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Potential of Honey in Sustainable Health Management

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ABSTRACT

Honey is a precious and sustainable natural resource. Ancient literatures belonging to early civilizations mention the use of honey by their physicians to treat patients with different symptoms, injuries or infections. The past few decades have witnessed various laboratories across the globe reporting their findings about the antimicrobial, wound-healing, anti-inflammation, anti-oxidant and anti-tumour effects of natural honey. Unfortunately, its use in modern medicine is severely limited. Scientific community is largely sceptical of its curative impact. The complex chemical composition and different beneficial properties of honey vary with its floral origin. Recently it has come into focus that honey has a very promising role in 'green nanotechnology'. Nanotechnology offers the opportunity to better exploit the potential of many natural compounds for application in nanomedicine. However, the classic nanotechnology synthetic approaches are often hazardous. Many nations are hence focusing on safer, easier, eco-friendly synthesis methods, using natural resources like honey. But in India, only a handful of laboratories are interested in exploring the immense valuable prospects of different varieties of honey available in different states.Every year only 30-40% of India's natural honey is harvested for sale and export, while the rest simply dries up. This is a huge waste of a valuable research resource that holds great promise for the pharmaceutical industry and in sustainable health management.

Key words : *Honey, Sustainable, Therapeutic, Green nanotechnology*

Introduction

It is crucial that we practise optimum, sensible, responsible usage and preservation of all global natural resources. India possesses a unique ecosystem, rich in diverse natural products. Sadly, many of these have not yet been characterized systematically for their various beneficial properties. This leaves a huge gap in understanding and appreciating their potential and delay their acceptance by the scientific community. Honey is one such natural renewable product. Its nutritional and medicinal properties have been valued by most ancient civilizations. In the recent decades, many researchers from different parts of the world have shown that honey samples with different floral origin possess varying but substantial degrees of anti-microbial, anti-oxidant, anti-tumour, anti-inflammation and wound healing properties. But, the health sector is yet to accept its therapeutic potential and put it to use (Eteraf-Oskouei and Najafi, 2013; Samarghandian *et al*., 2017). The composition and medicinal properties of different varieties of honey depend on botanical source, which is a very crucial criterion (Das *et al*., 2013; Goswami *et al*., 2017). India is gifted with vast expanses of diverse forest land, including ~40% of the largest mangrove of the world, the Sundarbans located in West Bengal. The country has about 500 flowering plant species and is home to four honeybee species. This offers an excellent opportunity for

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development of honey-based clinical research. Unfortunately, very few researchers are interested in exploring the immense valuable prospects of different varieties of honey available across the nation. On the other hand, many other countries have recently started focusing more on the use of their local honey, especially since it became known that honey can play a significant role in green and safe nanotechnology. The classical nanotechnology synthetic methods are usually costly, laborious, hazardous and are not eco-friendly. Scientists are hence opting for novel synthetic approaches using honey, which is proving to be safer, easier, non-hazardous with the nanoparticles often showing improved therapeutic efficacy (Balasooriya *et al.*, 2017).

This article discusses the great promises of honey for use in pharmaceutical industry and in nanotechnology, with a special focus on the anti-oxidant and anti-bacterial properties of honey from mangrove plants of Sundarban forest, West Bengal, India, as an example. It aims to encourage current researchers to investigate the potential of honey varieties available naturally in different parts of the country for drug development. This is in alignment with the 'Sweet revolution' initiative of the Government of India as part of Aatmanirbhar Bharat Abhiyaan (https://www.niti.gov.in/honeyed-shot-arm-aatmanirbhar-bharat).

Materials and Methods

Reagents were purchased from reputed companies like SRL/Himedia/Merck. Raw, natural honey samples (total 150) from different individual floral sources of Sundarban mangrove region like *Aegiceras corniculatum* or khalsi (K1H), *Sonneratia apetala* or keora (K2H), *Ceriops decandra* or goran (G1H), *Excoecaria agallocha* or gewa (G2H) plants, were purchased from licensed traders, bee keepers and local honey collectors. Samples were collected over three seasons, in three consecutive years. Commercially available, processed, muti-floral Sundarban honey samples were purchased from West Bengal Forest Department, Aranya Bhawan, Bidhan Nagar, Sector –III, Kolkata - 700 106 (sample labelled as BSH or 'Blended Sundarban Honey'), and also from South 24-Parganas Beekeeper's Cooperative Society located at Baruipur, West Bengal (labelled as BAH or Blended Apiary Honey). All the samples were stored at 0-4 ºC and analysed within three months. The honey samples were kept at room temperature overnight before each experiment. Experiments were performed with different percentage of aqueous solution of the honey samples (like 10%, 20% etc.) as mentioned.

