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Phytochemical screening and therapeutic potential of *Apis mellifera* propolis from India

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Abstract
Propolis is gaining popularity in the pharmaceutical sector as its numerous therapeutic activities become more widely recognised. The chemical composition of propolis varies depending on its geographical origin, resulting in differences in medicinal properties. Propolis from various geographical regions of India were evaluated for their pharmacological properties like antioxidant, antimicrobial and antidiabetic activities. The propolis extracts were evaluated for their antioxidant activities by chemical assays such as DPPH, ABTS, FRAP, and NO assays. Resazurin microtiter assay (REMA) was used to study the antibacterial activity of the propolis extracts. This study also investigated the antidiabetic activity of Indian propolis by α-amylase inhibitory assay and α-glucosidase inhibitory assay. The results of the various pharmacological assays demonstrated that all the propolis extracts showed significant antioxidant, antimicrobial and antidiabetic activities.

Keywords: propolis, antioxidant activity, antimicrobial activity, antidiabetic activity

Introduction
Owing to its various pharmacological activities and chemical diversity, propolis has gained wider appeal in diverse areas of scientific research that are focused on discovering novel therapies for the treatment of many diseases like diabetes, tumour, bacterial infections, allergic rhinitis, and ulcers [1]. Propolis is a resinous substance produced by honeybees from the selective collection of plant exudates that are subsequently mixed with beeswax and salivary bee secretions [2]. Honeybees use propolis to cover surfaces, to seal holes, creating a sterile atmosphere in the hive, which is why it is also known as "bee glue". Propolis is known to be a potential chemical weapon against bacteria, viruses, and other pathogens that can attack bee colonies. In addition, honey bees use it as an embalming agent to mummify intruders like other dead insects that are too heavy to remove from their hives. [3].
Propolis is generally composed of 50% plant balsam and resin, 30% beeswax, 10% essential and aromatic oils, 5% pollen, and 5% other organic and inorganic molecules. Until 2012, more than 500 chemical constituents of propolis were reported from various parts of the world. Between 2013 and 2018, at least 305 chemical compounds were isolated from propolis for the first time. Till 2018, more than 850 compounds have been isolated from propolis [4]. Variation in the chemical composition of different propolis samples arises due to differences in geographical vegetation, climate, honeybee species, and foraging season [5]. Regardless of the chemical variations, propolis from different geographical regions and chemical compositions are known to generally exhibit similar pharmacological activities [6]. Flavonoids, isoflavonoids, phenolic acids, terpenes, xanthones, propolones, and guttiferones are the most recognized bioactive chemical constituents in propolis and are responsible for their antimicrobial, anti-inflammatory, antiviral, antibacterial, anticancer, and wound healing properties [7].
The chemical diversity of various propolis samples has the potential to offer useful leads to active constituents. Thus, investigation of novel propolis types from unexplored geographical regions can lead to the rapid discovery of new compounds with significant pharmacological activities [8]. Indian propolis is believed to contain numerous bioactive compounds that vary from each territory and may prove to be a potential source of novel compounds that can be further evaluated for their therapeutic applications [1]. However, at present, there is very little information available on the total chemical composition of Indian propolis throughout the country, and the relation between its chemical composition and biological activities [9]. Thus, the present study aims to investigate and compare the phytochemical composition and pharmacological properties like antioxidant, antimicrobial and antidiabetic activities of propolis samples obtained from different geographical regions of India.

Materials and methods
Collection of Propolis Samples
Raw propolis samples were randomly collected from five different geographical regions of India namely Rohtas from Bihar (25.0686° N, 84.0167° E), Latur from Maharashtra (18.4088° N, 76.5604° E), Sawai Madhopur from Rajasthan (26.0378° N, 76.3522° E), Rewari from Haryana (28.1928°N, 76.6239° E) and Kota from Rajasthan (25.2138° N, 75.8648° E). The propolis samples were collected by scraping the frames of beehives from apiaries housing *Apis mellifera* colonies. The samples were stored in a cool place for further investigations.

