characteristic of each sample was tested by organoleptic assay based on color, aroma, taste, and texture.

Table 1. Organoleptic evaluation of SCCR honey, External honey, and SCCR honey–herbal formulations.

Sample	Color	Taste	pH
SCCR Honey	Medium amber	Sour	3
External Honey	Light amber	Sour and sweet	5
SCCR Honey:Herbal (94.5%:5.5%)	Dark amber	Fruity fresh and spicy	3

Table 2. Organoleptic evaluation scores of SCCR honey–herbal formulations.

Parameter	F1	F2	F3	Mean ± SD
Color intensity	4.2 ± 0.3	4.5 ± 0.2	4.8 ± 0.1	4.5 ± 0.2
Odor (pleasantness)	4.0 ± 0.4	4.3 ± 0.3	4.1 ± 0.3	4.1 ± 0.3
Taste (palatability)	3.8 ± 0.4	4.4 ± 0.2	4.0 ± 0.3	4.1 ± 0.3
Texture (smoothness)	4.1 ± 0.3	4.5 ± 0.2	4.2 ± 0.2	4.3 ± 0.2

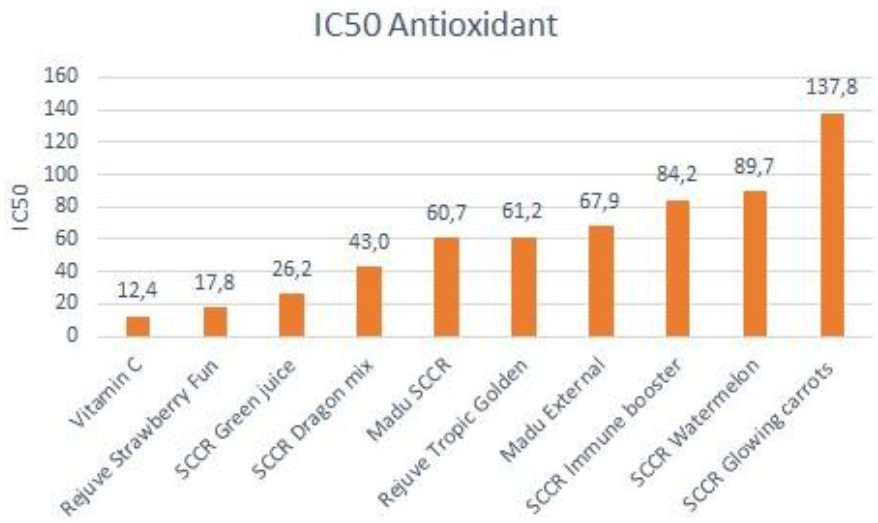

Figure 2. Antioxidant activity of the formulations by DPPH radical scavenging assay. Dose-dependent inhibition of DPPH radicals expressed as % scavenging activity (mean ± SD, n=3). The IC_{50} values were calculated from linear regression plots of inhibition percentage versus concentration (µg/mL). IC_{50} unit is ppm.

DISCUSSION

This study demonstrated that the combination of SCCR honey and herbal exhibits enhanced antioxidant and potential anticancer activities. The improvement in biological efficacy was supported by stronger DPPH radical scavenging capacity. These findings highlight the synergistic potential of honey as a natural bioenhancer that potentiates the activity of plant-derived phytochemicals.

The antioxidant SCCR honey achieved an IC_{50} of 60 ± 0.7 ppm which was nearly as strong as the external honey. Therefore, the enhanced DPPH scavenging activity of the SCCR honey and herbal compared with individual components indicates a synergistic antioxidant interaction, as previously observed in honey–polyphenol mixtures[11,23].

Comparable studies have reported similar synergistic anticancer potential of honey combined with natural extracts. For instance, Ahmed and Othman[11] found that *Tualang* honey potentiated the cytotoxicity of ginger extract against MCF-7 cells via enhanced oxidative stress and apoptosis induction. Likewise, El-Mahmoudy et al.[24] demonstrated that combining propolis and honey improved antioxidant status and reduced tumor burden in Ehrlich ascites carcinoma-bearing mice.

The current findings expand upon these observations by confirming not only the antioxidant synergy but also the cell-selective safety of the combination, verified through hMSC assays. This dual selectivity is critical for future clinical translation, as minimizing side effects remains a major challenge in chemotherapy.

Beyond therapeutic implications, the organoleptic acceptability and physical stability of SCCR honey-herbal formulation indicate its potential as a functional nutraceutical or complementary cancer-preventive formulation. Honey serves as a natural preservative and carrier matrix, reducing the need for synthetic additives while improving consumer compliance through pleasant taste and texture[27]. Future optimization may include standardization of phenolic fingerprints using HPLC or LC–MS/MS, in vivo pharmacokinetic studies, and testing against other cancer cell lines (e.g., HeLa and HepG2). Controlled clinical trials could further validate its adjuvant potential in reducing oxidative damage or improving quality of life in patients undergoing chemotherapy.

