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PHYTOCHEMICAL PROFILING AND ANTIBACTERIAL ACTIVITY OF PROPOLIS

Zoology

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ABSTRACT

Recently, drug resistance due to the extensive abuse and over-use of antibiotics has become an increasingly serious problem, making the development of alternative antibiotics a very urgent issue. In this study, crude propolis collected from honey comb was extracted with ethanol, chloroform and acetone based on solvent polarity. Preliminary qualitative phytochemical profiling was done, which revealed that ethanol extract showed the presence of acids, alkaloids, carbohydrates, coumarins, flavonoids, furanoids, phenols, quinones, saponins, steroids, tannins, terpenoids and triterpenoids, chloroform extract showed the presence of acids, coumarins, flavonoids, furanoids, phenols and saponins and acetone extract showed the presence of acids, coumarins, flavonoids, furanoids and phenols. The antibacterial activities of the extracts were determined by disc diffusion method. The results showed that the ethanol extracts possesses a broader antibacterial spectrum and greater antimicrobial activity against all of the tested clinical isolated bacteria, with varying concentrations tan that of the other extracts. The ethanol extract showed complete inhibition of pathogen growth due to the presence of phytoconstituents in the ethanol extract of propolis.

KEYWORDS

Propolis, phytochemical profiling, antibacterial activity.

INTRODUCTION

Propolis (bee glue) is the generic name for the resinous substance collected by honey bees (*Apis mellifera* L.) from various plant sources (substances exuded from wounds in plants: lipophilic materials on leaves and leaf buds, gums, resins, latices, *etc*.); it is used to seal holes in the honeycombs and smooth out the internal walls. Propolis is also used to protect the entrance against intruders; moreover, it contains antimicrobial agents active against a variety of pathogens [1]. In the temperate zone all over the world, the main source of bee glue is the resinous exudates of the buds of poplar trees, mainly the black poplar *Populus nigra*. European propolis contains phenolics: flavonoid aglycones (flavones and flavanones), phenolic acids, and their esters [2, 3]. Propolis from tropical regions has a different chemical composition; for example, Brazilian bee glue is harvested from the leaf resin of *Baccharis dracunculifolia* and it is composed of prenylated derivatives of *p*-coumaric acid and of acetophenone. Diterpenes, lignans, and flavonoids (different from those in "poplar type" propolis) have also been found [4]. The Cuban propolis from the floral resin of *Clusia rosea* is composed mainly of polyisoprenylated benzophenones [5].

The chemical composition of propolis is qualitatively and quantitatively variable, depending on the geographic and regional plant ecology. Propolis is widely used in traditional medicine and is reported to have a broad spectrum of pharmacological effects: antibacterial, antihepatotoxic, antioxidative, anti-inflammatory, and so forth. It has been demonstrated that flavonones, flavones, phenolic acids, and their esters of European propolis have antimicrobial, antiinflammatory, and antioxidant activity, whereas the antibacterial, antioxidant, and antitumoral activity of the Brazilian propolis are ascribed to prenylated p-coumaric acids, labdane diterpenes, and flavonoids. The antitumor activity of the European propolis is indeed attributed to caffeic acid phenethyl ester [5].

Propolis is a honeybee product, which is used to cover hive walls and fill gaps. Bees collect the resin-like product from cracks in the bark of trees and leaf buds [6, 7]. Thereby, propolis does not only act as a structural compound, but is mainly responsible as a chemical agent for the safety of honeycombs, especially against microorganisms [8-10]. The chemical composition of propolis is highly variable and depends on the local flora at the site of collection and on the season of collection [11-13]. It is a lipophilic, resinous material collected from living plants, which is mixed with the enzyme, beta-glycosidase, present in the bees' saliva, partially digested and added to beeswax to form the final product [14]. Despite potential intrinsic differences, which may depend on the origin, it has been proven that most propolis variants have a wide range of biological therapeutic effects, such as antimicrobial, antifungal and antiviral activity [15-17]. Therefore, sufficient evidences are available in the literature to justify propolis as a good candidate adjunct to prevent or treat infectious diseases [18].