Preparation of ethanolic extract of propolis (EEP)
The crude propolis extracts were prepared by extracting 20g of propolis with 100ml of 70% ethanol and placed on a magnetic stirrer for two days with intermittent shaking at room temperature. The extract was filtered by Whatman filter paper and the obtained extract was designated as Ethanolic Extract of Propolis (EEP). This extract was further evaporated to dryness under reduced pressure at 50°C by a rotary evaporator. The obtained dried extract was stored in air-tight vials in the refrigerator at − 20°C until further analysis.

Preliminary phytochemical screening

Preliminary phytochemical screening was performed to identify the presence of various chemical constituents in the propolis samples. Freshly prepared propolis extracts were screened by the procedure described [10].

Determination of antioxidant activity of propolis

DPPH radical scavenging activity

1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) scavenging activity was measured by the method described [11]. To various concentrations of the propolis extracts, 3ml of the 0.2mM DPPH solution was added. After 15 minutes of reaction in dark conditions, the absorbance of the solution was measured at 517nm. The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of a blank solution. The antioxidant capacity based was calculated from the linear calibration curve. DPPH radical scavenging activity was expressed as IC_{50} (µg/ml). The percentage of inhibition of free radical scavenging activity of each sample was calculated as:

% inhibition = [Abs (blank) – Abs (sample)/Abs (blank)] x 100

ABTS radical scavenging activity

Total antioxidant activity was measured using 2, 2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical scavenging activity method [12]. Sample stock solutions (10mg/ml) were diluted to various concentrations in methanol. Then, 3mL of ABTS+ solution was added to the different aliquots of methanolic extract of propolis samples. After 30 minutes of incubation in dark, the absorbance was measured at 734nm using a UV-Vis spectrophotometer. The antioxidant capacity was calculated from the linear calibration curve. ABTS+ free radical scavenging activity was expressed as IC_{50} (µg/ml). The inhibition percentage of free radical scavenging activity of each sample was calculated using the following formula:

% inhibition = [Abs (blank) – Abs (sample)/Abs (blank)] x 100

Nitric oxide radical scavenging activity

Nitric oxide (NO) radical scavenging potential was evaluated by the procedure described [13]. Various concentrations of the propolis extracts were mixed with 0.5ml of 10mM Sodium Nitroprusside in phosphate buffer saline (0.1M, pH 7.0). The samples were then incubated at 25°C for 3 hours. The incubated mixture was mixed with equal volumes of freshly prepared Griess reagent (Mix equal amounts of 1% Sulphanilamide in 2.5% phosphoric acid and 0.1% Naphthylethylenediamine dihydrochloride in 2.5% Phosphoric acid). The absorbance of these solutions was measured at 546nm against the reagent blank solution. The antioxidant capacity was calculated from the linear calibration curve.

The percentage and IC_{50} values of nitric oxide free radicals scavenging activity of each sample were calculated as:

% inhibition = [Abs (blank) – Abs (sample)/Abs (blank)] x 100

Ferric reducing antioxidant power (FRAP) assay

Reducing power was determined by the method described [14]. Various concentrations of the propolis extracts were mixed with a phosphate buffer (2.5ml, 0.2M, pH 6.6) and Potassium Ferricyanide (2.5ml, 1%), and the mixture was incubated at 50°C for 20 minutes. Thereafter, 2.5ml of Trichloroacetic acid (10%) was added to the reaction mixture, which was then centrifuged at 3000 RPM for 10 minutes. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and ferric chloride (0.5ml, 0.1%), and mixed thoroughly well. An intense blue color complex was formed when the Ferric tripyridyltriazine (Fe3+ TPTZ) complex was reduced to ferrous (Fe^{2+}) form and the absorbance at 700nm was recorded against the reagent blank. A graph was plotted between concentration and absorbance at 700nm and the results were compared from the graph. The percentage and IC_{50} values of the ferric ions of samples were calculated from the linear calibration curve.

