

## Potential biological activity of acacia honey

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### 1. ABSTRACT

Recent advances in functional foods-based research have increasingly become an area of major interest because it affects human health and activities. Functional foods are classes of foods with health promoting and disease preventing properties in addition to multiple nutritional values and of such type is honey. Acacia honey is a type of honey produced by bees (*Apis mellifera*) fed on Acacia flowers, hence the name. This review focuses on the potential biological activities of Acacia honey which includes quality, antioxidant, immuno-modulatory, antiproliferative and neurological properties at *in vitro* and *in vivo* levels. Based on our review, Acacia honey used from various researches is of high purity, contains some bioactive compounds ranging from vitamins, phenolics, flavonoids and fatty acids. It's highly nutritional with strong antioxidant and immuno-modulatory potentials which may therefore be considered a potential candidate for both cancer prevention and treatment. Neurologically, it may be considered as a viable therapeutic agent in the management of Alzheimer's disease.

### 2. INTRODUCTION

Functional foods are well characterized by their natural properties of health promotion, and disease prevention in addition to numerous nutritional values (1). One of the most consumed functional food is honey which has been defined as a natural product

produced by honeybees via a regurgitation mechanism of the plant parts (2). Documented biological activities of honey includes antioxidant, immuno-modulatory, cancer prophylactic and curative properties (3). In addition, experimental evidence indicates that honey from variety of floral and geographical sources like Manuka, Saudi sidr, Toulang, Chestnut, Rhododendron, Pasture, Jelly bush, Blossom, Sage and Neem could exert several health-beneficial effects which includes gastroprotective, hepatoprotective, reproductive, hypoglycemic, antioxidant, antihypertensive, antibacterial, anti-fungal and anti-inflammatory effects (4). This is because substances produced by honey bees (*Apis mellifera*), including propolis, honey, wax and venom have been used for their medicinal properties throughout history (5). Furthermore, antioxidant and other bioactive compounds are biosynthesized by a large number of plants that may be used by honeybees to collect nectar which consequently transfer a wide variety of phytochemicals to honey that are of biological importance to the ultimate consumers (4). Acacia is a genus of shrubs and trees belonging to the subfamily Mimosoideae of the family Fabaceae. Acacia honey is a type of honey produced by bees fed on Acacia flowers, hence the name.

The global prevalence of chronic diseases such as diabetes mellitus, hypertension, atherosclerosis,

**Table 1.** The physico-chemical properties of Acacia honey

Parameters	Amount	Standard amount (imposed limit)	References
Water content (g/100g)	18.3.9	NA	(6)
Electrical conductivity (mS/cm)	0.1.8	0.0.6-2.1.7	(6)
pH	4.0.7	3.5.0-5.5.0	(6), (9)
Total acidity (meq/kg)	16.6.5	Up to 50.0.0	(6), (8)
Fructose (g/100g)	43.5.1	NA	(6)
Glucose (g/100g)	29.6.8	NA	(6)
F/G ratio	1.5.7	Up to 1.6.4	(6)
F+G content (g/100g)	72.5.7	Up to 92	(9)
Maltose (g/100g)	3.0.8	NA	(6)
Sucrose (g/100g)	2.5.0	≤5.0.0	(6), (8)
Diatase activity (units/g of honey)	2.7.0	>8.0.0	(7), (8)
Hydroxymethylfurfural (mg/100g)	0.4.4	>15.0.0	(7), (8)
NA: Not available in the literature, F: Fructose, G: Glucose			

cancer and Alzheimer’s disease is on the currently rise. As a result of the participation of oxidative processes in the onset and development of degenerative diseases, much attention has been paid to the antioxidant properties of foods rich in polyphenols and other biologically important metabolites. At present, there are numerous experimental reports on the different biological activities of Acacia honey. However, a comprehensive review that collates all these experimental evidences, critically appraise the achievements documented so far as well as provide future research directions is lacking in the current literature. Therefore, this review will provide a critical appraisal on the available experimental data from *in vitro* and *in vivo* studies with respect to the biological activities of Acacia honey as functional food and a potential source of nutraceuticals.

