Antibacterial activity and medical properties of Witch Hazel *Hamamelis virginiana*

Talib F. Abbas [1*], Mosa Fadiel Abbas[2], Ali Jarad Lafta[3]

[1]Department of Clinical & Laboratory Sciences, Faculty of Pharmacy
University of Al-Muthanna, Iraq.
[2]Department of Surgery & Obstetrics, College of Veterinary medicine, University of Basra, Iraq.
[3]Department of internal & preventive medicine, College of Veterinary medicine, University of Basra, Iraq.
*Corresponding Email: tlb_abbas77@mu.edu.iq (Abbas)

Abstract: While the final proof of efficacy in common dermatitis such as atopic dermatitis was found still lacking, is the authors found fairly ample evidence for their activity against cutaneous inflammation in man, as may be deduced from experiments with normal volunteers and unwanted effects related to the drug are virtually absent.Witch hazel extractions applied to dermatological areas topically provide a calming effect through tannin extraction, therefore also utilized in cosmetics as well as therapeutically.Active components of hamamelis extractions may be traced to flavonoids, leucoanthocyanidins, tannins, and essential oils that provide benefit to blood circulation, antimicrobial activity, and antioxidant activity. This research aims to evaluate of biological activity of the herbal substances of *Hamamelis virginiana* L., bark, leaf and twigs and preparations thereof for the categorization as products under well-established use or traditional use and the establishment of the corresponding Community herbal monographs. Determining the best method of extraction, prepare the extracts, include milling,extraction of active compounds in soxhlets ,evaporation of the extract to form semi-solid extracts , then analyzing the functional groups in FTIR instrument, and determine the biological activity by measuring minimum inhibition concentration (MIC).The results illustrate that witch hazel extracts in three concentrations (10mg/ml,50mg/ml, 100 mg/ml) have an inhibition effect on 10 plates of *Candida albicans, with* optimum concentration (50mg/ml).*Staphylococcus aureus*showed that the higher concentration of 100 mg/ml has an optimum effect and wider radius of MIC (0.54cm±0.5). *E. coli* seems to have a similar effect to *S. aureus* biological test. The concentration of 100mg/ml showed great inhibition effect to the bacterial growth in 24 hours. Which hazel has a deteriorating effects on both Gram positive and Gram negative and fungi, a preferable actions are due to functional groups especially the carbonyl group, which has a strong effects since it's a form of aldehyde, with the alkyl and ketone groups all have the degradation effects on the microbial cell wall, chelating agents making them perfect disinfectant, in addition to its antioxidant effects, and blood veins constructive.

Keywords: Witch hazel, Pharmaceutical effects, FTIR

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1. Introduction

Although *Hamamelis virginiana* L. has long been used in the traditional treatment of skin diseases, there are few controlled clinical studies defining the extent of its anti-inflammatory action. Topical herbal drugs have for centuries been used for treating skin ailments. Although they are currently widely accepted by patients, their scientific esteem among dermatologists in particular is limited. Hamamelis preparations looked particularly well documented. The authors concluded that in particular dermatitis and related disorders that can be considered potential indications for topical herbal anti-inflammatory drugs [1,2].The antimicrobial activity of a distillate of *Hamamelis* (Aqua *Hamamelidis*), United States Pharmacopoeia (USP), and urea (5%) formulated as a topical dermatological preparation

was studied. The study was conducted in 15 healthy volunteers. In vivo, the occlusion and expanded flora tests produced consistent results.The distillate showed significant antimicrobial activity on aerobes. Formulations of hamamelis distillate and urea are mainly used for their anti-inflammatory, hydrating and barrier stabilizing effects in dermatitis maintenance therapy. The antimicrobial activity of such products is considered an added benefit. The antimicrobial activity is particularly welcome in the management of atopic dermatitis and intertrigo because the organisms involved in the pathogenesis of these conditions are susceptible to the hamamelis preparations; staphylococcus aureus and Candida albicans [3].

