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Physicochemical and antibacterial evaluation of herbal cough syrup containing combined stem bark extracts of *Prosopisa fricana* and *Anogeissus leiocarpus*

Olubunmi Jumoke Olayemi[*1], Mercy Itohan Aboh[2], Rashida Abdullahi[1], Christianah Yetunde Isimi[1]

[1]*Department of Pharmaceutical Technology and Raw Materials Development, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria;*

Email:** ***olubunmibiala@yahoo.co.uk

[2]*Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria;*

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ABSTRACT

Background & Aim: The use of medicinal plants in the treatment and management of various diseases is widely practiced in Nigeria. The stem barks of *Prosopisa fricana* and *Anogeissus leiocarpus* have been employed traditionally in the treatment of several respiratory diseases including cough. The aim of this study is to develop herbal syrup formulation from the combined aqueous stem bark extracts of *Prosopisa fricana* and *Anogeissus leiocarpus*which can be used in the treatment of cough.

Experimental: Pulverized stem bark was extracted using distilled water by hot maceration for 24 h and concentrated over a water bath. The resulting extract was used to prepare syrups using either honey, sugar or combination of both (1:1) as vehicles in the presence of preservatives and sweeteners where needed. Organoleptic and physicochemical properties such as odor, color, taste, presence of froth, pH and density were evaluated. Stability of the syrups at room temperature, in the refrigerator and at accelerated temperature was also evaluated. Interaction studies was investigated using Fourier Transform Infra-Red (FTIR) spectroscopy while Differential Scanning calorimetry (DSC) was used to evaluate thermal properties of the prepared syrups.

Results: The syrups were brown to dark brown in color, had characteristic smell with sweet to bitter taste. Viscosity was between 10.80 and 87.40 mpas, pH was between 3.92 and 4.90, density was between 1.39 and 1.52 mg/mL.No interaction was observed from FTIR spectra or DSC after thermal decomposition. *In vitro* antibacterial studies showed the syrups possess considerable inhibitory effect against tested microorganisms. Formulations PAH1 and PAH2 were found to be the most stable after 30 days' storage at different temperatures.

Recommended applications/industries: This study buttresses the potential of herbal extracts in developing stable syrup formulations using honey as a vehicle for treatment of cough and/or relieve of its associated symptoms.

1. Introduction

Herbal medicines have been in existence as long as mankind. The use and demand for herbal medicine is increasingly growing in the developing as well as developed countries. Various parts of plants (roots, leaves, fruits, buds, stems, flowers and barks) are composed of biochemically active constituents (secondary metabolites such as alkaloids, tannins, and flavonoids) that have been utilized globally in the

management of various diseases ranging from mild symptoms to very chronic diseases, and their use has been considered safe with limited side effects. Globally, 80 % of the world's population rely on herbal therapies for their primary healthcare needs and these numbers are postulated to increase in the near future due to the world's growing trend towards "green medicine" (WHO, 2004). Herbal medicine connotes a growing substitute to conventional medicine in managing health challenges and sicknesses. Reports show that about 25 % of contemporary medicines were developed from plants used traditionally for the treatment of various diseases (Arbab *et al.*, 2014). Recently, extensive attention has been shifted towards the use of plant-based products for the prevention, treatment and management of different diseases and disorders (Ibrahim *et al.*, 2018).

Cough is common but problematic upper respiratory tract infection which presents with and without mucus secretion. It can be acute or chronic depending on the duration, paroxysmal cough and psychogenic cough (Ankush *et al.*, 2020) and can be treated with a wide range of synthetic and natural medications.Cough is actually not a disease, but the symptom of a disease that occurs in respiratory tract indicating a more severe illness (Molyneux and Morice, 2011). Literature reveals scientific evidences of herbal cough formulations that have proven to be clinically efficacious in treating cough and managing its symptoms (Wagner *et al.*, 2015; Lawal *et al.*, 2020; Kumar and Parihar, 2022).

The use of medicinal plants in the treatment and management of various diseases is widely practiced in Nigeria. The stem barks of *Prosopisa fricana* and *Anogeissus leiocarpus*have been used traditionally in the treatment of several respiratory diseases including tuberculosis, asthma, cough, catarrh, chronic bronchitis, pneumonia, hay-fever, hemoptysis and pulmonary disorders (Mann *et al.*, 2008). The existence of secondary metabolites such as alkaloids, tannins, phenols in these plants are described to be responsible for their numerous medicinal properties.