### 3. PURITY AND QUALITY

Quality of honey depends on the plant source, the chemical composition of these plants as well as on the climatic conditions and soil mineral composition (6). Basically, Acacia honey is very pale like liquid glass. It has a mild, sweet, floral flavour, and is therefore, one of the most popular honey varieties. It is a good choice for mixing with beverages because it sweetens without changing the taste of the drink. It is also an excellent choice for cooking because of its mild flavour and because it mixes easily in liquids and batters. It has hints of vanilla flavour and no aftertaste. Acacia honey is slow to crystallize because it can remain in a liquid state for a long period of time due to its high concentration of fructose (7), (6). However, some experimental variables have also been identified and currently being explored (Table 1) in the determination of purity and quality of honey for commercial and

research purposes. These include the sugar contents, pH, diastase activity, hydroxymethylfurfural level, water activity, moisture contents, free acidity, electrical conductivity and microbial contamination (8)(9). Most of the aforementioned indices have been analyzed and reported in Acacia honey, all of which fall within the accepted limits (7), (6), which pave way for the frequent utilization of Acacia honey in biological research and commercial applications.

### 4. FLORAL IDENTIFICATION

The variability of honey types produced in a region depends upon the diversity of nectar sources present in the region (10). Microscopic analysis and other advanced techniques (11), (8,12,13) are currently being used for the analysis of the pollen in honey which may eventually determine its geographical origin. In addition, the pollen grain analysis of honey may provide information about the possible biological activity in terms of its prophylactic and therapeutic potential as the original residing plant may contain some certain phytochemicals. Using these procedures, the pollen grains from Acacia flower were largely identified in Acacia honey (2,14,15), hence the continuous usage of the terminology. However, other methods are also currently explored in addition to microscopic techniques (melissopalynology) to further validate the authenticity of floral sources (mono or polyfloral) which includes Near-Infrared spectroscopy, gas chromatography-mass spectrometry, amino acids and mineral analyses (9,11,16–19). Overall, the combination of these techniques will go a long way in improving the quality of honey not only on commercial basis but also generating unbiased and desirable outcomes with regards to scientific researches comprising *in vitro* and *in vivo* experiments.

**Table 2.** The biological activities of Acacia honey

Biological activities	Description of effects	References
Antioxidant	Suppression of ROS generation	(35), (15)
Mitogenic	Stimulation of lymphocytes proliferation	(21)
Immuno-modulatory	Regulating TNF- $\alpha$ , IL-1 $\beta$ and lymphocytes levels	(21), (14)
Antiproliferative	Cytotoxic and anticancer properties	(2), (14).
Neurological	Inhibition of acetylcholinesterase activity	(30)
Hepatoprotective	Down regulating the serum aminotransferases level	(7,32)
Nephroprotective	Down regulating the serum urea and creatinine levels	(7,32)
Haematological	Boosting of haemoglobin, RBC and WBC levels	(7,32)
Wound healing	Increased tissue granulation and collagen formation	(33,34)

## 5. ANTIOXIDANT AND IMMUNO-MODULATORY POTENTIAL

The *in vitro* antioxidant properties of natural or synthetic agents are measured in the form of antiradical activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay, oxygen radical absorbance capacity (ORAC) assay and ferric reducing antioxidant power (FRAP) assay (4,20). Other advanced protocols like luminol and lucigenin-amplified chemiluminescence assays are currently being explored in the study of an *in vitro* antioxidant activity of Acacia honey (21). Studies (Table 2) have demonstrated the antioxidant, mitogenic and immuno-modulatory properties of Acacia honey. A dose-dependent inhibition of the intensity of luminol and lucigenin-amplified chemiluminescence was observed with exceptional IC<sub>50</sub> values for whole blood, neutrophils and macrophages (21). Furthermore, Acacia honey was also found to possess a significant dose dependent inhibition of DPPH and superoxide anion radicals. The reducing power of honey was also found to proportionally increase with concentration. The foregoing *in vitro* data suggests that Acacia honey is endowed with potent anti-oxidative agents. On the other hand, Acacia honey was also reported to possess immuno-modulatory potential by increasing peripheral blood lymphocytes, mitotic index, and nuclear division and cytokinesis-block proliferation indices whereas cytotoxicity index was decreased by the honey (Table 2). Furthermore, research findings also indicate that Acacia honey was not able to induce chromosomal damage in lymphocytes suggesting a immune protective role of honey which may be due to the potent antioxidant activity observed (21). Mitotic index measures the proportion of cells in the M-phase of the cell cycle and its inhibition could be considered as cellular death or delay in the cell proliferation kinetics (22). The mitotic index used to characterize the proliferating cells and to identify compounds that inhibit or induce mitotic progression depends on the proportion of the cell population that participates in the whole cycle of interphase leading to division and the relative lengths