Antimicrobial activity of propolis extracts

Determination of antibacterial activity against various bacteria

Resazurin microtiter assay (REMA), was used to study the antibacterial activity of the propolis extracts as described [15]. Antibacterial activity was tested against the propolis extracts using ten strains of bacteria (Gram-negative strains: *Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Serratia marcescens, Klebsiella pneumoniae* and Gram-positive strains: *Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis, Streptococcus mutans, and Streptococcus oralis*). The potency of the propolis extracts against microorganisms was assessed by the minimal inhibitory concentration (MIC) values. Luria Bertani (LB) broth, a nutrient-rich growth media was used for bacterial growth.

Resazurin solution (10 %) was prepared in sterile water, stored at 20°C as a stock solution, and diluted to 1:10 with sterile water when required. The propolis samples were prepared in serially diluted concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.25, 7.81µg/ml). In each well of the microtiter plate, 100µl of sterilized Luria Bertani broth, 30µl of 0.1% resazurin solution, 100µl of serially diluted propolis extracts were added. Subsequently, 100µl of each bacterial culture was inoculated and 200µl of deionized water was added to prevent the sample from drying. The control wells were prepared with LB broth, resazurin dye, and deionized water. Blank wells were prepared only with broth and dye. The plates were sealed and incubated for 24hrs at 37 °C. After incubation, the colour change from blue to pink was observed which indicated the growth of bacteria. When there was no growth of organisms, the wells remained blue. Whereas, when there was the positive growth of organisms, the colour changed to Pink and Orange. The Minimum inhibitory concentration (MIC) was estimated by recording the lowest concentration of the propolis extract that prevented this change in colour indicating the inhibition of the bacterial growth. Ampicillin and Ethanol are used as positive and negative controls, respectively.

Antidiabetic activity of propolis

Alpha- amylase inhibitory assay

In vitro amylase inhibition of propolis extracts was carried out according to the standard method [16]. The enzyme solution was prepared by dissolving alpha-amylase in 20mM phosphate buffer (pH 6.9) at a concentration of 0.5mg/ml. The colorimetric reagent was prepared by mixing

equal volumes of sodium potassium tartarate tetrahydrate solution and 96mM 3, 5-Dinitrosalicylic acid (DNS) solution. 1ml of starch (0.5%) solution was mixed with various concentrations of the propolis extract (0.1-0.5mg/ml) and to this 1ml of the alpha-amylase solution was added and incubated at 25°C for 3 minutes to react with the starch solution. A 1ml of 96mM DNS reagent was added to the above solution and contents were heated for 15 minutes on a boiling water bath. The final volume was made up with distilled water and the absorbance was measured at 540nm using a spectrophotometer. Acarbose was used as the reference standard. The percentage inhibition and 50% inhibitory concentration (IC_{50}) of the alpha-amylase enzyme were calculated.

Alpha-glucosidase inhibitory assay

Alpha-glucosidase inhibitory activity of propolis extracts was carried out according to the protocol [17]. The lyophilized alpha-glucosidase enzyme was dissolved with distilled water and diluted to 0.006 to 0.022U/mL with 10mM Potassium phosphate buffer containing 1mg/ml BSA (pH 7.5). In a reaction mixture containing 50µl phosphate buffer (100mM, pH=6.8), 10µl alpha-glucosidase (1U/ml) and varying concentrations of propolis extract was pre-incubated at 37°C for 15 minutes. Then, 20µl P-NPG (5mM) was added as a substrate and incubated further at 37°C for 20 minutes. The reaction was stopped by adding 50µl Na_2CO_3 (0.1M). The absorbance of the released p-nitrophenol was measured at 405nm using a UV spectrophotometer. Acarbose at various concentrations (0.1-0.5mg/ml) was included as a standard. The percentage inhibition and 50% inhibitory concentration (IC_{50}) of the alpha-amylase enzyme were calculated.