Several laboratory studies exist, which assessed the antimicrobial potential of propolis using different *in vitro* test systems in order to evaluate the potential to inhibit the bacterial growth (bacteriostasis) or to induce bacterial death (bactericidal effect). To measure this potential, several evaluation methods are available, *e.g.,* simple agar diffusion tests or dissolution assays. The latter determines the minimum inhibitory (MIC), the minimum bactericidal concentrations (MBC) or agar diffusion tests, which, in principle, visually or optically assess the inhibition of bacterial growth. These tests are therefore commonly used to determine the potential of the agents and to classify them in relation to alternative chemical substances (controls). Antimicrobials are usually regarded as bactericidal, if the MBC is not more than four times the MIC [19]. Based on microbiological data derived from such assays, it has been suggested that propolis may even be of clinical value [20]. However, a systematic evaluation on the antimicrobial effects of propolis on bacterial species relevant to oral diseases and dentistry in general is still lacking. Keeping all this in mind we have decided to assess the phyto-constituents and antibacterial potential of the crude propolis extracts.

MATERIALS AND METHODS

Collection and Extraction of Propolis

Bee hives were collected from in and around areas of Udankudi village, Thoothukudi Dt., Tamil Nadu, India, and the collected hives were washed with running tap water and distilled to remove the debris and excess honey present in it. The washed combs were shade-dried in room temperature for ten days and then it was powdered using kitchen blender to powder form. The powdered honey comb (500 g) was put

into 1000 ml of respective solvents (acetone, chloroform and ethanol), covered and kept standing in the orbital shaker for three days and then the solvent was removed after squeezing the honey comb and filtered through Whatman filter paper No 1. The solvent was evaporated at low pressure by using a Buchi Rotavapor R-200 at 45° C. Pure semisolid propolis extracts were stored in a refrigerator until further use.

Qualitative Phytochemical Analysis

Qualitative phytochemical chemical tests were carried out for acetone, chloroform and ethanol extracts of propolis to identify different phytoconstituents present in them [21].

Test Organisms

Clinical isolates of bacteria *viz*., *Pseudomonas flurorescens, Pseudomonas citrea, Pseudomonas aeruginosa, Streptococcus aureus, Escherichia coli, Vibrio cholera, Vibrio parahaemlyticus, Vibrio harveyi, Vibrio fischeri* and *Bacillus substillus* were used for the present study.

Antibacterial Activity of Crude Propolis

Antibacterial activity against clinical isolates of bacteria was done according to the method of Chakraborthy (2008) [22].

RESULTS AND DISCUSSION

Qualitative Phytochemical Analysis

Qualitative phytochemical analysis of various solvent extracts such as ethanol, chloroform and acetone extract of propolis revealed that presence of various phytochemicals. It was found that ethanol extract showed the presence of acids, alkaloids, carbohydrates, coumarins, flavonoids, furanoids, phenols, quinones, saponins, steroids, tannins, terpenoids and triterpenoids, chloroform extract showed the presence of acids, coumarins, flavonoids, furanoids, phenols and saponins and acetone extract showed the presence of acids, coumarins, flavonoids, furanoids and phenols (Table 1). In this study, phytochemical screening revealed the presence of bioactive compounds like alkaloid in ethanol extract, which is consistent with the finding of Sharma *et al*. (2012) [23]. Oda *et al.* (2000) [24], stated that phytochemicals are chemical compounds formed during the plants normal metabolic processes; these chemicals are often referred to as secondary metabolites of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids. In this work, flavonoid was detected in the ethanolic extract of propolis, which is in agreement with the earlier investigation of Daniel *et al*. (2012) [25]. Methanol and ethanol have been proved as effective solvents to extract phenolic compounds as stated by Siddhuraju *et al*. (2002) [26]. According to Kumoro *et al*. (2009), [27] petroleum ether, n-hexane, ethyl acetate, acetone and chloroform might be the best solvents to remove unwanted components. Compounds other than phenolics present in the extracts might be due to higher solubility of proteins and carbohydrates in water and methanol than in ethanol and acetone. Our results are similar to those reported by Ghasemzadeh *et al*. (2011) [28], where the ethanol solvent was most effective in extracting phenolic compounds from propolis. Methanol and ethanol have been proven as effective solvents to extract phenolic compounds from different plants [29, 30]. Our findings are in agreement with a previous investigation by Sultana *et al*. (2009) [30] who reported that the higher extract yields were found in the 80% aqueous methanol of selected medicinal plants, such as *Moringa oleifera*, *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, *Eugenia jambolana* and *Aloe barbadenis*. Ethanol extraction product mainly contains high hydrophilic compounds such as polar neutral, basic, acidic compounds, amino acids, nucleotides, sugars and polysaccharides as opined by CDPH Public Health [31].