of interphase with recognizable mitotic stages (23). Lymphoid cells from organisms can be stimulated *in vitro* to synthesize DNA and undergo blastogenic transformation upon exposure to mitogens (24), and this might explain further the immuno-modulatory role of Acacia honey because of the reported induction of lymphocytes proliferation (Figure 1).

Studies (Table 2) using animal models indicate that honey, like other antioxidant agents, does protect against damage which is partly mediated via amelioration of oxidative stress in various cells and viscera (4). A study reported that sodium arsenite significantly suppresses the GSH level, SOD and catalase activities with simultaneous induction of lipid peroxidation and clastogenic effects whilst Acacia honey was able to increase the sodium arsenite-induced rise in GSH level, SOD and catalase activities with concomitant anti-clastogenic effects due to reduction in micronuclei which demonstrates that Acacia honey mitigates sodium arsenite induced-oxidative stress and clastogenicity in rats via an anti-oxidant dependent mechanism (15).

## 6. ANTIPROLIFERATIVE EFFECTS

Cancer, an abnormal cell growth characterized by high proliferative potential is a leading cause of death worldwide and its development is frequently associated with DNA damage, aneuploidy, and nonrandom chromosome aberrations all of which can result from exposure to environmental chemicals/genetic alterations (14,25,26). Cancer chemoprevention has been defined as the administration of agents to prevent induction or to delay the progression of cancer (25), or as the reversal of carcinogenesis at a premalignant stage (27). Interestingly, honey has gained vitality in this area since it is rich in phenolic compounds and other antioxidants like ascorbic acid, amino acids, and proteins. Some simple and polyphenols found in honey, namely, caffeic acid, caffeic acid phenyl esters, chrysin, galangin, quercetin, kaempferol, acacetin, pinocembrin,

