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Amber Tincture as a Source of Natural Medicine: Evaluation of Phytochemicals and Bioactivity.

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ABSTRACT

Amber is resin that formed over millions of years from ancient conifer trees. Amber has always been desirable for its appearance and health benefits. Amber naturally contains active compounds - such as succinic acid, terpenoids, flavonoids, and phenolic compounds - which may be implicated in its health effects, with the most important being attributed to the succinic acid. Succinic acid has been implicated in several beneficial health effects, such as decreasing inflammation, neutralizing oxidative stress, and providing pain relief. Historically, amber has been used in natural remedies in tincture, oil and powder. So, while amber has had traditional historical use in these forms and remains prominent in phytotherapy and as an adjunct alternative medicine, amber is also of exceptional importance in paleontology, as it preserves prehistoric organisms and may provide index of ancient biodiversity. The increased interest in obtaining amber presents significant ethical and environmental issues such as mining practices that are unsustainable and it also involves a great deal of fly by night substitute products.

Keywords: Amber Tincture, Succinic Acid, Kikar Amber (Acacia nilotica), Kamarkas Amber (Butea monosperma) , Pine Amber (Pinus succinifera)

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INTRODUCTION

Over the centuries, amber (fossilized tree resin), has been a source of fascination for the world's civilizations, as an aesthetic material, historical, and potential medicinal importance. Commonly known for its use in jewelry and various decorative objects, amber also has significance regarding medicinal use, since amber has active therapeutic components, mainly succinic acid. Succinic acid has anti-inflammatory, antioxidant, and analgesic properties, which make amber an attractive ingredient for practitioners of conventional and alternative medicine [1]. Historical uses demonstrate many world cultures have used similar amber resins to relieve pain, infection, stress, and inflammatory disease. In this study, we will be addressing three types of amber-coloured resins: Kikar Amber (Acacia nilotica), Kamarkas Amber (Butea monosperma), and Pine Amber (Pinus succinifera). All three types of amber resins and other known resins have traditional healing, detoxifying, and antimicrobial activities [2] Kikar Amber is a well-known natural material used in herbal medicine for treatment of wounds and digestive concerns, while Kamarkas Amber is well respected in the strength field and women's health. Pine Amber (commonly referred to as Baltic amber) has been heavily studied for its respiratory effects, and neuroprotective and immune support functions[3].The present study will analyze these resins using biochemical assays of their respective phytochemical properties and chemical profiling. Kikar amber is amber-like resin from the acacia nilotica tree, used in Ayurvedic and Unani medicine. Kikar amber contains a high degree of flavonoids, tannins, and polyphenols, granting strong antioxidant and wound healing properties. Traditionally, kikar amber has been applied to joint pain, skin infections, digestive disorders, and respiratory disorders. Furthermore, scientific studies such as the ABTS assay, have shown that kikar amber has strong free radical scavenging properties, and the ability to protect normal cells [4]

Additional antimicrobial work has shown kikar amber effectively inhibits pathogenic bacteria such as *E. coli,* and *staphylococcus aureus*, indicating kikar amber may be a potential natural remedy for skin infections as well as improving digestive health. Kamarkas amber is another powerful resin derived from the butea monosperma tree, also known as the flame of the forest. Kamarkas amber is a deep red resin, and has been traditionally used for postpartum recovery, menstrual recovery, and muscle strengthening [5]. Kamarkas amber has been traditionally used believed to detoxify, stimulate blood circulation, and enhance tissue repair due to its rich phytochemical constituents. Kamarkas amber has shown to possess anti-inflammatory and pain relieving effects, hence it is beneficial for arthritis treatment, skin conditions, and balancing hormones.Of all the amber variations, Pine Amber (Pinus succinifera) has attracted the most research because of the amount of succinic acid. It is often referred to as Baltic amber, its fossilized resin. It has been used for centuries for pain relief, respiratory health, and immune system enhancement [6].The major component in Pine Amber, succinic acid, is believed to be the main ingredient responsible for the medicinal properties because there is scientific evidence to confirm the anti-inflammatory antioxidant, and neuroprotective properties. Historically, the Pine Amber has been worn as amber necklaces to relieve teething pain in infants. However, it can still be found today as a part of alternative therapies aimed at stress relief and joint pain. In recent studies with FTIR spectroscopy and GC-MS analysis, Pine Amber has found bioactive terpenes and resin acids responsible for its potential therapeutic actions in chronic pain and inflammation as well as oxidative stress (Lambert et al., 1985).Amber tincture made in vodka is a time-honored extraction method and is believed to increase the bioavailability of bioactive substances contained within amber. Vodka, which has a higher ethanol content than most distilled spirits (40% average), has been shown to be a great solvent for any resin-like and phytochemical properties, such as succinic acid, terpenoids, flavonoids, and phenolic compounds. During the process, finely powdered amber, or pieces of amber, are submerged in vodka and allowed to steep for a designated period of time (weeks), while the alcohol is able to dissolve the active compounds into a concentrated medicinal solution. This process guarantees preservation and stabilization of the extract so that it can be used therapeutically and medicinally. With the recent surge in interest towards natural medicine and plant medicine, amber tincture with vodka may have considerable implications for future herbal pharmacology, alternative medicine, and nutraceutical work. Future research may pursue the standardization of the distinction preparation process to determine uniform concentrations of bioactive components at therapeutic doses, with the hope that this will lead to a larger medical response [7].