Solvents, such as methanol, ethanol, acetone, ethyl acetate, and their combinations have been used for the extraction of phenolics from plant materials, often with different proportions of water. Selecting the right solvent affects the amount and rate of polyphenols extracted [11]. In particular, methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols while the higher molecular weight flavonols are better extracted with aqueous acetone [12-15]. Ethanol is another good solvent for polyphenol extraction and is safe for human consumption [16]. In preparing anthocyanin-rich phenolic extracts from plant materials, an acidified organic solvent, most commonly methanol or ethanol is used. Dichloromethane was used as substitute for chloroform in DNA extraction procedures that usually requires phenol/chloroform mixtures for protein extraction. Dichloromethane is widely used as an extraction solvent in organic chemistry [32]. Acetone dissolves many hydrophilic and lipophilic components from propolis used, is miscible with water, is volatile and has a low toxicity to the bioassay used, it is a very useful extractant, especially for antimicrobial studies where more polyphenolic compounds are required to be extracted.

Saponins are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaked in methanol extract of propolis. According to Hu *et al*. (2002), [33] most saponins function as antioxidants, because they possess a special moiety (2,3-dihydri-2,5-dihyroxy6-methyl-4-pyran-4-one), which act by forming hydroperoxide intermediates thus removing free radicals. Saponins are known to possess hemolytic action on human erythrocytes [34]. Saponins with acyl residues or oxide-ring moiety tend to show haemolytic activity [24]. Antioxidant properties, reactive oxygen species scavenging, and cell function modulation of flavonoids could account for the large part of their pharmacological activity [35]. Phenolic alkaloids such as caffeic acid phenyl esters have been reported to possess beneficial effects such as anti-tumor property against human breast cancer and also in the treatment of acute inflammation as stated by Grunberger *et al*. (1988) [36] and Orban *et al*. (2000) [37].

Antibacterial Activity of Crude Propolis

As expected, ethanol extract of propolis showed relatively high antibacterial effect than chloroform and acetone extract of propolis as shown in the Table 2 and Fig. 1, 2 and 3. However, our finding suggests that the antibacterial action of the propolis as an adjuvant to therapy and it might be considered a potent candidate for treatment of several clinical scenarios. However, further studies should be done to achieve a superior dose to kill the target microorganisms. Therefore, our study believes that propolis promotes healing. On the other hand, propolis can be used as a natural alternative to antibiotics. The results obtained exhibited the presence of acids, alkaloids, carbohydrates, coumarins, flavonoids, furanoids, phenols, quinones, saponins, steroids, tannins, terpenoids and triterpenoids in the ethanolic extract, acids, coumarins, flavonoids, furanoids, phenols and saponins in chloroform extract and acids, coumarins, flavonoids, furanoids and phenols in acetone extract. On the other hand probably the bioactive property of propolis probably is related to flavonoid followed by tannins and steroids. In this regard, reports of Markham *et al*. (1996) [38], Milena *et al*. (2013) [39], Silvia *et al*.(2013) [40] and Manimaran *et al*. (2017) [41] supported our findings concerning to antibacterial property of flavonoids, polyphenol, tannins and steroid.

