Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

FORMULATION AND EVALUATION OF PROPOLIS EXTRACT AS A VAGINAL SUPPOSITORY

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ABSTRACT

The aim of this work is to formulate and evaluate ethanol extract of Propolis as a vaginal suppository. The suppository was formulated using the pouring method and cocoa butter as a base. The formulated suppositories were evaluated using physical appearance, crushing strength test, disintegration and dissolution time's tests, stability, as well as content uniformity. The visual examination showed brilliant and smooth surfaced, consistently bullet shaped, yellowish brown color and characteristic scented odor. The results of crushing strength (4.22 ± 0.92), disintegration time (7.25 ± 0.17) and content uniformity (99.16 ± 1.13) were within the normal limits. The result obtained from the dissolution time test showed that the suppositories dissolved within 35 min. The results indicated that ethanol extract of propolis can be formulated into a vaginal suppository.

KEYWORDS

Propolis, Extract, Suppository, Vaginal and Candidiasis.

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INTRODUCTION

Propolis is a sticky, gummy, resinous substance collected by honey bees (*Apis mellifera* and *Trigona sp*.) from tree exudates and secretions[1,2]. The word Propolis is derived from the Greek words "pro" meaning "in defense of" and "polis" meaning "city", referring to the defense of the city or the beehive[3]. It is a strongly adhesive substance collected and used by bees to seal the opening or holes of hives, to eliminate outside invaders[3] and to build aseptic

locals to prevent microbial infection of larvae[4,5]. Propolis has properties as bactericidal and fungicidal[6,7,] antioxidant[8,9], anti-inflammatory[10] and immunomodulatory[11,12], and it is used as an alternative treatment for infection. Other properties of Propolis, these are as a local anesthetic, reducing spasms, healing gastric ulcers, and strengthening capillaries. Propolis can be used by humans internally or externally[13]. Propolis has received greater attention due to its broad spectrum of biological and pharmacological properties[14,15], and it is an important product in alternative medicine in Japan nowadays[16]. Propolis is a complex of biologically active substances, more than 300 different compounds[17] including flavonoids, phenolics, aldehydes lipophilic, flavonoid aglycones and other compounds such as pollen, wax, vitamins, minerals and so on[18,19]. Flavonoids (flavonoles, flavones and flavanones) contained in the propolis may be responsible for the pharmacological and antioxidant activities including its antifungal properties[20]. Flavonoids were found to kill or inhibit many bacterial strains, inhibit viral enzymes, scavenge free radicals, etc.. [21,22]. Significant correlation was found between the flavonoid content in propolis and MIC[2,23]. Propolis inhibited the growth of both *C. albicans* and *C. glabrata* (MIC between 16 and 31 µg/ml)[25], Trichosporon spp. (MIC 0.1-0.4 µg/ml), and Rhodotorula spp. (MIC <0.01 µg/ml) and the most sensitive strain was Rhodotorula spp[26]. Bees significantly modify the original Propolis composition by mixing it with beeswax and salivary enzymes (β-glucosidase) [4,27] which they secrete during the Propolis collection to produce a cement-like substance that can be considered of both plant and animal origin. β - Glucosidase secreted by the bee during Propolis collection and processing hydrolyzes flavonoid heterosides into aglycones, which improves pharmacological properties of the product[28].

The first systematic investigation of antibacterial properties of Propolis was made by Kivalkina in 1940s[29], and since then, the antibacterial effect of Propolis has been demonstrated in a variety of Gram-positive and Gram-negative bacteria[30]. Activity of the propolis extract (PE) was checked against clinical yeast *C. albicans* and 31 non *C. albicans* (*C. glabrata, C. tropicalis, C. guilliermondii, and C. parapsilosis*) isolates in comparison with the main antifungal drugs used in the treatment of vulvovaginal candidiasis (VVC). All yeasts were inhibited by PE while *C. albicans* isolates showed resistance or dose dependent susceptibility for the azolic drugs and Amphotericin B[31].

Investigation of antifungal activity of Propolis against dermatophytes and yeast revealed that the percentage of inhibition being 100 % in each of the concentrations 10, 15, 20, 25µg/ml[32]. Also other studies show that the alcoholic Propolis extract has fungistatic and fungicidal activities similar to chlorhexidine and fluconazole at concentration 46-512 µg/ml respectively[33] and total flavonoid concentration of $5x10^{-2}$ mg/ml.

The mechanism of antibacterial effect of flavonoids is attributed to damage to the cytoplasmic membrane of bacteria and so increasing its permeability providing the leakage of the important intracellular solute potassium and causing damage to the permeability of the bacterial cell wall, microsomes, lysosomes because of interaction between flavonoids with bacterial DNA[34,35].