The plant *Prosopisa fricana* (Guill., Perrott. and Rich.) Taub. which belongs to the family Mimosaceae. It is also known as African Mesquite but in Nigeria, it is popularly called *Okpei* (Igbo), *Ayan* (Yoruba), *Okepghe* (Idoma and Tiv) and *Kiriya/Kiriaya* by the Hausas (Ezike *et al.*, 2010). The plant is broadly distributed in arid and semi-arid regions of the world but is native to intertropical Africa, from Senegal to Ethiopia, all over the Sudanese and Guinean Eco zones and the border of the Northern Sahel (Alagbe, 2020). *Prosopis* plant is a very hard wood tree which grows rapidly and is distinguished by its dark rough bark, pale dropping foliage with small pointed leaflets and sausage shaped fruits. The pods on the tree do not split open when dry but can be harvested by shaking off the ripe pods from the branches (Anyawuyi *et al.*, 2010; Olorunmaiye *et al.*, 2019). The aqueous extracts of the leaves and bark are traditionally used to cure mouth, throat infections, bronchitis, asthma as well as tremors, ulcers leprosy, and dysentery (Ishtiaq *et al.*, 2015). In Northern Nigeria, the hot water extract of a mixture of *Anogeissus leiocarpus* and *Prosopisa fricana* is traditionally used for the treatment and management of asthma (Isimi *et al.*, 2003).

The other plant explored in this study, *Anogeissus leiocarpus* belongs to the family Combretaceae, and is greatly distributed in Central, East, and West African countries. It is commonly called the "African Birch" and known in Nigerian local languages in Idoma as*Otra*, in Hausa as *Kwankila*, in Igbo as *Atara* and in Yoruba as *Orin-odan* (Mann *et al.*, 2008; Ichor *et al.*, 2011). Traditionally, the Hausas in Northern Nigeria used infusions of *Anogeissus leiocarpus* leaves in the treatment of cough, infant rash and wound infections (Shuiabu, 2008). Some rural communities in Nigeria use the bark of the plant for orodental hygiene; the end of the sticks are chewed into fibrous brush which is rubbed against teeth and gum (Ibrahim *et al.*, 2005).

The study by Lawal *et al.* (2017) demonstrated that the aqueous fractions of *Anogeissus leiocarpus* in combination with *Terminalia glaucescens* holds significant activity against selected micro-organisms implicated in respiratory diseases. Their study gives scientific credibility to the folklore belief of the use of these plants in treatment of respiratory tract infections. Another study reported the significant *in vitro* antibacterial effects of the extracts of *Anogeissus leiocarpus* against some bacterial strains including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigelladysenteriae*, *Escherichia coli* and *Klebsiella pneumonia* which are implicated in respiratory infections (Mann *et al.*, 2014; Gberikon *et al.*, 2019).

Some of these properties as revealed by folklore, anecdotal or scientific evidence point to the credence of using these two plants in the management of respiratory infections like cough.

Herbal medicines typically prepared by steeping, decoction or brewing do not usually have good aesthetic value or taste which directly affect patient's compliance to treatment. Consequently, it is very important to formulate these herbal extracts into dosage forms that will be readily acceptable by patients and encourage compliance. Syrup is a common dosage form which is used to in the treatment and management of cough and cold symptoms. The main advantages of the syrup as a dosage form are ease of administration, palatable taste and provide soothing effect on irritated tissues (Olayemi *et al.*, 2020).

Therefore, the aim of this study is to develop a suitable herbal syrup formulation from the combination of the aqueous stem bark extracts of *Prosopisa fricana* and *Anogeissus leiocarpus*, evaluate its physicochemical and antibacterial properties for possible use in the treatment and management of cough and its symptoms.

2. Materials and Methods

2.1. Materials

Dried stem bark of *Prosopis africana* and *Anogeissus leiocarpus* were prepared in the National Institute for Pharmaceutical Research and Development, Laboratory(NIPRD) Abuja laboratory, methyl paraben and propyl paraben (Tianjin ZhongxinChemtech Co., Ltd, China), sugar and sweetener were sourced from a local supermarket.Muller Hinton agar (MHA), Muller Hinton broth (MHB), Sabouraud dextrose agar (SDA), Sabouraud dextrose broth (Sigma-Aldrich, St. Louis, USA).