Statistical analysis

All the analysis was performed in triplicates and the data obtained from *in vitro* assays were expressed as mean ± SEM (Standard error of the mean). Statistical comparison was performed by SPSS version 17 software (SPSS Inc., Chicago, USA) to evaluate significant differences between groups with one-way ANOVA. The values were considered statistically significant when p values were less than 0.05 ($p< 0.05$).

Results

In the present study, the pharmacological activities like antioxidant, antibacterial and antidiabetic activities of propolis samples collected from five different geographical regions of India namely Rohtas from Bihar, Latur from Maharashtra, Sawai Madhopur from Rajasthan, Rewari from Haryana, and Kota from Rajasthan were investigated.

Preliminary phytochemical screening

The preliminary screening of the crude extracts of propolis revealed significant indication about the presence of secondary metabolites like alkaloids, carbohydrates, terpenoids, phenols, flavonoids, tannins, phlobatannins, cardiac glycosides, steroids, and saponins as shown in Table1. The results are expressed as +for the presence and– for the absence of phytochemicals.

Table 1: Result of phytochemical screening of ethanolic extract of propolis (EEP)

Sl. No.	Chemical constituents	Location of propolis collection				
		Rohtas, Bihar	Latur, Maharashtra	Sawai Madhopur, Rajasthan	Rewari, Haryana	Kota, Rajasthan
1.	Alkaloids	+	+	+	+	+
2.	Carbohydrates	+	+	+	+	+
3.	Terpenoids	+	+	+	+	+
4.	Phenols	+	+	+	+	+
5.	Flavonoids	+	+	+	+	+
6.	Tannins	+	+	+	+	+
7.	Saponins	-	+	+	+	+
8.	Amino acids	+	+	+	+	+
9.	Phlobatannins	+	+	+	+	-
10.	Steroids	+	+	+	+	+
11.	Cardiac glycosides	+	+	+	+	+

Antioxidant activity of propolis extracts

The propolis extracts collected from the different geographical regions of India were evaluated for their antioxidant activities by four different methods DPPH, FRAP, ABTS, and NO radical scavenging assays. The half inhibitory concentrations (IC_{50} values) of various propolis extracts determined by the radical scavenging assays were tabulated (Table 2& Figure 1). All the propolis extracts showed excellent free radical scavenging activities.

The DPPH radical scavenging activities of the propolis extracts ranged from 31.15µg/ml to 225.66µg/ml. Among all the propolis extracts, propolis sample from Rewari, Haryana showed the highest antioxidant activity with an IC_{50} value of 31.15µg/ml. Propolis sample from Sawai Madhopur, Rajasthan showed moderate antioxidant activity with an IC_{50} value of 130.90µg/ml. Propolis sample from Rohtas, Bihar exhibited appreciable free radical scavenging activities with an IC_{50} value of 179.392µg/ml. Propolis samples obtained from Latur, Maharashtra, and Kota, Rajasthan showed comparatively lesser antioxidant activities with almost similar IC_{50} values of 223.12µg/ml and 225.66µg/ml respectively.

ABTS assay of the propolis extracts showed potent radical scavenging activities with IC_{50} values ranging from 12.99µg/ml to 147.85µg/ml. The highest antioxidant activity was found in the propolis sample from Kota, Rajasthan with an IC_{50} value of 12.99µg/ml. Propolis sample from Sawai Madhopur, Rajasthan, and the propolis sample from Rewari, Haryana also exhibited very good antioxidant activities with IC_{50} values of 45.13µg/ml and 67.77µg/ml respectively. The propolis sample from Rohtas, Bihar showed moderate antioxidant activity with an IC_{50} value of 130.15µg/ml. The least antioxidant activity was reported in the propolis

sample from Latur, Maharashtra with an IC_{50} value of 147.85µg/ml.