The aim of this study is to formulate and evaluate Propolis extract as vaginal suppositories as an alternative treatment of vulvovaginal candidiasis.

MATERIAL AND METHODS

Propolis extract, and quercetin are obtained as a gift from Atos Parma, Egypt), Cocoa butter base purchased from local dealer, pH- meter (Mettler Tolido, Switzerland)Weighing balance (Adams, U.K.), Thermostat water bath (HH-S4, China), Disintegration Tester (Varian, USA), Dissolution tester (Varian 705 DS, USA), Spectrophotometer (UV/VIS 1800, Shimadzu, Japan), Stability Cabinet (Wise Cube® WTH-305 Temp./ Humidity Chamber, Germany), Hardness tester (Monsanto, SDT 1000, Mumbai), Potassium di-hydrogen orthophosphate (Scharlau, Spain).

Formulation of the Suppositories

The Suppositories were fabricated by molding method[36]. Propolis extract was incorporated as a

percentage of suppository base. Hence the displacement value was ignored[37]. Three grams suppository molds were used. The cocoa butter was weighed and placed in beaker on a digital water bath and constantly stirred with a glass rod until all the cocoa butter have melted, Propolis extract was then levigated with warm tween 80 and poured into the melted cocoa butter and stirred with a glass rod. Methyl paraben and propyl paraben were added to this mixture as preservative. Tween 80 was added to make the mixture homogenous and to enhance drug releasecharacteristics[36]. The mold was cleaned, dried and placed on ice. The mixture was poured into the molds to overflowing with constant stirring. The Suppositories were allowed to solidify by placing it in a refrigerator. The solidified Suppositories were then removed from the molds, wrapped in aluminum foil and stored in glass and kept in the refrigerator at a temperature (2- 8 °C) till further use of quality control tests.

Evaluation parameters

The formulated suppositories were evaluated physically: odor, color, shape, surface condition. It is important to check for the absence of fissuring, pitting, fat blooming, exudation, sedimentation, and the migration of the active ingredients[38]. Suppositories can be observed as an intact unit and also by splitting them longitudinally, the results shown in Table No.2. Suppositories also tested physicochemically including: Weight variation, Melting range, Liquefaction time, etc. and the results shown in Table No.3.

Weight variation

The weight variation test was determined according to the British Pharmacopoeia[39]. Twenty suppositories were selected at random and weighed. The average weight was calculated. Then all the suppositories were weighed individually and variation from the average was determined.

Melting range

The melting time or melting range is a critical factor in the determination of the release rate of the active ingredient(s) from the suppository[40]. The USP tablet disintegration apparatus was employed to measure the melting range[41] by measuring the time taken for the entire suppository to melt when immersed in phosphate buffer pH 7.2 at constant temperature bath maintained at 37±0.5˚C.

Liquefaction time

Liquefaction testing provides information on the behavior of a suppository when subjected to a maximum temperature of 37℃. Liquefaction time was measured using a burette having a broad opening on one side and a narrow opening on the other; suppository was pushed inside from the broad end to reach to the narrow end. Five (5) ml of phosphate buffer pH 7.2 was placed inside the burette, maintained at 37± 0.5 ℃. A thin glass rod was placed on the top of the suppository and the time at which the glass rod just inserts into the suppository was recorded as liquefaction time[42].

Hardness test

Hardness test is carried out to determine the tensile strength of the suppositories. The hardness of the formulated suppositories was tested using a Monsanto hardness tester. The hardness test also reveals the ability to withstand the hazards of packing and transportation[43].

Disintegration test

The disintegration time of the suppositories was determined by using the USP disintegration test apparatus and randomly selected six suppositories, each one was immersed in a cylinder of the apparatus containing 900 ml phosphate buffer pH 7.2 maintained at 37±0.5˚C.The time taken for the suppository to melt or disperse was recorded, which should not more than 30 minutes as per BP[39].

***In-vitro* release profile**

Calibration Curve

Total flavonoids were estimated as quercetin equivalent using Aluminium Chloride Colorimetric Method[44-47]. Therefore, standard calibration curve was constructed by dissolving 10 mg of quercetin in methanol followed by preparing serial dilutions 10-150 µg/ml, and measuring the absorbance of the dilutions at 415 nm (λ_{max} of quercetin) (Figure No.1).