2.2. Microorganisms

Typed bacterial and fungal cultures**:** *Escherichia coli* (NC 12923), *Bacillus subtilis* sbsp *spizenii* (NC 10400), *Staphylococcus aureus* (NCTC 6571), *Pseudomonas aeruginosa* (NC13628) and *Klebseilla pneumonia ATCC13883* were obtained from the Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.

2.3. Collection and Identification of Prosopis africana and Anogeissus leiocarpus stem barks

The stem bark of the plants *Prosopis africana* and *Anogeissus leiocarpus* were obtained from the botanical garden of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. Plant identification was done by the Institute's taxonomist (Mr. Akeem) and the specimen stored in the Institute's herbarium with voucher specimen reference NosNIPRD/H/7285 and NIPRD/H/7286 for *Prosopis africana* and *Anogeissus leiocarpus* respectively. The stem barks were washed, dried in a drying cabinet and pulverized using a mechanical grinder.

2.4. Preparation of Prosopis africana and Anogeissus leiocarpus extracts

An earlier method developed by Isimi *et al.* (2003) was adopted. Stem barks of *Prosopis Africana* were crushed, powdered and extracted using distilled water by hot maceration for 24 h with intermittent stirring in the ratio 1:20. The marc was filtered through a muslin cloth, the filtrate was concentrated over a water bath (Karl Kolb, Germany) at 70 ^{0}C and the resulting extract coded PA was pulverized, packaged in an air tight container and stored in a desiccator. The same procedure was repeated for the stem barks of *Anogeissus leiocarpus* and the resulting extract was coded AL

2.5. Preparation of the herbal syrups

The combined extracts of *Prosopis africana* and *Anogeissus leiocarpus* (PAAL) were used in the ratio 1:1according to the preliminary *in vivo* efficacy studies. Various formulations were prepared with different vehicles; honey alone, syrup B.P, combination of honey and syrup BP (1:1) as shown in the composition formula in Table 1.

Appropriate quantities of *Prosopis africana* and *Anogeissus leiocarpus* were weighed, placed in a mortar and the preservatives (methyl paraben and propyl paraben) and sweetener were mixed with the extract as required. A portion of the vehicle was

incorporated into the contents of the mortar to make a smooth paste. The paste was diluted with the appropriate vehicle (Table 1) triturated to obtain a consistent mixture. This was poured into the final container (bottle), made up to volume with the vehicle and stored until further use.

Table 1. Composition for the preparation of syrups containing *Prosopis africana* (PA) and *Anogeissus leiocarpus* (AL) stem bark extracts.

Ingredients/Batch	PAH1	PAH2	PAS1	PAS2	PAC1	PAC2
Prosopis Africana extract(g)	2.5	2.5	2.5	2.5	2.5	2.5
Anogeissus leiocarpus extract (g)	2.5	2.5	2.5	2.5	2.5	2.5
Methyl paraben (g)	0.07	0.07	0.07	0.07	0.07	0.07
Propyl paraben (g)	0.03	0.03	0.03	0.03	0.03	0.03
Water (mL)	20	20	20	20	20	20
Sweetener (g)	-	0.25	-	0.25	-	0.25
Honey to (mL)	100	100	-	-	-	-
Syrup BP to (mL)	-	-	100	100	-	-
Honey + Syrup BP (1:1) to (mL)	-	-	-	-	100	100

Key: PA=*Prosopisa fricana*, AL= *Anogeissus leiocarpus*, MPB= methylparaben, PPB= propylparaben, PAH1 = syrup prepared with honey alone, PAH2 = syrup prepared with honey and sweetener, PAS1 =syrup with syrup alone, PAS2 =syrup prepared with syrup and sweetener, PAC1 =syrup prepared with 1:1 honey and syrup, PAC2 =syrup prepared with 1:1 honey and syrup with sweetener

2.6. Evaluation of syrup formulations

2.6.1. Physical evaluation

The color, odor, taste, texture and the presence of froth on the syrups were evaluated.

