Antioxidant Potential And Functional Group Characterization Of Medicinal Plant Extracts: Application In Herbal Cream Development

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Abstract
The current work examines the phytochemical makeup, antioxidant capacity, and FTIR-based functional group characterisation of extracts from Tecoma stans, Rosmarinus officinalis, and Cinnamomum zeylanicum before incorporating them into an herbal cream formulation. Secondary metabolites, such as flavonoids, terpenoids, phenols, glycosides, and saponins, which are known to contribute to antioxidant activity, were found using qualitative phytochemical screening. When DPPH, ABTS, and nitric oxide radical scavenging assays were used to evaluate antioxidant capability, C. zeylanicum showed the best radical scavenging efficiency of all the extracts. In comparison to the separate extracts, the three extracts worked in concert to provide IC_{50} values that were noticeably lower in every assay. The phytochemical results were validated by FTIR spectral analysis, which also verified the presence of functional groups such hydroxyl, amine, carbonyl, and aromatic chemicals. After being tested for physicochemical characteristics such as pH, viscosity, spread ability, washability, and stability, a herbal cream made using these extracts was determined to be appropriate for topical use. Overall, the study shows how these medicinal plants can be used to create stable and appealing herbal formulations and highlights their potential as natural antioxidant sources.

Introduction

Antioxidants are essential biomolecules that protect the human body by neutralizing reactive species and minimizing oxidative damage. They play a crucial role in maintaining redox balance, thereby reducing the risk of chronic illnesses associated with oxidative stress [1]. Natural antioxidants are generally preferred over synthetic ones such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), as the latter are often unstable and may exhibit toxic effects upon prolonged use [2]. In contrast, plant-derived antioxidants are considered safer and more effective, which has increased global interest in herbal sources of therapeutic antioxidants [3].

Reactive oxygen species (ROS), including hydroxyl, nitric oxide, hydrogen peroxide, superoxide anions, hypochlorite, lipid peroxides, and singlet oxygen, are produced in the body during normal physiological metabolism [4]. Under controlled conditions, they play certain signaling roles; however, excessive ROS accumulation due to metabolic imbalance or inadequate antioxidant defences causes oxidative stress [5]. Elevated levels of ROS are directly implicated in the onset and progression of numerous degenerative diseases such as diabetes, cancer, atherosclerosis, cardiovascular disorders, arthritis, neurodegenerative conditions, and premature aging [6]. Moreover, external factors including radiation, pollution, smoking, and alcohol consumption further aggravate oxidative damage [7].

The plant-based phytoconstituents, which hare flavonoids, phenols, anthocyanins, iso-flavones, lignins, catechins, iso-catechins, and coumarins are the primary constituents that contribute to antioxidant potential. Assays for 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2-azinobis-ethylbenzothiozoline-6-sulphonic acid (ABTS) are used to measure the antioxidant effects [8]. Antioxidants are substances that may effectively hunt down and destroy free radicals, hence improving the sick state caused by ROS. Plants with strong antioxidant capacity have been shown to contain a wide variety of free radical hunting or antioxidant components, including

phenols, vitamins, terpenoids, and flavonoids [9]. Since plant-derived polyphenolic components have been shown to be more effective antioxidants than vitamin E or C in vitro, they may have more beneficial effects in vivo [10]. Due to their alimentary radical potential, certain medicinal herbs with antioxidant capacity have been effectively used to treat ROS and are very important. supplement that scavenges food. The abundance of phytonutrients and components such as phenols, flavonoids, and terpenoids found in medicinal plants is primarily responsible for their antioxidant activity. Numerous medicinal plants' antioxidant potential has been investigated for its hepatoprotective, immunomodulatory, anticancer, and hypolipidemic properties [11]. Therefore, the assessment of free radical scavenging ability (RSA) and the comparison of different medicinal plants using spectrophotometers with varied procedures are efficient methods for studying secondary metabolites in plants [12].