To measure the total polyphenol content of Sundarban mono-floral and multifloral honey samples, Folin–Ciocalteu assay was used following standard protocol (Beretta *et al.*, 2005; Dhar *et al.*, 2011; Das *et al.*, 2013; Goswami *et al.*, 2017). The calibration curve was plotted using gallic acid (0–100 mg/ml) as standard. The result of polyphenol content was represented as mg of gallic acid equivalents (GAE) per 100 g of honey.

To quantify total flavonoid content of each honey sample, Aluminium chloride method was employed following known protocol,using a standard curve of quercetin (0-50 mg/ml) (Beretta *et al.*, 2005; Dhar *et al.*, 2011; Das *et al.*, 2013; Goswami *et al.*, 2017). The results were expressed in terms of mg of quercetin equivalent (QE) per 100 g of honey.

To assess anti-oxidant activity, standard tests like FRAP (Ferric Reducing Antioxidant Power) assay and DPPH Free radical scavenging assay were carried out following standard protocols (Beretta *et al.*, 2005; Dhar *et al.*, 2011; Das *et al.*, 2013; Goswami *et al.*, 2017).

FRAP assay is a well-known test used for measuring total antioxidant capacity. It is based on the capability of the test sample to reduce Fe^{3+} to Fe^{2+}. For plotting calibration curve, ferrous sulphate was used at various concentrations (100-500 μM) as standard. The ferric reducing ability of honey sample was expressed as FRAP value (μM of FeII) of 10% honey solution.

Free radical scavenging activity was measured using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical assay following standard protocol.The assay measures hydrogen (or electron) donating ability of different concentrations of test samples thereby decolourising DPPH radical from purple to yellow and converting it to its reduced form. Results were expressed as % inhibition, which was calculated as [(Absorbance of Blank – Absorbance of sample)/ Absorbance of Blank] X 100. Distilled water served as control and ascorbic acid as the standard. Representative data from same working dilution is shown for all batches.

Batch-to-batch comparative study of above properties are reported. Experiments were repeated many times over three seasons.Values are represented as mean ± SD.

Anti-bacterial activity was measured using known assays and following standard protocol for each assay (Balouiri *et al.*, 2016; Wasihun and Kasa, 2016; Goswami *et al.*, 2017). Different working dilutions of each honey sample were prepared in sterile water along with suitable control sets. Bacteria used in the study were *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The first assay comprised of "Determination of zone of inhibition by agar well diffusion method". It tests a sample's potential to inhibit bacterial growth, thus giving rise to a zone of inhibition of variable diameter on Muller Hinton Agar plates. The second standard assay involved "Determination of MIC or minimum inhibitory concentration" using broth tube dilution method, to find the lowest concentration of a test sample that can inhibit visible bacterial growth (may be bacteriostatic or bactericidal effect). The third assay involved"Determination of MBC or minimum bactericidal concentration" to find the lowest concentration of a test sample required to kill a particular bacterium, thus showing no colony on agar plates (bactericidal).

Results and Discussion

All honey samples of Sundarban mangrove region collected over three seasons were found to contain substantial amounts of polyphenolic compounds and flavonoids (Figures 1, 2). The total phenolic and flavonoid content of uni-floral honey samples followed the order: khalsi > goran > keora > gewa.

Honey has a complex chemical composition and contains about 200 substances most of which are valuable and useful. Of these, polyphenols and flavonoids are crucial phytochemicals that can account for much of the antioxidant activity of honey (Das *et al.*, 2013; Goswami *et al.*, 2017). Their presence and concentration in honey can vary depending upon the floral source, geographical location, climatic conditions. Honey from different states of India, can thus be important sources of these beneficial compounds and need to be characterized systematically. For example, honey collected from individual Sundarban mangrove plants of West Bengal exhibit more or less consistent and stable polyphenol and flavonoid content, as is evident from the batch-wise study spread over three seasons.