2.6.2. Determination of density of syrup

The method of (1) was adopted with some modifications. An empty density bottle of 25 mL (Wv) capacity was weighed and noted as (W1), the bottle was then filled with syrup and weighed again (W2). The difference between the weight of the empty bottle and that of the bottle holding the syrup was noted (W3) and density was calculated using the formula;

$$Density = W3/Wv$$

2.6.3. Determination of pH

The pH of the syrup formulations at room temperature was determined using the digital pH meter (Mettler Toledo pH meter), three (3) determinations were made and the average calculated.

2.6.4. Determination of viscosity

The viscosity of each formulated syrup at room temperature was determined using a digital viscometer (Brookfield AMETEK) at 3 rpm.

2.6.5. Differential Scanning Calorimetry (DSC) Analysis

Samples of the vehicle (honey), the combined extract (PAAL) and syrup prepared with honey (PAH1) were placed in aluminum pans of the Differential Scanning Calorimetry; DSC (Model DSC 204 F1Netzsch, Germany) and scanned between 60 and 300 °C at a heating rate of 10 °C/min under constant nitrogen flow.

2.6.6. Fourier Transform-InfraRed (FT-IR) Spectra Studies

Samples of the vehicle (honey), the combined extract (PAAL) and optimized syrup prepared with honey (PAH1)were investigated for possible interaction. Eachsample was triturated with potassium bromide, made into pellets (1 ton/cm^2) and infrared (IR) spectra were obtained between scanning ranges of 4000 and 400 cm^{-1} using the Cary 630 Fourier transform infrared (FT-IR) Spectrometer (Agilent Technologies, USA).

2.6.7. Microbial load determination

The total aerobic microbial count (TAMC) for aerobic bacteria and Total combined yeast/ mold count (TYMC) and specific pathogens (*Staphylococcus aureus, Escherichia coli, Salmonella sp, Pseudomonas aeruginosa*) was done according USP (2020) for determination of quality of non-sterile preparations adopting the plate count method. One milliter of a 1:10 dilutions of the sample was dispensed in sterile Petri dish and 20 mL of molten tryptic soy agar (bacteria) and SDA (yeast and molds) were then poured into the petridishes, allowed to solidify, inverted and incubated at 30- 35 ^{o}C for 3-5 days for TAMC and 20- 25 ^{o}C for 5-7 days for TYMC respectively. The number of colonies was counted and the total viable count estimated. Bacteria and fungi contaminants were identified based on their biochemical and morphological characteristics.

2.6.8. Antibacterial susceptibility tests

The cup plate agar diffusion method was adopted according to CLSI (2017) with slight modifications. Simply, 100 µliters of the herbal cough mixtures were dispensed into bored holes on Muller Hinton agar seeded with 0.5 Mc Farland suspensions of the test bacteria and incubated at 35- 37 ^{o}C for 24 h. The zones of inhibition were measured using a meter rule and the test done in duplicates.

2.6.9. Evaluation of syrup stability under different storage temperature

Stability of the formulated syrups were investigated at different storage temperatures over 30 days; they were stored at room temperature, in the refrigerator (4 ^{0}C), and in a photo-stability chamber at accelerated temperature (40 ^{0}C/ 75% RH). Physical properties such as organoleptic properties, presence or absence of visible growth, pH and viscosity were reassessed after the storage period.

3. Results and discussion

3.1. Physical properties of syrup formulations

The physical properties of the formulated syrups are presented in Table 2. All the syrups were observed to be brown to dark brown in color due to the color of eth dried extracts, they formulations had honey-smell except those prepared with syrup BP (PAS1 and PAS2) which had characteristic smell. Although the extracts alone were bitter to taste, incorporation into honey-based vehicles as formulations PAH1, PAS1 and PAC1 were found to be sweet, while PAH2, PAS2, PAC2 had bitter after taste which was attributed to the presence of sweeteners. This shows honey-based vehicles without sweeteners produced better tasting formulations than the syrup-based formulations or those prepared with sweeteners.All the syrup formulations were homogenous and had smooth texture.

Table 2. Organoleptic properties of syrup formulations.