One of the most significant and widely used spices in the world, the bark of several cinnamon species is utilized in both traditional and modern medicine in addition to cooking. In general, Trees of the cinnamon genus are found all over the world, and there are over 250 species known to exist [13,14]. Because of its fragrance, which can be added to a wide range of foods, fragrances, and pharmaceutical products, cinnamon is primarily used in the aroma and essence industries [15]. Cinnamaldehyde and trans-cinnamaldehyde (Cin), the two most significant components of cinnamon, are found in the essential oil and contribute to the fragrance and different biological activities associated with cinnamon [16]. According to a study on Cinnamomum osmophloeum (C. osmophloeum), there is a significant amount of Cin in the essential oil extracted from cinnamon leaves. As a result, another spice substitute for Cassia is C. osmophloeum [17]. One of the key elements of essential oil extracted from C. zeylanicum termed (E)-cinnamaldehyde has an antityrosinase action [18], while cinnamaldehyde is the principal chemical responsible for this activity [19]. Procyanidins and catechins are found in cinnamon bark [20]. Procyanidin A-type and B-type linkages are among the constituents of procyanidins [21–23]. These procyanidins berry and cinnamon extracts have antioxidant properties as well. [21, 24]

The medicinal use of plants has been around for a while. Salvia rosmarinus Schleid. and Rosmarinus angustifolius Mill. are synonyms for Rosmarinus officinalis Linnaeus (1753, Lamiaceae). [25]. This species is a widely grown plant that is well-known in regional and traditional medicine for its pharmacological qualities and nutritional worth. Because of its antimicrobial and antioxidant properties, it is used as a food flavoring and preservative in the food industry. Cosmetic goods also contain R. officinalis [26]. The biological activities of R. officinalis essential oil (EORO) are ascribed to a number of compounds, primarily monoterpenes, including 1,8-cineole, borneol, pinene, limonene, camphene, camphor, and myrcene. [26,27] EORO is utilized as a supplemental treatment for inflammations, muscular or articular discomfort, circulatory abnormalities, and milder kinds of spasmodic gastrointestinal problems in addition to dyspepsia [28] An adaptive physiological state called inflammation is brought on by stress, infection, or tissue damage in an effort to restore tissue homeostasis. Nevertheless, inflammation-induced self-damage cannot be prevented, and its irreversibility causes harmful diseases [29,30], necessitating the creation of anti-inflammatory drugs to manage it. In addition to being used in traditional and local medicine to treat diseases linked to inflammation, EORO has been shown to have anti-inflammatory properties in both in vitro and in vivo tests [31].

One significant medicinal plant is tecoma stans. Alkaloids, phenols, terpenoids, glycosides, flavonoids, and saponins were among the main bioactive substances that had been separated from this plant. Traditional folk remedies are made from extracts of the biologically active compounds found in the leaves, bark, and roots. [32]. Phytoconstituents such as phytosterol, triterpene, glycosides, phenols, flavonoids, saponins, and tannins, either alone or in combination, may have a synergistic effect on wound healing.[33] tecoma stans has been shown to have anti-inflammatory properties in both in vitro and in vivo tests.

MATERIALS AND METHODS

Materials

Collection of plant material

Cinnamon, rosemary, tecoma are collected from the state forest research institute Jabalpur [M.P].

Preparation of extracts

Cinnamon barks were separately shade dried. Grinder is use for the pulverization of powder. In Soxhlet apparatus, the leave powder was extracted by ethanol and distilled water. After that the extract was hot filtered. By using distillation process solvents is removed. By reducing the pressure, the solvent is fully removed.

Rosemarry leaves were separately shade dried. Grinder is use for the pulverization of powder. In Soxhlet apparatus, the leave powder was extracted by ethanol and distilled water. After that the extract was hot filtered. By using distillation process solvents is removed. By reducing the pressure, the solvent is fully removed.