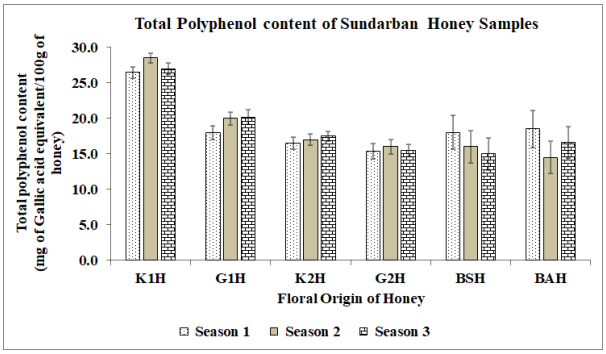

Fig. 1. A comparative analysis of Total Polyphenol content of Sundarban Honey Samples, collected over three seasons.[khalsi (K1H), keora (K2H), goran (G1H), gewa (G2H), Blended Sundarban Honey (BSH), Blended Apiary Honey (BAH)]

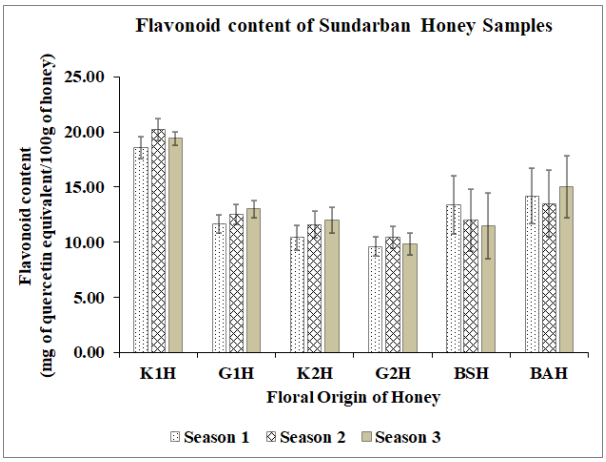

Fig. 2. A comparative analysis of Flavonoid content of Sundarban Honey Samples, collected over three seasons. [khalsi (K1H), keora (K2H), goran (G1H), gewa (G2H), Blended Sundarban Honey (BSH), Blended Apiary Honey (BAH)]

Reactive oxygen species, exogenous or produced inside cells, may induce oxidative damage to various bio-molecules leading to cardiovascular and neurodegenerative diseases, inflammation, aging and cancer. Since many decades, honey has been considered as a valuable dietary source of antioxidants that can help minimize these damages (Das *et al.*, 2013). Different botanical varieties of honey may exert variable anti-oxidant activities based on total phenolic composition, thus necessitating identification and characterization.

The antioxidant activity of each batch of different honey samples of Sundarban region seems to strongly correlate with the above batch-wise data on phenolic and flavonoid content, as is evident from the following results of FRAP assay (Figure 3) and DPPH assay (Figure 4).

The FRAP Test values reflect reducing activity and hence anti-oxidant property (Figure 3). All honey samples of Sundarban region, whether of single floral source or multi-floral, show substantial anti-oxidant activity in FRAP assay. The result also indicates that khalsi honey gives slightly higher (but reproducible) ferric reducing potential values in comparison to others, in agreement with its higher total phenolic and flavonoid content.

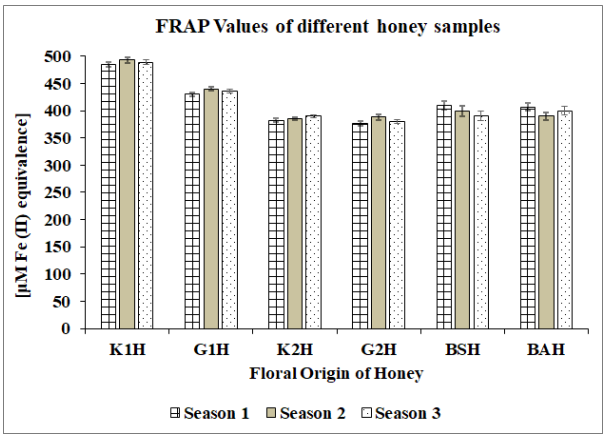

Fig. 3. Batch-wise study of FRAP values of honey samples, collected from Sundarban region over 3 seasons, to assess anti-oxidant potential. [khalsi (K1H), keora (K2H), goran (G1H), gewa (G2H), Blended Sundarban Honey (BSH), Blended Apiary Honey (BAH)]

Result of DPPH assay (Figure 4) suggests that all the honey samples of Sundarban have significant free radical scavenging activities, khalsi being the most effective, having % inhibition values closest to that of ascorbic acid which was used as standard.