Dissolution Time Test Procedure

In-vitro release study was performed using USP type I rotating basket apparatus. The dissolution medium used was 900 ml of phosphate buffer pH 7.2 maintained at 37±0.5˚C. The suppository was

placed in the metal basket at 50 rpm. Sample of 2ml was withdrawn every 5 minutes. After each withdrawal of the sample, same volume of the fresh dissolution medium was replaced. The aliquots were filtered through Whatmann filter paper. To 1 ml of sample 3 ml methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml potassium acetate (1M) and 5.6 ml of distilled water were added. Then the solution was incubated for 30 minutes at room temperature. The absorbance was measured at 415 nm using UV spectrophotometer against a blank[44-47].

Drug content studies

Drug Content was determined spectrophotometrically[44-47]. Ten individual suppositories were placed in 200ml of phosphate buffer pH 7.2 maintained at 37 ± 0.5 ˚C till it melted. One ml of the sample was withdrawn and diluted to 100 ml with phosphate buffer pH 7.2. The drug content was determined by using UV-Vis spectrophotometer (UV/VIS 1800, Shimadzu, Japan) by measuring absorbance of the filtered diluted sample at 415 nm.

Stability studies

The suppositories were also subjected to stability studies[48]. Suppositories were wrapped in the aluminum foil and kept in stressed conditions by six cycles of freeze (2-8°C) and thaw (25°C) process. Suppositories were also kept in accelerated condition, temperature (30°C) for 45 days.

Suppositories were examined visually and for percent drug content as per the procedure of content uniformity on the days 0, 15, 30, and 45, observations shown in Table No.4.

RESULTS AND DISCUSSION

The results of visual evaluation of the formulated suppositories as shown in Table No.2 indicated that they have good appearance with soft and smooth touch, yellowish brown color and a scented odor , their shape was consistently bullet shaped. On examination of the cross section of the formulated suppositories, there was no fissuring, pitting, fat blooming, exudation or migration of active ingredient.

The results of physicochemical evaluation are shown in Table No.3. Evaluation of suppositories weight revealed that not more than two of the individual weights deviated from the average weight by more than 7.5% and none deviated by more than 5%. This means that they were similar with little variation.

The results of melting and liquefaction times (Table No.3) indicated that the suppositories melted and liquefied at 32.38 ± 1.25 and 3.64 ± 0.53 min respectively. These measures the time necessary for suppositories to melt and liquefy under pressure similar to those found in the vagina in the presence of water at body temperature[49]. The crushing strength of suppositories was found to be 4.22 ± 0.92 KgF showing good mechanical strength for handling and transportation and within specified limits of 3 - 6KgF[49]. The disintegration time test measures the time required under a given conditions for a group of suppositories to disintegrate into particles which mimics the time for suppositories to completely disintegrate when it enters the body. The result shows the suppositories disintegrated within 7min., which was less than the 30 minutes required by official books[39].

Dissolution is the time it takes a suppository to go into solution. Figure No.2 shows the percentage concentration of the drug released at different times. The highest concentration of the drug was 35 minutes. This means that in 35 minutes after administering the drug, the maximum concentration in the body is reached.

The drug content of all the suppositories was within the permissible limits (98-102%) indicating the uniform dispersion of drug in a cocoa butter base.

Stability studies show that there is no significant change in physical and percent drug content, which means that suppositories are stable at freeze and at accelerated temperature and there is no need for it to be refrigerated to prevent melting at storage.

Table No.1: Working Formula for the Formulation of Propolis Extract Vaginal Suppositories

S.No	Ingredient	Quantity/Suppository
1	Propolis Extract (g)	0.12 (4%)
2	Tween 80 (g)	0.4
3	Methyl paraben (g)	0.03
4	Propyl paraben (g)	0.02
5	Cocoa Butter (g) to	3

Table No.2: Results of visual examination of the suppositories

S.No	Test	Observations
1	Shape	Consistently bullet shaped
2	Surface condition	Smooth
3	Color	Yellowish brown
4	Odor	Characteristic scented odor
5	Fissuring	No
6	Pitting	No
7	Fat blooming	No
8	Exudation	No
9	Migration of Active Ingredient	No

Table No.3: Physicochemical properties of Propolis Extract Vaginal Suppositories

S.No	Properties	Results
1	Weight Variation (g) ± SD	2.93 ± 0.043
2	Melting Range at 37 ± 0.5 °C (min)	32.38 ± 1.25
3	Liquefaction Time at 37± 0.5 °C (min)	3.24 ± 0.53
4	Hardness (Kg/cm2)	4.22 ± 0.92
5	Disintegration Time (min) ± SD	7.25 ± 0.17
6	Drug Content (%) ± SD	99.16 ± 1.13