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Qualitative phytochemical screening

Presence or absence of different Phyto-constituents (qualitative tests for phytochemicals) in the prepared extracts of important selected medicinal plants C. zeylanicum, Rosmarinus officinalis, tecoma stans. Different chemical reagents were prepared. These tests were performed according to the standard procedures with minor modification [32-34].

Test for terpenoids [33]

The reddish-brown color showed the presence of terpenoids when 0.2 g of each sample was mixed with 3mL of conc. H2SO4.2 mL chloroform.

Test for glycosides [32]

Molisch's reagent test: 5mL of Molisch's reagent and concentrated H2SO4 were added to the extract. The glycosides were indicated by violet color.

Test for reducing sugar [33]

1mL of water and 5–8 drops of Fehling's solution were added to 0.5 mL of plant extract and was heated. The appearance of brick red precipitation indicated the presence of reducing sugar.

Test for quinine [33]

The ammonium thiocyanate and freshly prepared FeSO4 solution (1mL) were added to the extract, and then conc. H2SO4 was added drop by drop. The presence of quinine was indicated by deep red color.

Test for saponins [32]

20 mL of dH2O was boiled with 2 g powdered sample. 5mL of dH2O and 10 mL of filtrate were quivered vigorously. The presence of saponins was indicated by the appearance of frothing.

Dil. NH3 tests [34]

To the extract, 5mL of dilute NH3 solution was added along with conc. H2SO4. The flavonoids were indicated by the appearance of yellow-color.

Test for flavonoids [34]

Shinoda test: 4mL of extract solution, 1.5 mL of 50% methanol solution, and a small magnesium chunk were warmed. 5–6 drops of con. HCl were added. Red color was observed for flavonoids.

Test for volatile oils [32]

A small quantity of dilute HCl and 0.1 mL of NaOH were shaken with 2mL of extract. The presence of volatile oil was indicated by the white precipitate.

Dragendroff's reagent test [32]

2% H2SO4 and 2mL of extract were warmed with the addition of few drops of Dragendroff's reagent. The presence of alkaloids was observed by the orange-red color.

Test for alkaloids: Meyer's test [33]

1mL of Meyer's reagent was added to 2mL of extract. The presence of alkaloids was indicated by the pale-yellow precipitate.

Test for steroids [34]

A few drops of acetic acid and a drop of conc. H2SO4 were added in 1 g plant extract. The presence of steroids was indicated by the appearance of green color.

Test for cardiac glycosides [34]

2mL of glacial acetic acid with one drop of FeCl3 solution was used to treat the 5mL of plant extract. The presence of cardiac glycosides was indicated by the appearance of a violet ring or a greenish ring.

Antioxidant assays

To determine the antioxidant effects of plant extracts, different antioxidant assays for the selected plant extracts were performed according to standard procedures with minor modifications.

DPPH antioxidant assay

By calculating the DPPH radical's scavenging capability, antioxidant activity was evaluated. With some adjustments, the DPPH test is conducted using the methodology outlined by [35] 2 ml of a methanolic solution of DPPH (0.006%) is combined with 2 ml of the methanolic solutions of M. vulgare L. leaf extracts made from a stock solution (10 mg/ml) at various concentrations (200 µg/ml, -1000µg/ml). A UV spectrophotometer was used to measure the absorbance at 517 nm against a blank after 30 minutes. Using Eq. (1), the proportion of DPPH inhibition was determined.

% Inhibition ¼ [(Abscontrol-Abstest)/Abstest] *100 (1)

The results were expressed as IC50. The lower IC50 value is an indication of a more potent antioxidant activity.

ABTS assay

With certain adjustments, the ABTS test was assessed in accordance with [36]. Two solutions are prepared: the potassium persulfate (K2S2O8) solution (2.45 mM) and the ABTS solution (7 mM). The Abts cation radical solution was created by combining the two solutions at a ratio of (1:0.1). After 12 to 16 hours in a dark environment, the stock solution was diluted using a spectrophotometer set to 734 nm to achieve an absorbance of 0.70 to 0.02. After adding 200 µl of M. vulgare L. extract at varying concentrations (200–1000 µg/ml) to 1 ml of the prepared solution, the absorbances of the extract were measured at 734 nm after three minutes in comparison to a blank. Using Equation (2), the ABTS scavenging percentage was determined.