Tannin is extracted from bark and leaves with strict storage specifications for preservation. Extractions of tannins require a water and acetone and are store in an aqueous state with a protein affinity. Tannins are attributed for their astringent nature and therapeutically, "they waterproof the external layers of the skin and mucosa's, thus protecting the underlying layers." Through tannin extracts, witch hazel utilization heals small cuts and superficial burns. This occurs because tannins exert a vasoconstrictor effect on vessels closer to the dermis through allowing tissue regeneration and fluid retention [4]. The antimicrobial and anti-carcinogenic properties of witch hazel may be attributed to tannins (tannic acid). At different doses, tannins activate physiological activity by accelerating blood coagulation, causing hypotension, decreasing the serum lipid level, producing of liver necrosis, and control immune responses. Their antimicrobial property is used to preserve foods such as catfish fillets, extending their shelf life [5]. Cosmetically, tannin extracts are utilized for their anti-microbial and antioxidant actions for antiseptics and anti-aging products, respectively.

Witch hazel extracts locally applied in therapeutic amounts do not penetrate into the deeper layers of the skin because of the astringency of their ingredients, and they are therefore not absorbed into the blood circulation.However, the fermentation of aqueous extracts gave clear evidence on conversion process result in gallic acid, glycosides, and kaempferol. In particular, the analogy between the microbial metabolisms of phenolics from fermented Hamamelis extracts [6].The flowers, bark and leaves of the common, colorful American witch hazel shrub provided tonics and remedies to Native Americans. Today, natural witch hazel is considered as one of the few plant products that meet FDA standards for safety and effectiveness. The plants of the genus *Hamamelis* are unusual because they bear their blossoms and fruits together, at the very same time of the year, usually in autumn or winter. Flowers of *Hamamelis virginiana* L. are ribbon-like clusters of yellow, orange, or red petals; the adjacent seed capsules, from the previous year's blossoms, which eject two black seeds when they burst.

The different origins (leaf, bark, and twig) of extraction target specific systems and method of application whether it is applied orally or topically. Flavonoids present in many fruits, flowers, and vegetables provide pigmentation through a rudimentary structural formula, 2-phenylchromane [7]. The structural properties of flavonoid subgroups such as "the presence of an oxygen group at position 4, a double bond between carbon atoms 2 and 3, or a hydroxyl group in position 3 of the C (middle) ring" are properties that are required for the antioxidant effect [8]. Flavonoids are characterized by their yellow, orange, or red pigmentation in autumn, the source of the yellow flowers blooming in witch hazel. Flavonoid structure resembles that of tocopherols (vitamin E)[9]. The antioxidant, anti-inflammatory, antihistamine, antiviral, antithrombotic and anti-carcinogenic activities of flavonoids have been documented, as well as structures resembling Vitamin P, decreasing capillary permeability. Cosmetically, hair color protection traces back to the antioxidant property of flavonoid extraction from witch hazel, through inhibition of enzymes during the oxidative process. The oxidative process gives flavonoids their antioxidant property, through combination of their iron chelating organic structure and scavenging ageing-inducing free radicals. Flavonoids inhibit oxidases, which prevent formation organic hydroperoxide and reactive oxygen species [10].