The Ferric reducing antioxidant power (FRAP) of the propolis extracts showed extremely high radical scavenging activities. The IC_{50} values varied among the different samples, it ranged from 41.67µg/ml to 166.51µg/ml. Among all the propolis samples, the propolis sample from Latur, Maharashtra exhibited the maximum capacity to reduce ferric ions of the sample with an IC_{50} value of 41.67µg/ml. Potent antioxidant activity was also exhibited by propolis samples from Sawai Madhopur, Rajasthan, and Rohtas, Bihar with IC_{50} values of 62.28µg/ml and 83.09µg/ml respectively. On the other hand, propolis sample from Rewari, Haryana also exhibited moderately high reducing power with an IC_{50} value of 99.76µg/ml. The propolis sample from Himachal Pradesh exhibited the least reducing power compared to other samples with an IC_{50} value of 166.51µg/ml.

The NO radical scavenging activities of the propolis extracts varied between 193.59mg/ml to 2069.66mg/ml. Among all the propolis samples, the sample from Rohtas, Bihar with an IC_{50} value of 193.59mg/ml was found to be the most potent in inhibiting the nitrite formation than all the other samples. Propolis sample obtained from Rewari, Haryana exhibited low radical scavenging activity with an IC_{50} value of 552.59mg/ml. Significantly lower radical scavenging activity was exhibited by the propolis sample from Sawai Madhopur, Rajasthan with an IC_{50} value of 805.42mg/ml. Propolis samples obtained from Latur, Maharashtra, and Kota, Rajasthan exhibited the least radical scavenging activities with IC_{50} values of 1810.26mg/ml and 2069.66mg/ml respectively.

Table 2: Antioxidant activity of various extracts of Indian propolis

Sl. No.	Location of propolis collection	Free radical scavenging assay (IC_{50} values in µg/ml)			
		DPPH Assay	ABTS Assay	FRAP Assay	NO Assay
1.	Rohtas, Bihar	179.39	130.15	83.09	193.59
2.	Latur, Maharashtra	223.12	147.85	41.67	1810.26
3.	Sawai Madhopur, Rajasthan	130.90	45.13	62.28	805.42
4.	Rewari, Haryana	31.15	67.77	99.76	552.59
5.	Kota, Rajasthan	225.66	12.99	166.51	2069.66

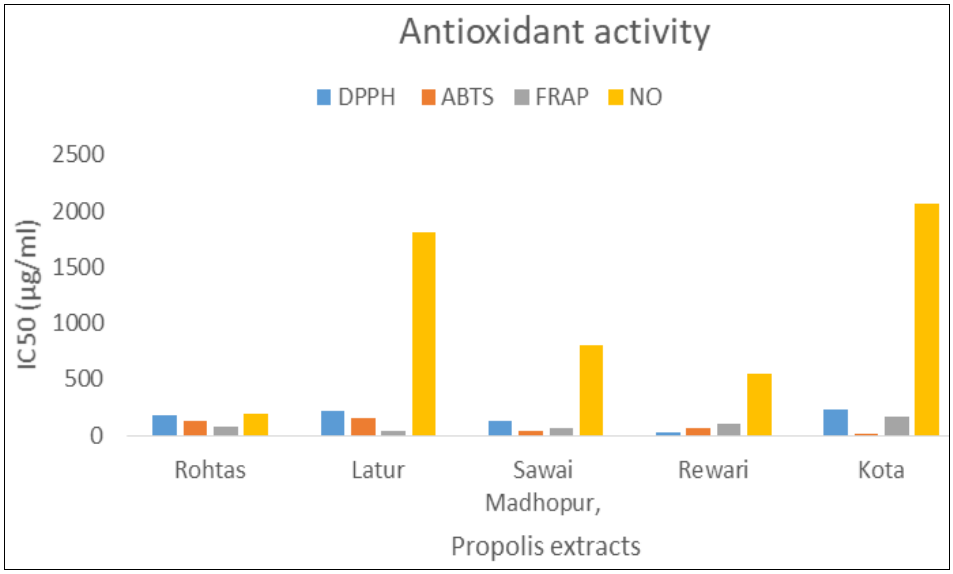

Fig 1: Comparison of antioxidant activity of propolis with DPPH, ABTS, FRAP, and NO assay