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MATERIALS AND METHODS

Samples Collection

Amber resin samples were collected from the trunks of three plant species known for their medicinal resinous exudates: *Acacia nilotica* (commonly referred to as Kikar Amber), *Butea monosperma* (Kamarkas Amber), and *Pinus succinifera* (Pine Amber).The dried resin samples were collected, cleaned, and ground into a fine powder using a mortar and pestle.

Tincture Preparation

To make the amber tincture, Precisely weighed amounts (25g) of the powdered resin from each species—*Acacia nilotica*, *Butea monosperma*, and *Pinus succinifera*—were transferred into separate amber glass containers.Then 100 ml of plain vodka were added to beaker as a solvent. The contents were stirred vigorously to disperse the resin into the solvent. Then the beaker was covered in aluminium foil to prevent light exposure and placed in a cool, dry location. The mixture was allowed to macerate over the course of 21 days at room temperature (~25°C), with occasional shaking to enhance solvent penetration and extraction efficiency. After the extraction period, the macerated mixtures were filtered using Whatman No. 1 filter paper to remove undissolved particles. The filtrates were then collected as tinctures, labeled accordingly.

Analytical test for Amber Tincture

Density Test

Density testing is done using agraduated cylinder and digital balance. Density is expressed as Density = Mass (g) / Volume (mL), which indicate the concentration of the dissolved compounds[8].Density testing provides assurance that a tincture is made the same way consistently since density is an indicator of purity. Density should remain relatively consistent when multiple tests are done. A difference in density between samples may indicate that the material was not mixed correctly or that the tincture has degraded.

$$Density = \frac{Mass}{Volume}$$

pH Test

pH testing indicates the acidity or alkalinity of the amber tincture, affecting its stability and medicinal compatibility. A pH meter is calibrated with standard buffer solutions of pH 4, 7 and 10, and the electrode is dipped into the tincture A pH of 2.5 to 6.5 is advantageous to medicinal uses indicating mild acidity.[9]

Solubility Test

The solubility assessment investigates the tincture's solubility in multiple solvents, including water, ethanol, and oil. Assessing solubility tests ensures that the tincture will dissolve in whatever it is intended, will remain a homogeneous product, and assists in product development and stability. The tincture ought to dissolve completely in the solvents it is intended to be used with without any precipitation. The formation of anything from insolubility or separation is a sign of formulation trouble can pose some difficulty [10].

Viscosity Test

The viscosity test either using a viscometer, or using a more simple flow test (drop method) for assessing the thickness of the tincture. It is essential for a formulation to have consistent viscosity to maintain formulation consistency, meaning it continues to be easy to use and properly mixed. It is important that the viscosity remains consistent and uniform when tested multiple times, any variance may indicate phase separation or degradation [11].

Temperature Test of Amber Tincture

The temperature testing will take advantage of the variable temperature conditions to test the tincture: cold storage (4°C); room temperature (25°C); and very high temperature (40°C or above, during accelerated aging studies). The tincture will be studied for changes. Changes indicate potential temperature sensitivity, including (but not limited to) phase separation, changes in viscosity, evaporation of solvents or compounds within the tincture, and/or chemical degradation. pH testing, while not required for this test, can confirm any acid-base shifts. Overall, this test verifies that the tincture will remain stable when stored and transported under ambient storage conditions, or temperature extremes including cold or high. The tincture should not show any significant physical or chemical changes across the different temperature conditions. Any adverse or marked instability indicates temperature sensitivity and may be needed for formulation changes.[12]

Antimicrobial and Gut Health Analysis of Amber Tincture

Agar Well Diffusion Assay for Antibacterial Activity:

The antibacterial potential of amber tincture was evaluated using the agar well diffusion method against *Escherichia coli* (E. coli), a common pathogenic bacterium. Nutrient agar plates were prepared and uniformly inoculated with an overnight culture of *E. coli*. Wells of 6 mm diameter were aseptically punched into the agar and filled with defined volumes of amber tincture. Plates were incubated at 37°C for 24 hours. Post-incubation, the diameter of the clear inhibition zones surrounding each well was measured in millimeters. The presence of a zone of inhibition indicates antibacterial activity, with zones exceeding 10 mm signifying significant efficacy [3].This assay provides a qualitative and semi-quantitative measure of the tincture's ability to inhibit bacterial growth.

Probiotic Compatibility Assessment

To evaluate the compatibility of amber tincture with beneficial gut micro biota, probiotic strains *Lactobacillus spp.* were cultured on De Man, Rogosa, and Sharpe (MRS) agar supplemented with varying concentrations of the tincture. Inoculated plates were incubated at 37°C for 24 to 48 hours under anaerobic conditions optimal for probiotic growth. The extent of colony formation and growth patterns were compared against control cultures grown without tincture. Unaffected or enhanced growth of probiotic colonies indicates that the amber tincture does not exert inhibitory effects on these beneficial bacteria and is thus considered gut-friendly. This test is crucial for determining the suitability of amber tincture for applications involving gut health modulation [13].

Phytochemical Analysis

For phytochemical screening 3 different amber samples was selected to determine the presence of several bioactive agents.

Flavonoids Test (Alkaline Reagent Test): A few drops of sodium hydroxide (NaOH) were added to the extract. The appearance of yellow coloration was indicative of positive flavonoid response.

Tannins Test (Ferric Chloride Test): The extract was treated with 1% ferric chloride solution. The appearance of blue, dark blue, black, or green coloration was indicative of positive tannin response.

Saponins Test (Foam Test): A small amount of the extract was added to distilled water and vigorously shaken for a few minutes. The presence of stable foam lasting for a few minutes was indicative of positive response for saponins.

Phenols Test (Alkaline Chloride Test): The extract was treated with alkaline chloride reagents. The color change was considered a positive response for presence of phenolic compounds.

Terpenoids Test (Salkowski's Test): The extract was treated chloroform and concentrated sulfuric acid (H_2SO_4). Reddish-brown coloration at the interface indicated positive response for terpenoids.

Carbohydrates Test (Molisch's Test): Add a few drops of Molisch's reagent to the extract, then add concentrated sulphuric acid (H2SO4) down the side of the test tube. The formation of a purple ring at the interface of the two layers indicates the presence of carbohydrates or glycosides.

RESULTS AND DISCUSSION

Analytical test for Amber Tincture

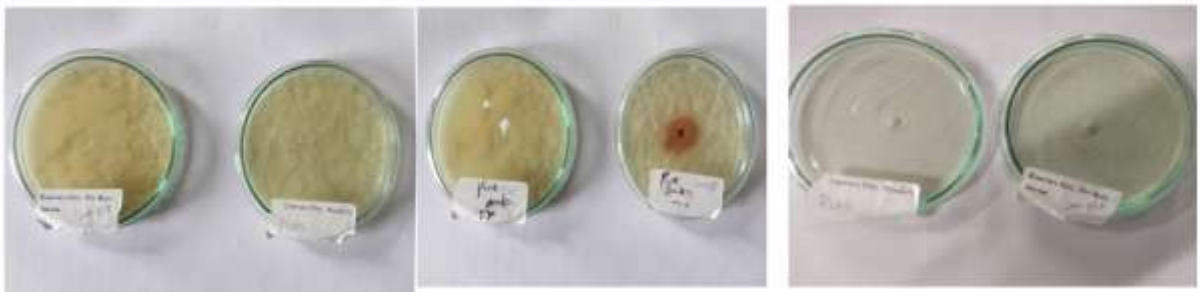
Table1. Comprehensive Analytical Evaluation of Amber Tinctures from Three Plant Sources:

Sr. No.	Analytical Parameter	Kikar Amber (*Acacia nilotica*)	Kamarkas Amber (*Butea monosperma*)	Pine Amber (*Pinus succinifera*)
1	**Density (g/mL)**	~0.98 – 1.00	~0.99 – 1.01	~0.97 – 0.99
2	**pH**	5.6	4.2	2.7
3	**Solubility in Ethanol**	Moderately soluble	Highly soluble	Moderately soluble
4	**Viscosity** *(qualitative)*	Moderate	High	Moderate
5	**Thermal Stability (4–40°C)**	Stable; slight alcohol loss at 40°C+	Stable; slight alcohol loss	Stable; slight alcohol loss
6	**Boiling Temperature (°C)**	92	74	82