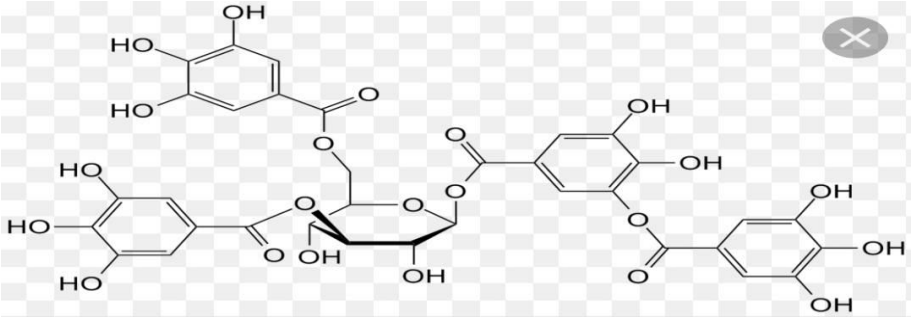


Figure 1. illustrate the tannin chemical structure

The oral administration of 10-20 g*Hamamelis virginiana* L. distillate (single dose) of hamamelis preparation (not specified) showed no toxic effect in mice and rats. The LD_{50} (rats) on oral administration could not be found. A daily oral intake of 100 mg/kg body weight for three months produced no abnormalities in rats [11]. The *IV* administration of different aqueous solutions of a liquid extract of Hamamelis (unknown declaration) 0.2-0.4 g extract/kg bw to dogs (8.5 kg) has shown a marked arterial hypotension, with lethal doses from 0.6 to 0.8 g/kg bw. The lethal dose after a slow administration in the intrafemoral arteria was of 0.5 to 1.2 g/kg bw [12].Its utilization is often documented in homeopathic medicine, cosmetic industry, and Over-the-Counter (OTC) pharmaceutical products. Witch hazel extracts are obtained through leaves, twigs, and bark, each serving a specific therapeutic effect, most commonly in the treatment of hemorrhoids and cosmetically in the form of an antimicrobial and antioxidant through tannin and flavonoid extractions [13].
The aim of this research is to evaluate of biological activity of the herbal substances of *Hamamelis virginiana* L., bark, leaf and twigs and preparations thereof for the categorization as products under well-established use or traditional use. Determining the best method of extraction, prepare the extracts, considering its chemical properties by FT-IR.

2. Materials & Methods

All the chemical materials had been treated and preserved under the standard conditions authorized by the faculty of pharmacy, ministry of higher education and scientific research, accordance with ministry of health parameters, supervised by the laboratory advisors. The Witch hazel has collected from the local markets,methanol 700ml (Srlchem.co), Potato dextrose agar, MacConkey agar, and Muller-Hinton agar were from (Micromedia.co); Econazole antifungal standard disk, Meropenem antibiotic standard disk, and Gentamycin antibiotic standard disk. All Instruments manipulated within the laboratory conditions inside the laboratory, collaborated before the experiments, using Hood, balance, pestle and mortar, grinder, soxhelt extraction, evaporator, autoclave, petri dishes, bunsen burner and Fourier- transform Infrared spectroscopy (FT-IR Burker-10).

Milling; in this experiment, the witch hazel leaves, stems, and seeds had been grinded by mortar and pestle and then softened by grinder 0.05 mm. taking an appropriate amount of witch hazel (in this experiment we used 26 g).

Extraction using soxhelt apparatus;Normally a solid material containing the desired compound is placed inside a thimble made from thick filter papers, which is loaded into the main chamber of the soxhelt extractor. In this experiment we used witch hazel plant, the plant material can be dried. It needs to be crushed using pestle and mortar to provide sufficient surface area. The plant material should be sufficient to fill the porous cellulose thimble. The soxhlet extractor is placed onto a flask containing the extraction solvent, which is methanol (700 ml).The temperature is set at 40c.Over 4 hours for 3 days a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

After extraction the solvent is removed, typically by means of a **rotary evaporator**, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, figure 1.a shows the whole parts of herb, 1.b the extracted condenseddistillate[6].

A B

Figure 2. A.shows the whole parts; park, leaves, and seeds of Witch hazel.B. shows the distillated Witch Hazel, semi-solid dark green colure and high viscosity.

FT-IR Spectroscopy;Organic molecules can absorb IR radiation between 4000 cm^{-1} and 400 cm^{-1} which corresponds to an absorption of energy between 11 kcal/mole and 1 kcal/mole. This amount of energy initiates transitions between vibrational states of bonds contained within the molecule.IR spectroscopy is a very powerful method for the identification of functional groups. The most important regions of the IR spectrum are >1650 cm^{-1}, whereas the fingerprint region (600 -1500 cm^{-1}) of the spectrum cannot easily be used for identification of unknown compounds. Many references exist which tabulate the IR frequencies for various functional groups and organic compounds, table 1. If the instrument has just been turned on, then its necessary to run a test to be sure that all components are ON. The instrument Burker FT-IR spectro supplied by OPUS computer program of scanning. Sacn background was performed with blank IR plate chamber, then the scan performed in the sample, repeated the scan three times for the same sample, detecting the best zone that display the spectra with better views of peaks absorbance at the clear wave lengths base line, saving the results and comparing with library data to get the best expectations for distilled [14].