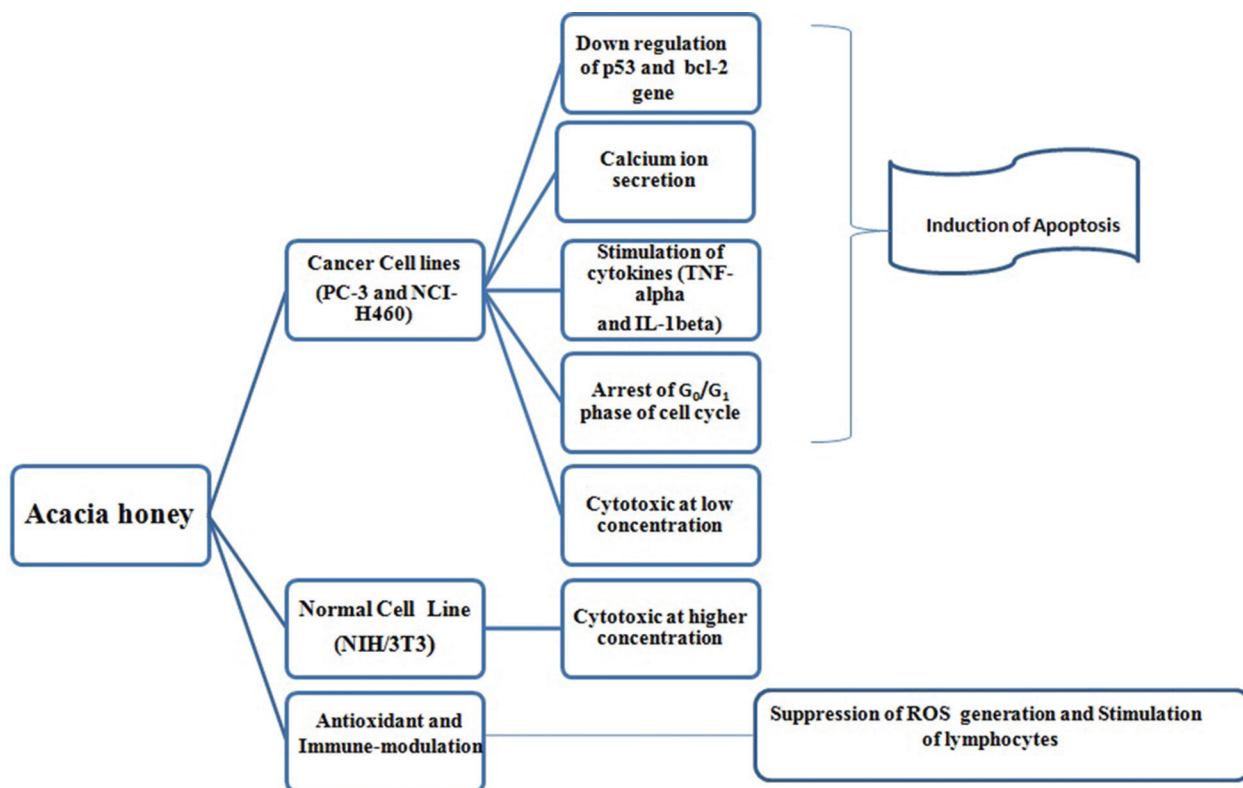


Figure 1. Summary of the biological activities of Acacia honey.

pinobanksin and apigenin, have evolved as promising pharmacological agents in treatment of cancer (4,25). The 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, trypan blue exclusion test, cell cycle, flow cytometry, gene expression analysis by conventional and real time polymerase chain reactions have been utilized as viable tools to underscore the antiproliferative effects of Acacia honey (Table 2). It was reported that Acacia honey was able to induce an antiproliferative effect on melanoma cells in a dose and time-dependent manner. Flow cytometry analysis indicated that cytotoxicity induced by honey was mediated by  $G_0/G_1$  cell cycle arrest and induction of hyperploids progression (2). Other findings showed the anti-proliferative effects of Acacia honey on NIH/3T3 and PC-3 cell lines with exceptional  $IC_{50}$  values and simultaneous increase in the secretion of calcium ion and down regulation of prostate specific antigen. Moreover, a significant dose-dependent modulation of  $G_0/G_1$  phase was observed as compared with the control. There was also an inverse relationship between  $TNF-\alpha$  and  $IL-1\beta$  with latter being up regulated. Mitotic index of PC-3 cells inversely varies with the honey concentration (28). Based on these findings, it was proposed (Figure 1) that apoptotic role of Acacia honey on PC-3 cell line may be due to modulation of  $G_0/G_1$  phase, pro-inflammatory cytokines, calcium ions secretion and down regulation of prostate specific antigen *in vitro*. Furthermore, Acacia

honey was reported to inhibit cells proliferation, arrested  $G_0/G_1$  phase, stimulated cytokines, calcium ion release as well as suppressed p53 and bcl-2 expressions in NCI-H460 cells (14). Overall, Acacia honey may therefore be considered a potential candidate for both cancer prevention and treatment (Figure 1).

## 7. NEUROLOGICAL ROLES

Honey polyphenols were found to be useful in improving memory deficits and can act at the molecular level by involving in apoptotic activities while attenuating microglia-induced neuroinflammation (29). Thus, honey might reduce the risk of some neurodegenerative diseases like Alzheimer's disease (29,30). It was reported (Table 2) that Acacia honey significantly decreased acetylcholinesterase (AChE) activity in the brain which was potentiated in the presence of sodium arsenite. Furthermore, strong correlation was observed between the sodium and calcium ion levels with acetylcholinesterase activity in the brain and the findings suggest that Acacia honey modulates acetylcholinesterase activities which may be explored in the management of Alzheimer's diseases (30). High activities of AChE in the brain have been implicated in the pathogenesis of the disease and its inhibition is considered as a viable therapeutic strategy in the management of the disease (31).