Batches/Parameter	Color	Odor	Taste	Texture
PAH1	Brown	Honey	Very sweet	Smooth
PAH2	Brown	Honey	Very sweet with bitter after taste	Smooth
PAS1	Dark brown	Characteristic	Sweet	Smooth
PAS2	Dark brown	Characteristic	Sweet with bitter after taste	Smooth
PAC1	Brown	Honey	Very sweet	Smooth
PAC2	Brown	Honey	Very sweet with bitter after taste	Smooth

3.2. Chemical properties

Density of the syrups was observed to be between 1.39 and 1.52 mg/mL as shown in Table 3.

Table 3. Chemical properties of syrup formulations.

Parameter/Batches	Density (g/mL)	Viscosity (mpa.s)	pH
PAH1	1.50	87.40	3.92 ± 0.01
PAH2	1.52	86.40	3.94 ± 0.01
PAS1	1.39	17.40	4.75 ± 0.02
PAS2	1.39	10.80	4.90 ± 0.00
PAC1	1.45	28.00	4.37 ± 0.05
PAC2	1.46	28.20	4.22 ± 0.05

Formulations prepared with honey as vehicle (PAH1 and PAH2) had highest densities (1.50 and 1.52 respectively) which is attributable to the high viscosity of honey alone. Those containing the combination of honey and syrup as vehicle (PAC1 and PAC2) showed intermediate densities (1.45 and 1.46 respectively) while formulations prepared with syrup BP (PAS1 and PAS2) showed the least density (1.39). Similarly, viscosity of PAH1 and PAH2 (86.40 and 87.4 mpa.s respectively) were the highest while those of PAS1 and PAS2 (17.4 and 10.8 mpa.s, respectively) were the lowest.

For liquids, density is said to be directly related to viscosity in that an increase in density could imply an increase in viscosity which implies that preparations with higher density would have higher resistance to flow which could affect the manner in which the preparations are dispensed from the final container. However, our results show that in spite of the difference in densities, all of the formulations had ability to freely flow out of the container orifice which will not have a negative impact on dosing accuracy of the formulation.

The pH of all the syrup formulations was observed to be acidic; between 3.92 and 4.90 depending on the pH of the vehicles used for preparation (Table 3). Formulations PAH1 and PHA2 with pH 3.92 and 3.94

respectively had similar pH with honey alone (3.81) and is within the range of honey (3.4 and 5.5) as revealed by literature (Sereia *et al.*, 2017; Yakubu *et al.*, 2021). The highest pH range of 4.75 and 4.90 was observed for formulations prepared with syrup BP (PAS1 and PAS2 respectively) while those of PAC1 and PAC2 were 4.37 and 4.22 respectively. The pH of a mixture is critical as its influences the physical and microbiological stability of the formulation which in turn could impact on the activity of the formulation (Vázquez-Blanco *et al.*, 2018). The acidic nature of these formulations could be a positive factor in maintaining its stability over time. More so, pH within acidic ranges of oral medications like syrups have been found to be acceptable even for children (Zupanets *et al.*, 2021).

3.3. Microbiological evaluation

Microbial load of the prepared syrups as shown in Table 4 was between 1.0×10^0 and 3.9×10^1 cfu/mL for total viable count (TVAC) and absence of yeast or mold, respectively. These values are within the acceptance limit for oral aqueous preparation as specified by the USP (2020) portraying that the syrups do not contain are relatively free of contaminants.

Table 4. Microbial load of prepared syrups.

Sample	TVAC (cfu/mL)	TYMC (cfu/mL)	Remark
PAH1	2.7×10^1	$<1.0\times10^0$	Passed
PAH2	3.9×10^1	$<1.0\times10^0$	Passed
PAS1	1.0×10^0	$<1.0\times10^0$	Passed
PAS2	3.6×10^1	$<1.0\times10^0$	Passed
PAC1	8.1×10^1	$<1.0\times10^0$	Passed
PAC2	2.2×10^1	$<1.0\times10^0$	Passed

From the measurements of zone of inhibition resulting from the antibacterial susceptibility of the syrups against the different microorganism (Table 5), all the syrups can be said to possess inhibitory effect but to varying degrees. The inhibitory activity of the syrups was found to be greater than the extracts alone (PA or AL) or in combination (PAAL) except against *Pseudomonas aeruginosa* and *Klebseilla pneumonia* where PAAL showed greater inhibition zone. However, there was no significant difference at $P>0.05$ between the antibacterial activity of the syrups against each other. Thus, incorporation of these extracts into syrup formulations containing different vehicles did not obliterate the inherent antibacterial activity of the extracts.