Table 1. Represent the functional groups and their absorbency by type of vibration

Functional groups	frequencies cm-1	Type of vibration
OH	3750	Stretch
CH	3313	Aromatic weak
C=C	1717	Stretch
CH	1626	Binding
Co	1200	Stretch

Determination of Biological Activity; Minimum Inhibition concentration *(MIC)* values are used to determine the susceptibilities of bacteria to drugs and also to evaluate the activity of new antimicrobial agents.Agar dilution involves the incorporation of different concentrations of the antimicrobial substances into a nutrient agar medium followed by the application of standardized number of cells to the surface of the plate agar. For broth dilution, bacteria are inoculated into liquid growth medium in the presence of different concentrations of an antimicrobial agent. Growth is assessed after incubation

for a defined period of time (16-20 h) and the MIC value were read [5]. For agar dilution, solutions with defined numbers of bacterial cells are spotted directly onto the nutrient agar plates that have incorporated different antibiotic concentrations.

After incubation, the presence of bacterial colonies on the plates indicates growth of the organism. Broth dilution uses liquid growth medium containing geometrically increasing concentrations of the antimicrobial agent, which is inoculated with a defined number of bacterial cells.Breakpoints (particular MIC that differentiates susceptible, and assumingly treatable, from resistant and assumingly untreatable organisms) can vary according to the particular species being examined and the particular antimicrobial agent. Features that define these breakpoints are MIC distributions of the relevant species, pharmacodynamics and pharmacokinetics of the antimicrobial agent, and clinical outcome data. Resistance is associated with a high likelihood of therapeutic failure, whereas susceptibility is associated with a great probability of therapeutic success.

In laboratory experiments, there were three types of agar medium; Potatoes dextrose agar for cultivation of fungi, Mueller-hinton agar for antibiotic resistance testing and McConkey and Nutrient agars and broths for cultivations. It have prepared 30 petri dishes, 10 petri dishes of Mueller-Hinton agar type for susceptibility tests for both *E. coli* and *Staphylococcus* species, and 10 petri dishes of potatoes dextrose agar (PDA) for fungal susceptibility tests for *Candida* species, standardized disks were of meropenem, gentamycin and econazole.

3. Statistical analysis

Standard statistical methods were used to determine the meanand standard deviation (SD).Unpaired t-test was used to comparethe results of different biological activity parameters with the controls. P-value ≤ 0.005 was considered to be statisticallysignificant [15].

3.1 The results

The results of witch hazel grinding demonstrated that the components of this dried parts has green color with brownish colored seeds, the plant was softened using mortar and pestle instrument to get a homogenous powder, then the resultant powder was further softened using a grinder of 0.5 mm, obtaining about 26 g of the powder. By using soxhelt apparatus with medium sized thimble, extraction with a methanol as a solvent showed that we obtained an extract of witch hazel about 200ml. extraction process was repeated several times,3-4 hours for 3 days in a row. Semi-solid and liquid preparations containing the equivalent 5-10% of bark; diluted tincture of hamamelis (1:10, 45% ethanol), have been widely used for lotion and mouthwash, expressing their safety.precipitated these compounds from the solvent methanol by rotary evaporator to obtain 7g of witch hazel, dark green high viscous semi sold substance; figure 2B.The quantification of witch hazel through infrared spectroscopy was developed and validated for pharmaceuticals. The method involves the extraction of the active ingredient with methanol and the measurement of the area of the infrared band corresponding to the carbonyl group centered at 1670-1820cm^{-1}, alkyl group C-F, C-Br centered at 500-1400 $cm^{-1}$and many functional groups as in the table 2. The specificity, linearity, detection limits, precision and accuracy of the calibration curve, witch hazel extraction, infrared analysis and data manipulation were determined in order to validate the method, figure 3.