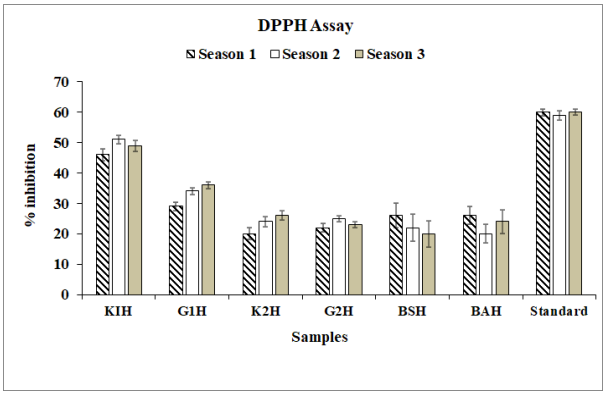

Fig. 4. Batch-wise DPPH Assay result of honey samples collected from Sundarban, over 3 seasons. [khalsi (K1H), keora (K2H), goran (G1H), gewa (G2H), Blended Sundarban Honey (BSH), Blended Apiary Honey (BAH); Standard = Ascorbic acid]

Synthetic antioxidants have been recently reported to be harmful for human health. Raw, natural honey can play a major role as a therapeutic natural antioxidant, as is evident from the anti-oxidant and free radical scavenging abilities of honey samples of Sundarban.

Antibiotic-resistant microbial species is currently a global threat (Alanis, 2005). The current deadlock in new antibiotic production is another great concern that adds to the menace. This has led to a renewed interest in the therapeutic potential of ancient remedies like honey. Bacterial resistance to honey has not yet been reported. This has encouraged many modern researchers to re-evaluate this natural product. Different varieties of honey from across the world have been reported to inhibit different species of bacteria, fungi and even some viruses, to varied extents (Wasihun and Kasa, 2016; Goswami *et al.*, 2017). The variation is most likely due to difference in honey composition and properties which are determined by specific geographical location, seasonal variety and botanical source. The antibacterial nature of honey is said to be dependent on various factors working either singularly or synergistically, like its high sugar content, low pH, high viscosity, hydrogen peroxide, phenolic compounds and osmotic pressure (Goswami *et al.*, 2017). For local honey to be used as an antimicrobial alternative, it has to be first tested in laboratory to determine its antimicrobial spectrum, stability and safety. This aspect further necessitates detailed research using the varied types of Indian natural honey obtained in different seasons to assess for consistent and reliable anti-microbial efficacy. For example, raw honey samples collected from individual mangrove plants of Sundarban region of West Bengal over three seasons show stable antibacterial activity across all batches, as suggested by the results obtained in the following assays.

The assay for "Determination of zone of inhibition by agar well diffusion method" showed that all the honey samples exhibited antibacterial properties, the effect being most pronounced against *E. coli* and lesser against *Staphylococcus aureus*. Figure 5 shows representative data from same working dilution for all batches. Khalsi honey gave excellent result, almost comparable to values obtained from respective antibiotic standard dose (drug) used.

Some honey may be bacteriostatic, some bactericidal. Effect may also depend on honey concentration. Well established assays like MIC assay or MBC

assay can help determine the antibacterial potential of a test sample. All Sundarban honey samples exhibited bacteriostatic and bactericidal activities in different dilutions, against the bacterial species tested, the effect being greatest against *E. coli* and less pronounced against *Staphylococcus aureus* (Tables 1, 2), suggesting considerable antibacterial potential, especially for khalsi honey.

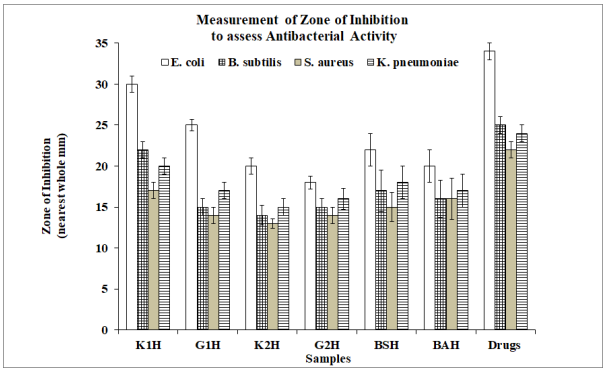

Fig. 5. Assessment of antibacterial activity of honey samples from Sundarban by measurement of zone of inhibition (representative data from one working dilution). [khalsi (K1H), keora (K2H), goran (G1H), gewa (G2H), Blended Sundarban Honey (BSH), Blended Apiary Honey (BAH); Drugs= known standard doses of antibiotics]

Thus, local natural raw honey from Sundarban forest region of West Bengal seem to have potential to be regularly used in therapeutics or to make honey-based value-added products. The four tested mono-floral raw honey samples possess significant and consistent antioxidant and antibacterial properties, stable over different seasons, the most promising being khalsi honey. However, the processed multi-floral honey samples mostly show lower efficacy with greater deviation between samples, thus making them less reliable.