The antibacterial activity of the various extracts varied with the solvents used. Like in the present study the best result was obtained with ethanol extract followed by acetone and chloroform extracts. The extraction of the antibacterial compounds depends on the polarity of the compounds. The variation in the antimicrobial activity of the various solvents is due to the nature of the polarity of the solvent. Ethanol is having higher polarity and thus it tends to dissolve different compounds from the propolis materials dipped in them. Karaman *et al*. (2003) [42] and Wei *et al*. (2008) [43] reported that ethanol and methanol are commonly used solvents for extraction of antibacterial compounds. Jayakumar *et al*. (2008) [44] stated that solvents such as ethanol, methanol, ethyl acetate and hexane could be used for extraction of antibacterial and antifungal compounds from *M. citrifolia*.

Most importantly, the antibacterial mechanism of the active components from propolis ethanolic extract might be due to action on the cell wall and cytoplasmic membrane. These results are in agreement with the findings of Hu *et al*. (2002) on other plant studies [33]. Similar observations were also reported by Manimaran *et al*. (2017) [41] on the antimicrobial effect of resveratrol compound against *Staphylococcus aureus*, *Klebsiella pneumonia, Escherichia coli, Proteus mirabilis, Micrococcus species, Bacillus cereus, Pseudomonas aerogenosa , Proteus vulgaris, Aspergillus niger, Aspergillus fumicatus , Aspergillus flaves, A.terreus* and *Rizhopus oryzae*.

Khan *et al*. (2009) [45] reported that the ethanol extract of *Achyranthus aspera* was much effective in Gram-negative bacteria than Gram-positive one, and these observations might be attributed to the nature of

biological active components, whose activity increases in the presence of ethanol. Barnabas and Nagarajan (1988) [46] reported that phytochemicals such as alkaloids, glycosides, steroids and proteins possess antibacterial activity. Hence the higher antimicrobial activity might be due to the presence of phytochemcials present in propolis and their ability to penetrate through the cell wall and cytoplasmic membranes of the microbes, thus causing growth inhibition in the microbes, which finds support from the above authors.

CONCLUSION

The present investigation had shown that propolis possesses remarkable antibacterial properties, which lend support to use of propolis in folklore as an antibacterial agent. Further studies are required to determine the active principles.

Table 1 : Qualatative phytochemical analysis of propolis ethanol, chloroform and acetone extracts

S. No.	Phytocompounds	Ethanol	Chloroform	Acetone
1	Acids	+	+	+
2	Alkaloids	+	-	-
3	Anthocyanins and Betacyanins	-	-	-
4	Carbohydrates	+	-	-
5	Cardiac Glycosides	-	-	-
6	Coumarins	+	+	+
7	Flavonoids	+	+	+
8	Furanoid	+	+	+
9	Glycosides	-	-	-
10	Phenols	+	+	+
11	Proteins	-	-	-
12	Quinones	+	-	-
13	Saponins	+	+	-
14	Starch	-	-	-
15	Steroids	+	-	-
16	Tannins	+	-	-
17	Terpenoids	+	-	-
18	Triterpenoids	+	-	-