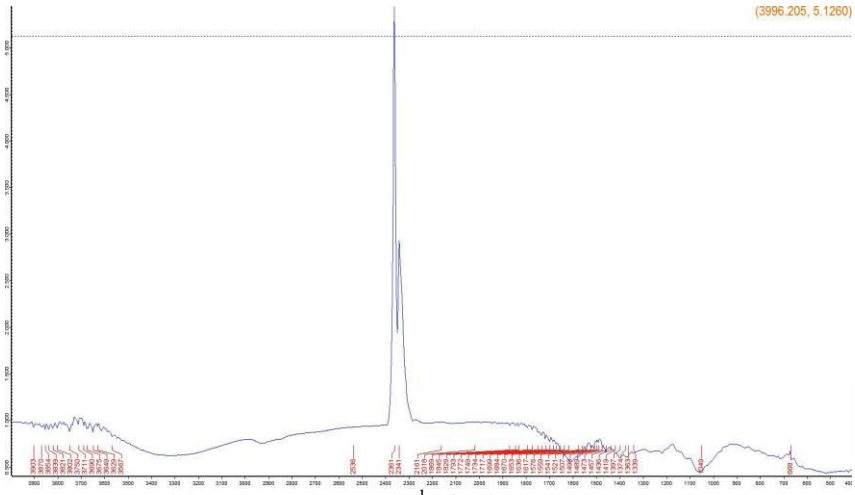
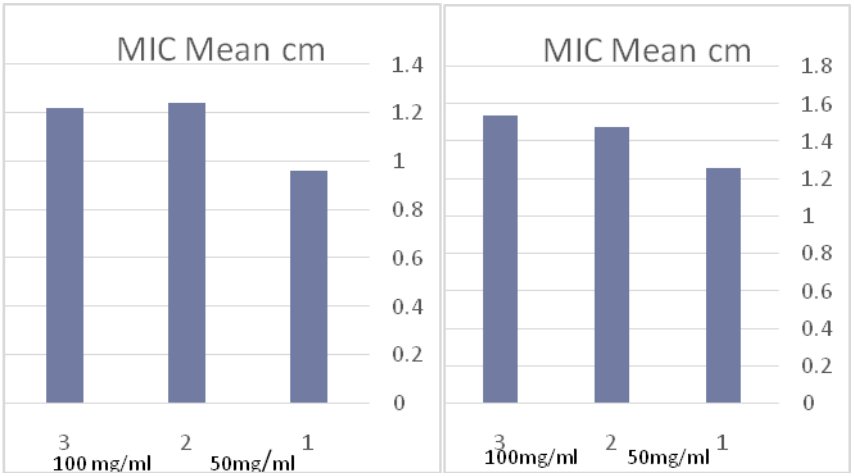


Figure 3. illustrate (FTIR Chart) the IR-absorbance cm^{-1} of Witch hazel extracts by FT-IR Burker-10 instrument.

Table 2 show functional groups in witch hazel by using FTIR

Functional group	Absorption cm^{-1}	Intensity& Vibration
Alcohol -OH	3200-3600 3500-3700	Strong brand&stretch Strong sharp & stretch
Alcane–CH	2850-3000 1350-1480	Bending& stretch Strong and variable
Carbonyl-C=O	1670-1820	Strong & stretch
Alkyl halide-C-F Alkyl-C-Br	1000-1400 500-600	Strong & Stretch Strong & stretch
Aromatic-C-H	2500-3000	Strong two bands