**Table 3.** The bioactive compounds of Acacia honey

Bioactive compounds	References
Vitamins (A, C and E)	(30)
p-hydroxybenzoic acid	(6,30)
Cinnamic acid	(6,30)
9-octadecanoic acid-2-hydroxy-1-(hydroxymethyl) ethyl ester	(6,30)
Chrysin	(6,30)
2-hydroxypentadecanone	(6,30)
Hydrofol acid	(6,30)
1, 6-anhydro-beta-D-glucofuranose	(6,30)
5-hydroxymethylfurfural	(6,30)
Pyrazol-3-one	(6,30)
Pinobanksin	(6,30)
Apigenin	(6,30)
Pinocembrin	(6,30)
Acacetin	(6,30)
Abscisic acid	(6,30)
Ferullic acid	(6,30)
2, 4-dihydroxy-5-methylpyrimidine	(6,30)
Amino acids (arginine, aspartic acid, cystein, glutamic acid and proline)	(17)

### 8. OTHER BIOLOGICAL EFFECTS

Other effects of Acacia honey reported (Table 2) include hepatoprotective by decreasing the level of serum aminotransferases and maintaining the integrity of hepatocytes, and nephroprotective by decreasing the level of serum urea and creatinine as well as boosting of haematological parameters (white blood cells, red blood cells e.t.c.) corroborating its role as functional food (7,32). A study was also conducted to assess the wound-healing activity of Acacia honey using incision, excision, burn and dead-space wound models in rats. Different formulations of honey were used and rats were treated topically as well as orally in which both the higher and lower doses of honey produced a significant effect on healing. The area of epithelisation was found to increase, followed by an increase in wound contraction, skin-breaking strength, and tissue granulation. The hydroxyproline content also increased in the rats treated with higher doses of honey compared to control, indicating an increase in collagen formation (33). Furthermore, a wound healing ability of Acacia honey was also reported in which corneal keratocytes cultured in media supplemented with 0.025% Acacia honey showed an increase in proliferative capacity while retaining their morphology, gene and protein expressions with normal cell cycle after incubation in Dispase solution confirming its promising

role in accelerating the initial stage of corneal wound healing (33,34).

### 9. BIOACTIVE COMPOUNDS

Acacia honey has been fully characterized (Table 3), and the bioactive compounds which include vitamins, phenolics, flavonoids and fatty acids. The vitamins identified so far are vitamin A, C and E (30). p-hydroxybenzoic acid, cinnamic acid, 9-octadecanoic acid-2-hydroxy-1-(hydroxymethyl) ethyl ester and flavonoids like chrysin have been identified. Others include 2-hydroxypentadecanone, hydrofol acid, 1, 6-anhydro-beta-D-glucofuranose, 5-hydroxymethylfurfural, pyrazol-3-one, pinobanksin, apigenin, pinocembrin, acacetin, abscisic acid, ferullic acid and 2, 4-dihydroxy-5-methylpyrimidine were also determined (6,35). Furthermore, some amino acids of physiological significance were also reported in Acacia honey which include arginine, aspartic acid, cystein, glutamic acid and proline (17). The presence of these compounds may go a long way in further underscoring the potential biological activity of Acacia honey. This is because most of them have been analyzed and reported in literature to possess strong biological activities (4,20).

### 10. CONCLUSION

Acacia honey used from various researches is of high quality by virtue of the applied melissopalynology. It's highly nutritional with strong antioxidant and immunomodulatory potentials which may therefore be considered a potential candidate for both cancer prevention and treatment. Neurologically, it may be considered as a viable therapeutic agent in the management of Alzheimer's disease.

### 11. FUTURE RESEARCH

The role of Acacia honey in cancer epigenetics needs to be investigated and further studies are needed to validate the contribution of Acacia honey in tumor therapy *in vivo*. Its role in the management of Alzheimer's disease using the real experimental models both at cellular and molecular levels should be conducted for further validations. The correlation between its physico-chemical and antimicrobial properties should also be looked into. Furthermore, its toxicology effect needs to be fully investigated at the molecular level.

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