Table 5. Antibacterial susceptibility of prepared syrups.

Microorganisms	Average Diameter Zones of Inhibition (mm)								
	1	2	3	4	5	6	A	B	C
B. subtilis NC 03179	18.0	20.0	20.0	17.0	16.0	16.0	15.0	15.0	16.0
S. aureus NC 10788	20.0	20.0	23.0	21.0	20.0	23.0	14.0	15.0	15.0
E. coli NC 12923	20.0	23.0	21.0	19.0	20.0	22.0	14.0	20.0	16.0
P. aeruginosa NC13628	15.0	14.0	17.0	15.0	17.0	19.0	15.0	21.0	22.0
K. pneumonia ATCC BAA 1705	11.0	12.0	11.0	11.0	11.0	11.0	15.0	22.0	19.0
C. albicans NCPF03179	15.0	21.0	19.0	19.0	16.0	20.0	14.0	19.0	18.0

Key: 1 = syrup prepared with honey alone (PAH1), 2 = syrup prepared with honey and sweetener (PAH2), 3 = syrup with syrup alone (PAS1), 4 = syrup prepared with syrup and sweetener (PAS2), 5 = syrup prepared with 1:1 honey and syrup (PAC1), 6 = syrup prepared with 1:1 honey and syrup with sweetener (PAC2), A = 20 mg of *Prosopisa fricana* extract (PA), B = 20 mg of Anogeissus leiocarpus extract (AL), C = 20 mg of the combined extracts (PAAL).

In view of the fact that all the syrups possess good antibacterial activity; the physicochemical properties, stability studies under storage and acceptable, soothing taste was used to select the formulation prepared with honey alone as the optimized formulation. Therefore, thermal analysis and interaction studies were carried out on only the optimized formulation (PAH1).

3.4. Differential Scanning Calorimetry (DSC)

DSC is a thermal analysis technique in which the heat flow into or out of a sample is measured as a function of temperature or time. It is a very powerful technique used to evaluate material properties of a wide range of materials, including polymers, pharmaceuticals, organic materials, and lots more. With DSC, it is possible to determine purity and thermal stability of materials.The advantage of the DSC in thermodynamic analysis is that it is fast and completely automated (Fernandes *et al.*, 2013).

The thermogram of the vehicle, combined extracts alone and optimized syrup formulation are presented as Figures 1a, 1b and 1c. Table 6 shows onset of thermal processes for PAAL at 60.02 ^oC and ended at 300.48 ^oC

while the peak temperature was 238.39 ^{o}C which corresponds to its melting point. The peak and conclusion melting temperature for the optimized formulation (PAH1) was found to be lower than those of PAAL but similar to those of honey (Table 6) which could be attributed to the influence of incorporation the extract into the vehicle (honey).

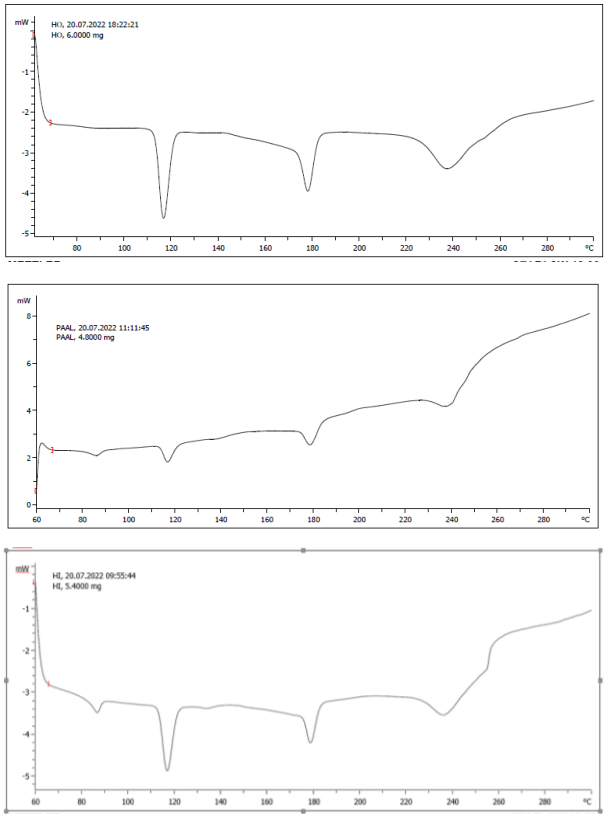

Fig. 1. Thermogram showing Honey alone (H0), combination of *Prosopis africana* and *Anogeissus leiocarpus* aqueous bark extract (PAAL) and the optimized syrup formulation (H1=PAH1).