Determination the biological activity through the calculation the minimum inhibition zone (MIC) had been done in the well-known pathological microorganisms, by testing *Candid albicans*, *Staphylococcus aureus*, and *Escherichiacoil*. Witch hazel extracts holding on paper disk in three concentrations (10mg/ml,50mg/ml, 100 mg/ml) had an inhibition effects on 10 plates of *Candida albicans*,The radius of MIC were (10 mg/ml) (o.96 cm±0.2), (50mg/ml. (1,24cm±0.1) and (100mg/ml(1,22cm±0,3); For the *candida albicans* seems that optimum concentration of high MIC was (50mg/ml), figure 4A. The other biological indicator in the experiment was Staphylococcus aureus, while the results obtained have a different way of effect depending on the concentration, which means the higher concentration had more effect.The columns showed that the higher concentration of 100 mg/ml has an optimum effect and wider radius of MIC of (0.54cm±0.5). In negative gram stain bacteria, *E. coli*, the results showed a similar effect to *S. aureus* biological activity. The concentration of 100mg/ml showed great inhibition effect to the bacterial growth in 24 hours.



A B

Figure 4.A explain the susceptibilitytests of various concentrations of witch hazel in *Candida albicans*culture media, B- in *Staphyllococcus aureus and E.Coli.*

4. Discussion

The Native Americans produced witch hazel extract by boiling the stems of the shrub and producing a decoction, which was used to treat inflammatory conditions. Early Puritan settlers in New England adopted this remedy from the natives. A missionary, Dr. Charles Hawes, eventually learned of the preparation's therapeutic properties and further determined that the product of the plant's twigs was even more efficacious. From the middle of the 19th century onwards, Hamamelis finally became a constituent of the official American and European medicine. The leaves (Hamamelidis folium) as well as the bark (Hamamelidis cortex) of the plant are used. The main constituents of the extract include: Tannin, gallic acid, catechins, proanthocyanins, flavonoids (kaempferol, quercetin), essential oils (carvacrol, eugenol, hexenol), choline, and saponins. It is a strong anti-oxidant and astringent, which makes it useful as a natural remedy also for acne, psoriasis, eczema, aftershave applications, ingrown nails, cracked or blistered skin, and for treating insect bites [16].

Quality plays an important role. Commonly used Hamamelis distillate (HMM-Water) elicits only low peaks for the active-principles. To achieve a rapid regeneration of the scalp and a positive course of the entire therapeutic process, the use of high-grade active principles from Hamamelis obtained from wild stocks and sustainable production, is, therefore, essential. Today, the following commercially used medicinal preparations are obtained from Hamamelis, with the corresponding ingredients and applications. Erol® Energy hair care products,(Apomedica, Switzerland), based on Virginian witch hazel, with the botanical name H. virginiana, have been specially developed for the care and treatment of the sensitive scalp. The shampoo is composed of extracts of H. virginiana and a shampoo base of mild tensidic character, free of cocamidopropyl betaine and parabens [17].Witch hazel with other herbs cloves, tea, cinnamon like phenolic in nature improve glucose metabolism via other mechanisms or that this in vitro screening is not a reliable predictor of hypoglycemic effects. They have positive effects of specific plant extracts on insulin activity suggest a possible role of these plants in improving glucose and insulin metabolism [18].

By using FT-IR, found many functional groups; carbonyl groups in 1670-1820 cm^{-1}which consider active compound in witch hazel in main structure (tannin). When its compare the curve with functional groups it will appear the main structures, which are contain form tannin,alkyl and flavonoid. The effects of witch hazel are come from tannin. This functional groups have many action as antioxidant,anti-fungal, anti-bacterial and as anti-septic and many other activities.The leaf contains 3-10% tannins (a mixture of catechins, gallotannins, plus cyanidin and delphinidin type proanthocyanidins), mainly hamamelose; catechins, mainly (+)-catechin, (+)-gallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate; phenolic acids (caffeic and gallic acids); flavonoid galactosides and glucuronides; flavonoids such as kaempherol, quercetin, quercitrin, and isoquercitrin.

0.01-0.5% volatile oil, among which 40% are aliphatic alcohols, 25% carbonyl compounds, 15% aliphatic esters, and a maximum of 0.2% safrol[19].