Antibacterial activity against various bacteria

Resazurin microtitre assay (REMA) was employed to reveal the antibacterial activity of the propolis samples collected from various geographical regions of India against five Gram-positive bacteria (*Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis, Streptococcus mutans, and Streptococcus oralis*), five Gram-negative bacteria (*Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Serratia marcescens, Klebsiella pneumoniae*). MIC values for different propolis extracts obtained for the various bacteria representing antibacterial activity are shown in Table 3& Figure 2. For the antibacterial activity of propolis samples collected from various geographical regions of India, it was found that the propolis samples inhibited the growth of Gram-negative bacteria. The sensitivity of Gram-negative bacteria to propolis extracts varied among the bacterial strains tested and the propolis samples used. However, the propolis extracts failed to inhibit the Gram-positive bacteria. Gram-positive bacteria required a higher concentration of the extract than that of Gram-negative bacteria.

The results showed that all the propolis samples failed to exhibit inhibitory effects against the two Gram-negative bacteria *Pseudomonas aeruginosa* and *Salmonella typhi*. On the other hand, *Escherichia coli, Klebsiella pneumoniae,* and *Serratia marcescens* were found to be susceptible against all the propolis extracts at various concentrations. The observed minimum

Inhibitory concentrations (MIC) against the Gram-negative Bacteria were found to be ranging from 7.81µg/ml to 62.5µg/ml.

All the propolis samples showed susceptibility towards *Escherichia coli.* The least antibacterial activity was exhibited by the propolis sample from Kota, Rajasthan with

a MIC at 62.5µg/ml against *Escherichia coli* while the propolis obtained from Sawai Madhopur, Rajasthan also showed significantly lower antibacterial activity towards *Escherichia coli* with a MIC at 31.25µg/ml. Propolis samples collected from Rohtas, Bihar; Latur, Maharashtra and Rewari, Haryana showed potent antibacterial activities against *Escherichia coli* with a MIC value of 7.81µg/ml. All the five propolis samples showed promising antibacterial activity towards *Serratia marcescens* with a MIC at 7.81µg/ml. Propolis samples from Rohtas, Bihar, and Rewari, Haryana showed significant antibacterial activity against *Klebsiella pneumoniae* with a MIC at 7.81µg/ml whereas propolis samples from Latur, Maharashtra; Sawai Madhopur, Rajasthan and Kota, Rajasthan failed to inhibit *Klebsiella pneumoniae.*

After the overall observation of the MIC results, it was concluded that the ethanol extracts of Indian propolis showed a broad range of antibacterial activity and could be a potential source of antimicrobial compounds.

Table 3: Minimum Inhibition Concentration against gram-negative bacteria

Sl. No	Location of propolis collection	Minimum Inhibition Concentration (MIC) in µg				
		Escherichia Coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Serratia marcescens*
1.	Rohtas, Bihar	7.81	7.81	ND	ND	7.81
2.	Latur, Maharashtra	7.81	ND	ND	ND	7.81
3.	Sawai Madhopur, Rajasthan	31.25	ND	ND	ND	7.81
4.	Rewari, Haryana	7.81	7.81	ND	ND	7.81
5.	Kota, Rajasthan	62.5	ND	ND	ND	7.81

Where ND – Not determined

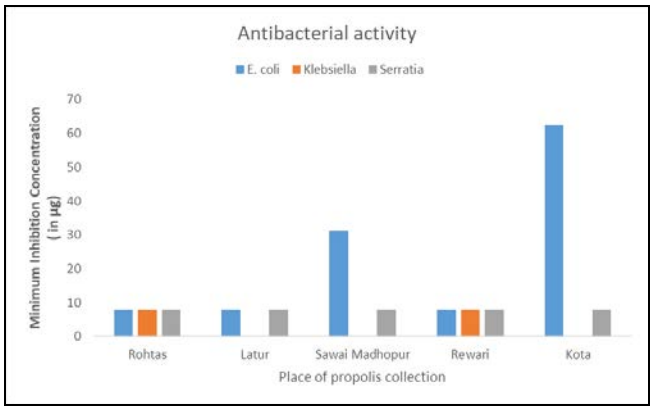

Fig 2: Comparison of antibacterial activity of different propolis extracts

Antidiabetic activity of propolis extracts

To assess the anti-diabetic potency of propolis extracts, the extracts were tested for their alpha-amylase inhibitory assay and alpha-glucosidase inhibitory assay**.** The results of *in vitro* anti-diabetic activity of propolis extracts are given in Table 4& Figure 3.