Table 6. Thermal properties of optimized syrup formulation, extracts and vehicle (honey).

Parameter	H0	PAAL	PAH1
Onset temperature (^{o}C)	110.61	60.02	107.76
Peak temperature (^{o}C)	116.66	238.39	116.81
Conclusion temperature (^{o}C)	124.39	300.48	127.77
Enthalpy of crystallization (J/g)	388.95	94.73	592.78

The thermal decomposition of PAAL produced two endothermic peaks between 80 and 100 ^{o}C and between 110 and 130 ^{o}C which were observed to be retained in the thermogram of PAH1 at the same temperature ranges. A weak peak in PAAL observed between 220 and 250 ^{o}C was also found to be retained in PAH1. Enthalpy of crystallization characterizes sensitivity of materials to thermal changes; materials not susceptible to thermal changes usually exhibit low enthalpy. The effect of thermal changes was observed in PAH1 with high enthalpy of crystallization which suggest higher energy requirement in disentangling bonds present in the formulation; this infers higher stability of the formulation (Bhupender *et al.*, 2013; Pauliuc *et al.*, 2022). However, there was no new peak formed nor the complete absence of pre-existing peaks of the combined extract in the optimized formulation (Figure 1c), showing that there is no molecular interaction between the extract and the other materials used in the formulation of the syrup.

3.5. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of honey alone (H0), combined extracts (PAAL) and the optimized syrup formulation (PAH1) are presented in Figure 2a, 2b and 2c respectively.

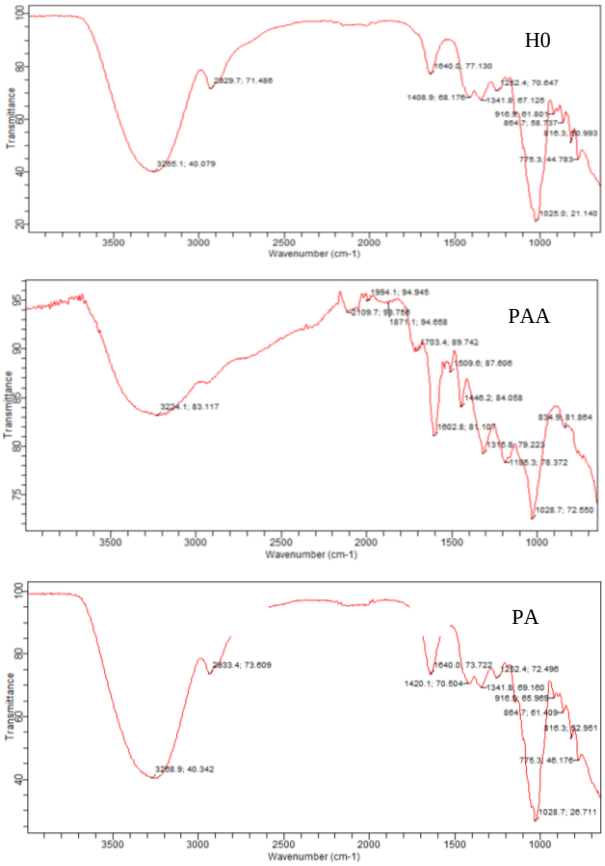

Fig. 2. FTIR spectra showing Honey alone (H0), combination of *Prosopis africana* and *Anogeissus leiocarpus* aqueous bark extract (PAAL) and the optimized syrup formulation (PAH1).