Bioactivity and Compatibility Evaluation

Antimicrobial Activity (Agar Well Diffusion Method)

The antibacterial activity of the amber extract was evaluated using the Agar Well Diffusion Test and showed some form of bacterial inhibition. The extract caused a clear zone of inhibition around the wells with the amber extract, demonstrating its antibacterial potential. The size data of the inhibition zone varied based on the bacteria type that was being tested, suggesting that amber extract may have different antibacterial activity. The positive control (antibiotic solution) showed a larger inhibition zone, validating the method while the negative control (70% ethanol alone) showed no inhibition, confirming that the antimicrobial effect was provided due to the amber extract. The results demonstrate that the bioactive compounds located within the amber extract may have antibacterial properties.



a **b** **c**

Figure 1: Zones of inhibition against *E. coli of a.* Kikar Amber, b. Pine Amber and c. Kamarkas Amber, respectively.

Table 2. Antibacterial Activity of Amber Tinctures

Sr. No.	Sample	Zone of Inhibition (mm)	Activity Level
1	Kikar Amber (*Acacia nilotica*)	10 mm	Moderate
2	Kamarkas Amber (*Butea monosperma*)	14 mm	Strong
3	Pine Amber (*Pinus succinifera*)	12 mm	Moderate

Probiotic Compatibility (Gut Health Assessment)

To assess the gut-friendliness of the amber tinctures, probiotic compatibility was evaluated using *Lactobacillus* spp. plated on MRS agar. The tinctures showed no inhibitory effect on probiotic colony formation. CFU counts were consistent with control plates, suggesting the tinctures do not adversely affect beneficial gut flora. No zones of inhibition were observed near the amber extract application site, indicating compatibility with probiotic organisms. These findings support the potential of amber tinctures for oral formulations that maintain gut microbiota balance.

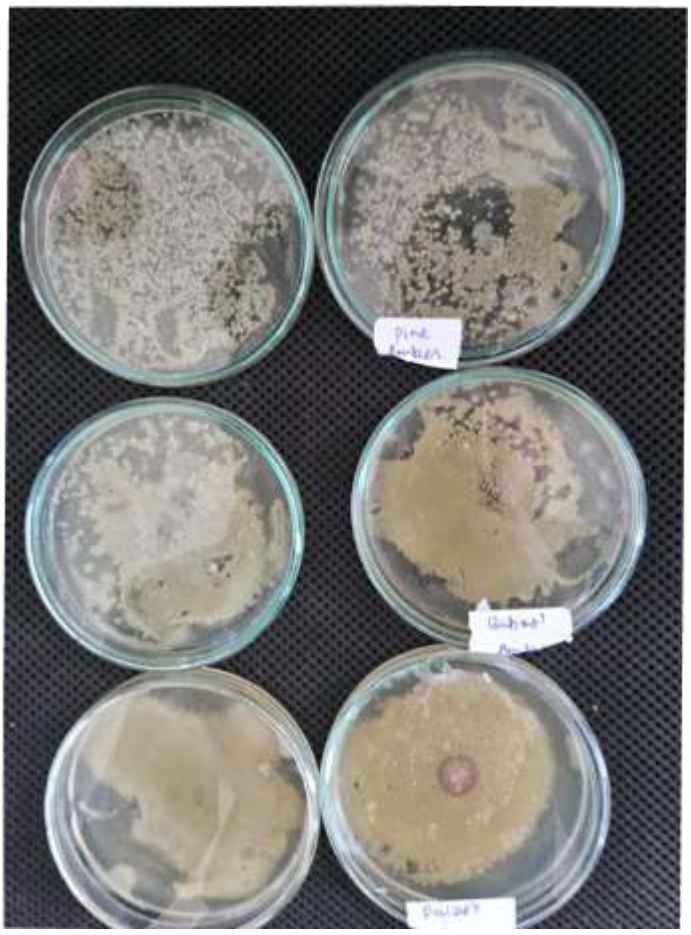


Figure 2: Compatibility Testing of Amber Extracts with Probiotic *Lactobacillus* spp

Phytochemical Analysis

A preliminary phytochemical screening was conducted to identify the presence of various bioactive compounds in the ethanol-based amber tinctures