Witch hazel bark contains 8-12% tannins (minimum content recommended of 4% tannins). Cortex tannins are qualitatively similar to folium tannins, but have a higher content of hamamelitannin (1-7%), followed by monogalloylhamamelose, free gallic acid, condensed catechin tannins, and small amount of flavonols; approximately 0.1% volatile oil with a very complex composition. The bark contains significantly higher levels of phenylpropanoids and sesquiterpenoids in the volatile fractions compared to the leaves, which contain higher amounts of monoterpenoids. The bark is richer in hydrolysable tannins and the leaves mainly contain condensed tannins [20].

Tannins are a broad class of complex phenolic compounds of molecular weight between 500 and 3000. The biological importance of tannins is attributed to their ability to bind and precipitate mainly proteins but also alkaloids. Tannins are generally extracted with a water and acetone mixture. The polymeric proanthocyanidins and high molecular weight gallotannins remain in the aqueous phase. The therapeutic activity of tannins is mainly due to the astringency, and result from their affinity for proteins. Externally, they waterproof the external layers of the skin and mucosa's, thus protecting the underlying layers; they also have a vasoconstrictor effect on small superficial vessels. By limiting fluid losses, tannins enhance tissue regeneration in case of superficial burn or wound [9].

Generally speaking, tannins are enzyme inhibitors. They block 5-lipoxygenase; they inhibit angiotensin converting enzyme, hyaluronidase activation, and the glucosyl-transferases of microorganisms involved in the formation of cavities; ellagitannins and complex tannins inhibit protein kinase C [5].

Tannins especially the hydrolysable ones inhibit the peroxidation induced by ADP and ascorbic acid in rat hepatic mitochondria. In vitro they are radical scavengers and inhibitors of superoxide ion formation, and some of them inhibit lipoxygenase in rat peritoneal granulocytes [21]. The main characteristic constituent of *Hamamelis virginiana* L. is hamamelitannin, a mixture of the α- and β-forms of (2´, 5-di-O-galloyl-hamamelose), its molecular structure bears two gallate moeities and a sugar unit, hamamelose. Wang et al (2003) developed an HPLC method for the determination of hamamelitannin, catechins, and gallic acid from witch hazel bark, twig, and leaf. The concentrations in the bark for hamamelitannin, gallic acid, (+)-gallocatechin, and (+)-catechin were 4.77, 0.59, 0.22, and 0.39% (wt/wt), respectively. Hamamelitannin and catechins were also detected in the leaves at concentrations of < 0.04% (wt/wt) [20]. Polymeric proanthocyanidins were isolated from an acetone-water (7:3) extract from hamamelis bark in yields of about 5% by Dauer et al (2003)[22], Touriño et al [16]. Terminal chain units were catechin and gallocatechin in a constant ratio of 95:5. All chain extension units were completely galloylated at position 0-3, while chain terminating units were not galloylated. Predominant interflavan linkages were 4→8-bonds.

5. Conclusion

A traditional use for witch hazel is to soothe infections like poison ivy, and heal bruises and cuts, which are caused or exacerbated by viruses and bacteria. This astringent has an overall anti-inflammatory effect and an indirect antibacterial effect that makes it an ideal choice for minor cuts and bruises. Thisstudy found significant antimicrobial activity for witch hazel when investigating its effect on bacteria and fungi such as Staphylococcus aureus, Candida albicans and E. coli in vitro. This could help prevent bacterial colonization, which plays a crucial role in atopic dermatitis and intertrigo dramatis, including other microbial skin conditions. Witch hazel can help treat poison ivy, chicken pox, and heal bruises and cuts exacerbated or originated from viruses and bacteria [23]. From results of susceptibility tests of witch hazel on bacterial in two types gram positive (*staphalocucausaerues*) and gram negative *E.coli* and fungi as *Candia albicans* , comparing with FT-IR results that show the active compounds of witch hazel; the effects of witch hazel inhibited the growth of these organisms reasoning out the action of this extracts were due to the functional groups in main structure. Specially, the carbonyl group which have strong effect, comprised of aldehydes and ketones. Aldehydes can be formed by amino acid deamination or transamination, Strecker degradation, microbial activity during fermentation, and fatty acid oxidation, chelating agents making them perfect disinfectant, in addition to its antioxidant effects, and blood veins constructive.

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