Alpha -amylase inhibitory activity

The propolis extracts revealed a considerably significant alpha-amylase inhibitory activity. The alpha-amylase inhibitory activities resulting from propolis extracts ranged from IC_{50}values of 9.53µg/ml to 177.57µg/ml. It was found that out of all the propolis extracts, the propolis sample from Latur, Maharashtra exhibited the highest alpha-amylase inhibitory activity with an IC_{50} value of 9.53µg/ml. Significantly higher alpha-amylase inhibitory activity was also exhibited by the propolis sample from Rohtas, Bihar with an IC_{50} value of 12.36µg/ml. Considerable higher alpha amylase inhibitory activity was shown by the propolis sample from Sawai Madhopur, Rajasthan with an IC_{50} value of 38.56µg/ml. The propolis sample obtained from Rewari, Haryana exhibited low inhibitory activity on alpha-amylase with an IC_{50} value of 114.28µg/ml. The least inhibitory alpha amylase inhibitory activity was shown by the propolis sample from Kota, Rajasthan with an IC_{50} value of 177.57µg/ml.

Alpha-glucosidase inhibitory assay

The studied propolis extracts showed significant antidiabetic activity by alpha-glucosidase inhibitory assay in a concentration-dependent manner. The alpha glucosidase inhibitory activities resulting from propolis extracts ranged from IC_{50} values of 22.68µg/ml to 98.12µg/ml. Among all the extracts, propolis from Rewari, Haryana showed the highest alpha glucosidase inhibitory activity with an IC_{50} value of 22.68µg/ml. Propolis samples from Sawai Madhopur, Rajasthan and Rohtas, Bihar inhibited the alpha-glucosidase activity potentially with IC_{50} values of 55.2µg/ml and 77.14µg/mlrespectively. The least alpha glucosidase inhibitory activity was shown by propolis samples from Kota, Rajasthan and Latur, Maharashtra with IC_{50} values of 90.71µg/ml and 98.12µg/ml respectively.

Table 4: Anti-diabetic assays of various extracts of Indian propolis

Sl. No.	Location of propolis collection	Enzyme inhibitory activity (IC_{50} values in µg/ml)	
		Alpha-amylase activity	Alpha-glucosidase activity
1	Rohtas, Bihar	12.36	77.14
2	Latur, Maharashtra	9.53	98.12
3	Sawai Madhopur, Rajasthan	38.56	55.2
4	Rewari, Haryana	114.28	22.68
5	Kota, Rajasthan	177.57	90.71

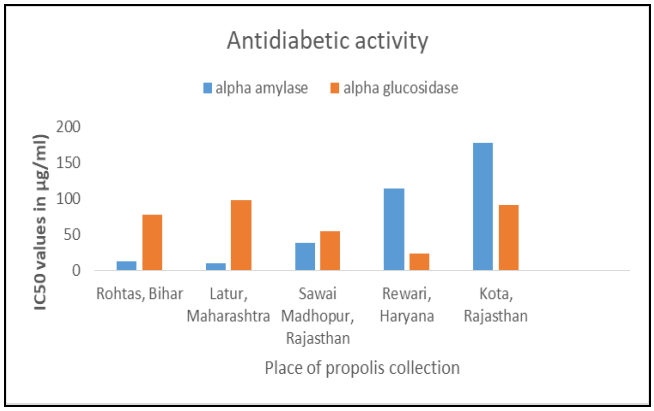

Fig 3: Comparison of antidiabetic activity of propolis with alpha-amylase and alpha-glucosidase assay

Discussion

Propolis has numerous pharmacological activities and its mechanisms of action have been widely investigated *in vitro* and *in vivo* in the last years. Researchers have focused on the study of its constituents and therapeutic properties [1]. In the present study, the phytochemical and pharmacological investigations were carried out with the ethanol extracts of propolis samples obtained from different regions of India. The findings of the present study, are in agreement with the previous research studies that have demonstrated significant pharmacological activities of propolis samples obtained from various other geographical regions of India.