Phytochemical Test	Result
Flavonoids Test (Alkaline Reagent Test)	+
Tannins Test (Ferric Chloride Test)	+
Saponins Test (Foam Test)	-
Phenols Test (Alkaline Chloride Test)	-
Terpenoids Test (Salkowski's Test)	++
Carbohydrates Test (Molisch's Test)	+

Here, ++ indicates high amount of the presence of the respective compounds, + indicates Moderate amount of presence of the respective compounds while – indicates complete absence of the respective compounds

CONCLUSION

Amber has historically been valued in traditional medicine, and contemporary studies are starting to substantiate its therapeutic benefits. This research indicated that amber tinctures obtained from *Acacia nilotica, Butea monosperma, and Pinus succinifera* are abundant in bioactive constituents, including succinic acid, flavonoids, phenolics, and terpenoids. These compounds contribute to its properties as an antioxidant, antimicrobial, and anti-inflammatory, indicating potential applications for immune support, digestive health, skincare, and wound care. The tincture's compatibility with probiotic strains points to its potential benefits for gut health, while its antimicrobial properties suggest wider applications, such as food preservation and wellness drinks. As the interest in plant-based therapies continues to grow, amber emerges as a promising candidate for incorporation into herbal and pharmaceutical products. Further investigation is necessary to standardize extraction techniques, optimize dosage, and verify clinical effectiveness. Additionally, sustainable sourcing needs to be prioritized to ensure long-term availability and ethical utilization.

REFERENCE

[1] Zeikus J, Jain M, Elankovan P. Biotechnology of succinic acid production and markets for derived industrial products. Appl Microbiol Biotechnol. 1999;51:545–552. https://doi.org/10.1007/s002530051431

[2] Bhushette PR, Annapure US. Comparative study of Acacia nilotica exudate gum and acacia gum. Int J Biol Macromol. 2017;102:266–271. https://doi.org/10.1016/j.ijbiomac.2017.03.178

[3] Kang S, Kim J, Lee S, et al. Anti-inflammatory activities of amber extract in LPS-induced macrophages. Int Immunopharmacol. 2021;96:107611. https://doi.org/10.1016/j.intimp.2021.107611

[4] Wang H, Xia B, Lin M, et al. Succinic acid inhibits CYP450 enzyme activity. Pharm Biol. 2020;58(1):1150–1155. https://doi.org/10.1080/13880209.2020.1839110

[5] Helmy YA, Taha-Abdelaziz K, Hawwas HAE-H, et al. Antimicrobial resistance and recent alternatives to antibiotics. Antibiotics. 2023;12:274. https://doi.org/10.3390/antibiotics12020274

[6] Ferone M, Raganati F, Olivieri G, Marzocchella A. Bioreactors for succinic acid production. Crit Rev Biotechnol. 2019;39(4):571–586. https://doi.org/10.1080/07388551.2019.1592105

[7] Lambert JB, Santiago-Blay JA, Anderson KB. Chemical signatures of fossilized resins. Angew Chem Int Ed Engl. 2008;47(50):9608–9616. https://doi.org/10.1002/anie.200705973

[8] Benning JW, Moeller DA. Microbes, mutualism, and range margins: Testing the fitness consequences of soil microbial communities across and beyond a native plant's range. New Phytol. 2021;229:2886–2900. https://doi.org/10.1111/nph.17102

[9] Duffin CJ. Historical survey of the internal use of unprocessed amber. J Hist Med Allied Sci. 2015;70(3):420–443. https://doi.org/10.1093/jhmas/jrv003

[10] Sik B, Hanczné Lakatos E, Kapcsándi V, et al. Investigation of the long-term stability of various tinctures belonging to the Lamiaceae family by HPLC and spectrophotometry. Chem Pap. 2021;75:5781–5791. https://doi.org/10.1007/s11696-021-01755-z

[11] Ferone M, Raganati F, Olivieri G, Marzocchella A. Bioreactors for succinic acid production. Crit Rev Biotechnol. 2019;39(4):571–586. https://doi.org/10.1080/07388551.2019.1592105

[12] Lambert JB, Frye JS, Poinar GO Jr. Amber from the Dominican Republic: NMR analysis. Archaeometry. 1985;27(1):43–51. https://doi.org/10.1111/j.1475-4754.1985.tb00315.x

[13] Beauprez JJ, De Mey M, Soetaert WK. Microbial succinic acid production: Natural vs metabolic engineered producers. Process Biochem. 2010;45(7):1103–1114. https://doi.org/10.1016/j.procbio.2010.03.035