Figure 2b shows a broad band spectrum around 3224.1 cm^{-1} in PAAL assigned to alcohol and hydroxyl

group (OH) stretching was also observed in the spectra of PAH1 at 3256.8 cm^{-1}. A sharp peak around 1602.8 cm^{-1} associated with N-H stretching of primary amine groups was also observed to be retained in PAH1 at 1640.0 cm^{-1}. Characteristic peaks for honey were also observed in the spectrum on PAH1.There were no new peaks or major shifts of pre-existing peaks indicating no new bands or bonds were formed in the optimized syrup corroborating the result of DSC that there is no interaction between the ingredients used in preparation of the optimized syrup (PAH1).

3.6. Stability studies

Stability studies of the formulated syrups under the different storage conditions are presented in Table 7.

Table 7. Organoleptic and physicochemical evaluation of formulated syrups after storage.

Batch	Color	Odor	Growth	Viscosity	pH
4 ^{0}C					
PAH1	NC	NC	NG	143.2	3.95 ± 0.1
PAH2	NC	NC	NG	158.6	3.89 ± 0.1
PAS1	NC	NC	NG	21.00	4.59 ± 0.1
PAS2	NC	NC	NG	17.40	4.64 ± 0.1
PAC1	NC	NC	NG	36.80	4.02 ± 0.1
PAC2	NC	NC	NG	38.40	4.02 ± 0.1
Room temperature					
PAH1	NC	NC	NG		3.94 ± 0.1
PAH2	NC	NC	NG		3.93 ± 0.1
PAS1	NC	NC	NG	21.20	4.77 ± 0.1
PAS2	NC	NC	NG	30.40	4.73 ± 0.1
PAC1	NC	NC	NG	71.40	4.08 ± 0.1
PAC2	NC	NC	NG	40.40	4.13 ± 0.2
40 ^{0}C					
PAH1	NC	NC	NG		3.87 ± 0.1
PAH2	NC	NC	NG	124.8	3.96 ± 0.1
PAS1	NC	NC	NG	32.00	4.55 ± 0.1
PAS2	NC	NC	NG	27.80	4.72 ± 0.1
PAC1	NC	NC	NG	49.60	4.09 ± 0.1
PAC2	NC	NC	NG	48.80	4.15 ± 0.1

It shows all the formulated syrups retained their physical properties; color and odor, it also shows no visible growth was observed in all the formulations. These could be attributed to the presence of preservatives included in the formulations in addition to the preserving ability of honey and the bacteriostatic activity of simple syrup BP used for the preparations (Mali *et al.*, 2019; Olayemi *et al.*, 2020).

Viscosity of the syrups stored at 4 ^{0}C was observed to be more significantly increased which may be due to possible crystallization of honey resulting in thickening than those stored at 40 ^{0}C. Viscosity of those stored at room temperature was not found to be as markedly increased as the others and was readily removed from the packaging bottle. On the other hand, increase in viscosity of syrups on storage has been suggested to improve its stability (De Rodriguez *et al.*, 2004) which may be an advantage for the formulated herbal syrup.

The pH of the syrups was found to be affected to varying degrees upon storage. Formulations PAH1 and PAH2 showed no significant changes in pH ($P>0.05$), however, pH of PAS1 and PAS2 were found to slightly decrease upon storage at 4 ^{0}C and 40 ^{0}C while PAC1 and PAC2 showed significant reduction in pH ($P<0.05$) under all the storage conditions. Decreasing pH of syrups has been associated with hydrolyzing rate of sucrose which may change the quality of the formulations (Farrokhi *et al.*, 2012). This suggest that formulations PAC1 and PAC2 may be unstable and highlights the importance of controlling pH upon storage. Some other have also reported similar reduction in pH of syrups upon storage (Glass and Alison Haywood, 2006; Ghaderi *et al.*, 2015; Mukantwali *et al.*, 2017).

From this thermal stability studies, we can deduce that PAH1 and PAH2 are the most stable of all the

syrup formulations and that the optimum storage condition for these herbal syrups is at room temperature.

4. Conclusion

This study shows that the aqueous extract of combined stem bark of *Prosopis africana* and *Anogeissus leiocarpus* can be developed into stable syrup formulations with acceptable organoleptic and physicochemical properties.Syrup formulation prepared with honey alone and stored at room temperature showed the best physicochemical properties in addition to good antibacterial activity against selected microorganisms implicated in cough infections. Therefore, syrup formulations of these extracts could be developed for possible commercialization for relieve of cough and its symptoms.

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