Nanotechnology is currently the most happening field with numerous applications in diverse areas, including the health sector. However, the classical nanotechnology synthesis methods often need hazardous, toxic chemical compounds and apply dras-

Table 1. MIC% (vol/vol) of Honey Samples [lowest concentration of honey that inhibits visible growth of bacteria] + indicates visible growth; - indicates no visible growth

Honey Sample	Bacteria	Honey concentration (%)					
		100	50	25	12.5	6.25	3.125
K1H	*E. coli*	-	-	-	-	-	+
	B. subtilis	-	-	-	-	-	+
	S. aureus	-	-	-	+	+	+
	K. pneumoniae	-	-	-	-	+	+
G1H	*E. coli*	-	-	-	-	+	+
	B. subtilis	-	-	-	-	+	+
	S. aureus	-	-	-	+	+	+
	K. pneumoniae	-	-	-	+	+	+
K2H	*E. coli*	-	-	-	-	+	+
	B. subtilis	-	-	-	-	+	+
	S. aureus	-	-	+	+	+	+
	K. pneumoniae	-	-	-	+	+	+
G2H	*E. coli*	-	-	-	-	+	+
	B. subtilis	-	-	-	-	+	+
	S. aureus	-	-	+	+	+	+
	K. pneumoniae	-	-	+	+	+	+
BSH	*E. coli*	-	-	-	-	+	+
	B. subtilis	-	-	-	+	+	+
	S. aureus	-	-	+	+	+	+
	K. pneumoniae	-	-	+	+	+	+
BAH	*E. coli*	-	-	-	-	+	+
	B. subtilis	-	-	-	+	+	+
	S. aureus	-	-	+	+	+	+
	K. pneumoniae	-	-	+	+	+	+

tic physical conditions which may be unsafe for human beings, ecosystem and environment. These techniques are also costly, complex, time consuming. Green and safer synthesis methods using natural resources can be used to rule out such menaces. Many countries are using their local honey to synthesize different nanoparticles as a hazard-free, cost effective, sustainable, simple, rapid, eco-friendly alternative method and have met with considerable success (Philip, 2009; Philip, 2010; Haiza *et al.*, 2013; Balasooriya *et al.*, 2017). Moreover, often such nanoparticles are showing improved characteristics, for example, better anti-microbial property, probably due to a synergistic effect due to honey's own beneficial medicinal properties (Sreelakshmi *et al.*, 2011, Neupane *et al.*, 2019). This offers a novel option which may give sustainable, safe, economic yet efficient treatment results. However, it is absolutely essential to understand the choice of correct honey for a specific nanomaterial, according to its intended applications. This is still a developing field and remains to be fully optimized. In this respect, natural honey varieties of India (like Sundarban khalsi honey), with significant anti-bacterial and anti-oxidant properties, hold great promise and should be explored for their potential application in nanomedicine.

In conclusion, natural honey has great potential, as is obvious from its excellent therapeutic abilities and recent application in nanomedicine, as described above. Moreover, it is an absolutely sustainable resource, available globally. India is rich in natural honey varieties. While our government is trying to promote the use of honey, the sad reality is that the scientific community and health industry still do not sufficiently appreciate or favour its potential. It is high time that we re-evaluate and respect the potential of natural honey.

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Table 2. MBC% (vol/vol) of Honey Samples[lowest concentration of honey that can kill bacteria and give no colony] - indicates no colony; + indicates few and ++ indicate many colonies

Honey Sample	Bacteria	Honey concentration (%)					
		100	50	25	12.5	6.25	3.125
K1H	*E. coli*	-	-	-	-	+	++
	B. subtilis	-	-	-	+	+	++
	S. aureus	-	-	+	+	++	++
	K. pneumoniae	-	-	-	+	++	++
G1H	*E. coli*	-	-	-	+	++	++
	B. subtilis	-	-	-	+	++	++
	S. aureus	-	-	+	++	++	++
	K. pneumoniae	-	-	-	+	++	++
K2H	*E. coli*	-	-	-	+	++	++
	B. subtilis	-	-	-	+	++	++
	S. aureus	-	-	+	++	++	++
	K. pneumoniae	-	-	+	+	++	++
G2H	*E. coli*	-	-	-	+	++	++
	B. subtilis	-	-	+	++	++	++
	S. aureus	-	-	+	++	++	++
	K. pneumoniae	-	-	+	+	++	++
BSH	*E. coli*	-	-	-	+	+	++
	B. subtilis	-	-	+	++	++	++
	S. aureus	-	-	+	++	++	++
	K. pneumoniae	-	-	+	++	++	++
BAH	*E. coli*	-	-	-	-	+	++
	B. subtilis	-	-	-	+	++	++
	S. aureus	-	-	+	++	++	++
	K. pneumoniae	-	-	+	+	++	++

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Conflict of Interest

The author declares that there is no conflict of interest.

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