Phytochemical studies of the propolis extracts revealed that Indian propolis is a good source of important secondary metabolites like alkaloids, carbohydrates, terpenoids, phenols, flavonoids, tannins, phlobatannins, cardiac glycosides, steroids, and saponins. Similar results about the phytochemical screening of Indian propolis were also reported in the previous work [18] where the presence of all the given bioactive constituents in the propolis samples except steroids were reported.

Strong radical scavenging activities of the propolis extracts were produced in DPPH, ABTS, FRAP and NO assays in the present study and the values were comparable with the previous studies [9]. The antioxidant activity of Indian *mellifer*a propolis (IMP) samples collected from 13 different states was evaluated by DPPH, ABTS, and FRAP assays. The samples showed strong and considerable antioxidant potential in different assays. The findings of the present studies are also in agreement with the earlier work such as [19] in which the radical scavenging potential of ethanol extracts of propolis collected from ten different Indian states were assessed like Haryana, Himachal Pradesh, Uttaranchal, Punjab, Delhi, Maharashtra, Tamil Nadu, Karnataka, Kerala, and Andhra Pradesh by DPPH, ABTS, Nitric oxide, and Hydrogen peroxide scavenging assays. The highest antioxidant activity was found in Tamil Nadu, Kerala, Karnataka, and Haryana propolis samples. Similar results were also reported by studies like [20]. Where the antioxidant activity of ethanolic, aqueous, and hydro-alcoholic extract of propolis sample obtained from Pune, India was measured. Amongst the various extracts of propolis, ethanolic extract showed more potent activity followed by hydro-alcoholic extract and then aqueous extract.

The findings in the present study are contradictory to the earlier studies like [21] in which propolis was found to be more active against Gram-positive bacteria (*Staphylococcus aureus*) than Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and yeasts. However, the results in the current research demonstrated that propolis samples inhibited the growth of Gram-negative bacteria. The sensitivity of Gram-negative bacteria to propolis extracts varied among the bacterial strains tested and the propolis samples used. However, the propolis extracts failed to inhibit the Gram-positive bacteria. Gram-positive bacteria required a higher concentration of the extract than that of Gram-negative bacteria.The results of the present study also indicated a potent anti-diabetic activity of Indian propolis. Current findings are compatible with the previous studies like [22]where the anti-diabetic potential of Indian propolis was investigated by the inhibition of alpha-amylase enzyme and non-enzymatic glycosylation of haemoglobin assay. The propolis samples were collected from four Southern states of India like Tamil Nadu, Karnataka, Kerala and Andhra Pradesh. It was found that all the propolis samples possessed significant anti-diabetic activity.

Conclusions

Propolis due to its various antioxidant and poly-phenolic compounds has a role in control and treating some of the chronic diseases. Our current work unveiled the pharmacological efficacy of propolis extracts collected from different geographical regions of India. The phytochemical analysis of propolis extracts showed the presence of a significant amount of various bioactive components in Indian propolis like alkaloids, flavonoids, glycosides, saponins, and tannins which may be responsible for the observed biological activities.

The results suggested that Indian propolis is a rich source of natural antioxidants and may prove to be beneficial in the prevention of various free radicals-related diseases. Also, Indian propolis has promising antibacterial activity which may vary according to the geographical location and bacterial species. It was found that the propolis extracts produced significant antibacterial effects at different doses. The findings also indicated that Indian propolis offers a promising therapeutic value in prevention of diabetes. Further advanced investigations would be required to understand the underlying molecular cellular mechanism as well as to isolate bioactive compounds responsible for each pharmacological activity.

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