+ denotes presence of phytochemicals ; - denotes absence of phytochemicals

Table 2: Antibacterial activities of propolis extracts

S. No.	Microorganisms	Concentration (mg/ml)	Zone of inhibition (mm)		
			Ethanol	Chloroform	Acetone
1	*Pseudomonas fluoresces*	5	5	4	5
		10	6	6	6
		15	7	6	6
2	*Pseudomonas chlororaphis*	5	6	5	5.7
		10	6	5	5
		15	7	5	6
3	*Pseudomonas aeruginosa*	5	7	5	5
		10	6.5	6	6.5
		15	6.7	6	6.8
4	*Staphylococcus aureus*	5	6	5.9	6.9
		10	4	6	6.5
		15	6	5	5.3
5	*Escherichia coli*	5	5	4	5.1
		10	4	4	5.7
		15	6	5	6.2
6	*Vibrio cholerae*	5	7	5	5.2
		10	6	5	6
		15	8	3	7
7	*Vibrio parahaemolyticus*	5	6	4	6
		10	7	6	5
		15	7.1	5	5
8	*Vibrio harveyi*	5	5.7	4	4
		10	6	5	7
		15	6.2	5.1	5.6
9	*Vibrio fischeri*	5	5	5	4.3
		10	5.1	4.6	6.6
		15	6	5.8	5.2
10	*Bacillus subtilis*	5	4	3.5	3.2
		10	4.3	3	6.2
		15	5	4.7	7

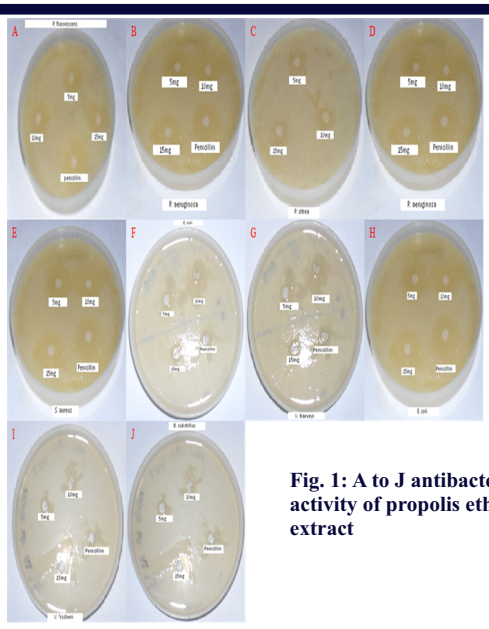

Fig. 1: A to J antibacterial activity of propolis ethanol extract

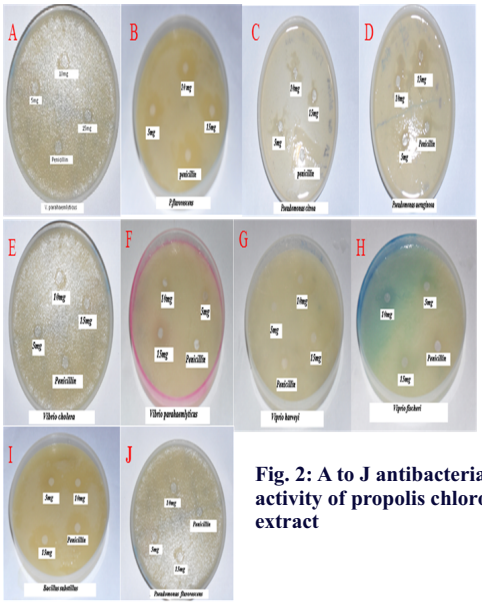

Fig. 2: A to J antibacterial activity of propolis chloroform extract

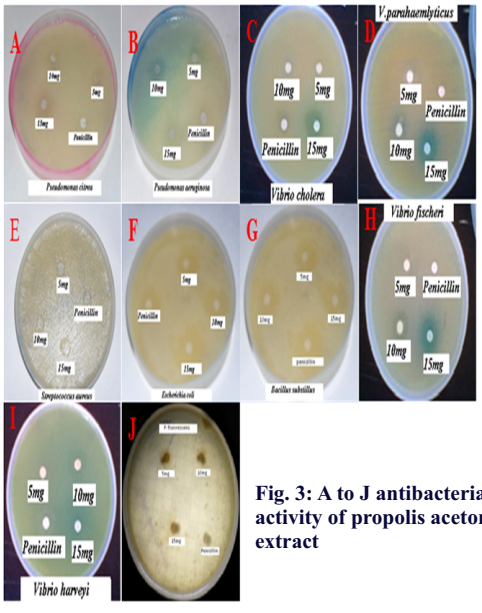

Fig. 3: A to J antibacterial activity of propolis acetone extract

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