Data represented by means ±standard deviation of triplicate experiments

Table No.4: Results of stability studies

Freeze and thaw (six cycles)			Accelerated temperature (30°)		
S.No	**Days**	**Physical Changes**	**% drug Content ± S.D.**	**Physical Changes**	**% drug Content ± S.D.**
1	0	No significant changes were seen	98.54±0.15	No significant changes were seen	98.13±0.12
2	15	No significant changes were seen	99.12±0.23	No significant changes were seen	99.00±0.34
3	30	No significant changes were seen	99.11±0.31	No significant changes were seen	98.19±0.71
4	45	No significant changes were seen	98.32±0.25	No significant changes were seen	98.16±0.31

Data represented by means ±standard deviation of triplicate experiments

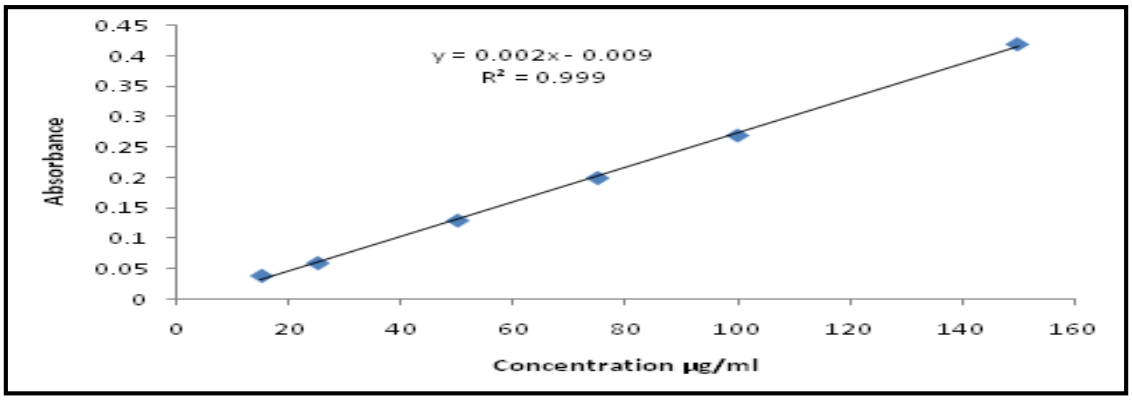

Figure No.1: Standard Calibration Curve of Quercetin
Data represented by means ±standard deviation of triplicate experiments

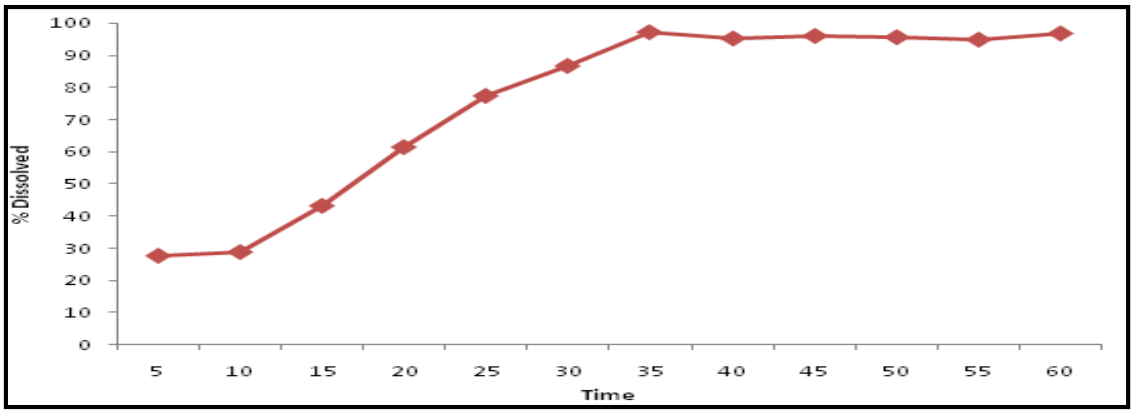

Figure No.2: Dissolution profile of formulated suppositories
Data represented by means ±standard deviation of four experiments

CONCLUSION

Propolis extract can be formulated into stable vaginal suppositories which pass all official quality control tests. Propolis is natural, non-toxic product and the highest antifungal activity of it holds a promise for application as an alternative treatment for infections caused by fungi as vulvovaginal candidiasis after further clinical studies.

ACKNOWLEDGMENT

I am grateful to Advanced Research Center (ARC) for their help and for allowing us to use their labs and equipment.

CONFLICT OF INTEREST

There is no any conflict of interest.

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Please cite this article in press as: Kamal A Badr. Formulation and evaluation of Propolis extract as a vaginal suppository, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 5(2), 2017, 60-68.