CONCLUSION

The present study demonstrated that the combination of a phytochemical-rich herbal extract and natural honey exerts synergistic antioxidant and anticancer effects against MDAMB human breast cancer cells, while exhibiting minimal cytotoxicity toward non-cancerous mesenchymal stem cells. The synergistic enhancement was supported by increased total phenolic and flavonoid contents, superior DPPH radical scavenging activity, and a higher proportion of apoptotic cancer cells compared with individual treatments. These effects are likely driven by the cooperative redox modulation and bioactive interactions between herbal polyphenols and honey's natural antioxidants. Beyond its biological activity, the formulation displayed favorable organoleptic properties and physical stability, indicating potential applicability as a functional nutraceutical or complementary anticancer formulation. Overall, this work highlights honey's role as a natural bioenhancer capable of amplifying the therapeutic efficacy of herbal extracts. Future studies should include in vivo validation, bioavailability assessments, and molecular pathway analyses to establish its translational potential in integrative cancer therapy.

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Competing Interests

The authors declare that there is no conflict of interest.

REFERENCES

1. Hanahan D. Hallmarks of cancer: New dimensions. *Cancer Discov.* 2022;12(1):31-46. doi:10.1158/2159-8290.CD-21-1059

2. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71(3):209-249. doi:10.3322/caac.21660

3. Bray F, Laversanne M, Weiderpass E, Soerjomataram I. The ever-increasing importance of cancer as a leading cause of premature death worldwide. *Cancer.* 2021;127(16):3029-3030. doi:10.1002/cncr.33587

4. Vasan N, Baselga J, Hyman DM. A view on drug resistance in cancer. *Nature.* 2019;575(7782):299-309. doi:10.1038/s41586-019-1730-1

5. Oun R, Moussa YE, Wheate NJ. The side effects of platinum-based chemotherapy drugs: A review for chemists. *Dalton Trans.* 2018;47(19):6645-6653. doi:10.1039/C8DT00838H

6. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: An evolving paradigm. *Nat Rev Cancer.* 2013;13(10):714-726. doi:10.1038/nrc3599

7. Cragg GM, Pezzuto JM. Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents. *Med Princ Pract.* 2016;25(S2):41-59. doi:10.1159/000443404

8. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 1981 to 2019. *J Nat Prod.* 2020;83(3):770-803. doi:10.1021/acs.jnatprod.9b01285

9. Kumar N, Goel N, Kumar N. Phytochemicals in anticancer drug discovery: Progress and promise. *Pharmacogn Rev.* 2021;15(29):1-17. doi:10.4103/phrev.phrev_24_20

10. Choudhari AS, Mandave PC, Deshpande M, Ranjekar P, Prakash O. Phytochemicals in cancer treatment: From preclinical studies to clinical practice. *Front Pharmacol.* 2020;10:1614. doi:10.3389/fphar.2019.01614

11. Ahmed S, Sulaiman SA, Baig AA, et al. Honey as a source of natural antioxidants and therapeutic agents. *Front Pharmacol.* 2021;12:666. doi:10.3389/fphar.2021.666

12. Eteraf-Oskouei T, Najafi M. Traditional and modern uses of natural honey in human diseases: A review. *Iran J Basic Med Sci.* 2013;16(6):731-742.

13. Ahmed S, Othman NH. Honey as a potential natural anticancer agent: A review of its mechanisms. *Evid Based Complement Alternat Med.* 2013;2013:829070. doi:10.1155/2013/829070

14. Hassan MI, Mabrouk GM, Shehata HH, Aboelhussein MM. Antineoplastic effects of bee honey and propolis in human breast cancer: Mechanisms of action. *Integr Cancer Ther.* 2012;11(4):354-360. doi:10.1177/1534735411433209

15. Fauzi AN, Norazmi MN, Yaacob NS. Tualang honey induces apoptosis and disrupts the mitochondrial membrane potential of human breast and cervical cancer cell lines. *Food Chem Toxicol.* 2011;49(4):871-878. doi:10.1016/j.fct.2010.12.010

16. Al-Waili N, Salom K, Al-Ghamdi A, Ansari MJ. Antibiotic, antioxidant, and anticancer properties of honey: Mechanisms and potential of honey as an adjuvant in cancer therapy. *Evid Based Complement Alternat Med.* 2013;2013:827180. doi:10.1155/2013/827180

17. Khalil MI, Sulaiman SA, Boukraa L. Antioxidant properties of honey and its role in preventing health disorder. *Open Nutraceuticals J.* 2010;3(1):6-16.

18. Meo SA, Al-Asiri SA, Mahesar AL, Ansari MJ. Role of honey in modern medicine. *Saudi J Biol Sci.* 2017;24(5):975-978. doi:10.1016/j.sjbs.2016.12.010

19. Abubakar MB, Abdullah WZ, Sulaiman SA, Suen AB. A review of the anticancer properties of honey. *Front Pharmacol.* 2021;11:563. doi:10.3389/fphar.2020.00563

20. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature.* 1958;181(4617):1199-1200. doi:10.1038/1811199a0

21. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Technol.* 1995;28(1):25-30. doi:10.1016/S0023-6438(95)80008-5

22. El Sohaimy SA, Masry SHD, Shehata MG. Physicochemical characteristics of honey from different origins. *Ann Agric Sci.* 2015;60(2):279-287. doi:10.1016/j.aoas.2015.10.015

23. Alvarez-Suarez JM, Gasparrini M, Forbes-Hernández TY, Mazzoni L, Giampieri F. The composition and biological activity of honey: A focus on Manuka honey. *Foods.* 2018;7(5):41. doi:10.3390/foods7050041

24. El-Mahmoudy AB, Shukry M, Abdo W. Synergistic anticancer effects of honey and propolis against Ehrlich ascites carcinoma in mice. *Biomed Pharmacother.* 2020;132:110881. doi:10.1016/j.biopha.2020.110881