Scanning activity % ¼ [(Abs Control – Abs sample) /Abs Control] _ 100 (2)

The results were expressed as IC50. The lower IC50 value is an indication of a more potent antioxidant activity

Scavenging of nitric oxide radical

From sodium nitroprusside, nitric oxide is evaluated and it was measured by Griess reaction. Different concentration of extract was incubated in in sodium nitroprusside and in phosphate buffer. Tubes are incubated into 25^0C temperature for 5hr. In identical manner the test control was conducted in identical manner. After completion of 5h 0.5ml of incubation solution is removed and Griess solution is use for dilution. At diazotization of nitrite, the chromophore of absorbance with sulphanilamide with coupling of naphthylmethyl diamine at 546nm [37].

Analysis by FTIR

Spectrum was used to scan the various extracts in the wavelength range of 8,300 to 350 cm-1 with a resolution of 0.5 cm-1 in order to investigate the chemical functional groups of l extracts of Cinamon, Rosemary, Tecoma that were collected from two distinct geographic locations. Using two PerkinElmer FTIR spectrometers, distinctive peaks and their functional groups were found. Peak FTIR readings were noted. Each study was conducted three times to confirm the spectrum.

2. Preparation of polyherbal cream

To begin the cream formulation, the oil phase was prepared by heating liquid paraffin and beeswax in a borosilicate glass beaker at 75 °C, maintaining the temperature to ensure complete melting and uniform mixing. Simultaneously, the aqueous phase was prepared by dissolving borax and methylparaben in distilled water in a separate beaker. This solution was also heated to 75 °C until both borax and methylparaben were fully dissolved, yielding a clear solution.

Once both phases reached the desired temperature, the aqueous phase was slowly added to the oil phase with continuous stirring. This gradual addition helps in the formation of a stable emulsion. After the emulsion began to form, a pre-measured amount of herbal extracts Cinnamomum zeylanicum, Tecoma stans, and Rosmarinus officinalis were incorporated into the base. The mixture was stirred vigorously to ensure uniform dispersion of the extracts and to develop a smooth consistency. A few drops of rose oil were then added to impart a pleasant fragrance. To refine the texture, the cream was transferred onto a glass or ceramic slab, where a few drops of distilled water were added if necessary. The cream was then mixed using the geometric dilution method, ensuring all ingredients were thoroughly.[38] (ingredient represented the table 1).

Table1: Formulation of cream

S. No.	Ingredients	Quantity
1	Rosemarry extract	1 ml
2	Tecoma extract	1ml
3	Cinnamon extract	1ml
4	Beeswax	3gm
5	Liquid paraffin	10ml
6	Borax	0.2gm
7	Methylparaben	0.02gm
8	Distilled Water	q.s

Evaluation of herbal cream

Physical evaluation [39]

In this test, the cream was observed for color, odor, texture, state

Wash ability [39]

A small amount of cream was applied on the hand and it is then washed with tap water.

PH [40]

0.5 g cream was taken and dispersed in 50 ml distilled water and then PH was measured by using digital PH meter.

Viscosity [39]

Viscosity of cream was done by using Brooke field viscometer at a temperature of 25 ℃ using spindle No. 63 at 2.5 RPM.

Phase separation [39]

Prepared cream was kept in a closed container at a temperature of 25-100 ℃ away from light. Then phase separation was checked for 24 h for 30 d. Any change in the phase separation was observed/checked.

Spread ability [39]

The spreadability was expressed in terms of time in seconds taken by two slides to slip off from the cream, placed in between the slides, under certain load. Lesser the time taken for separation of the two slides better the spreadability. Two sets of glass slides of standard dimension were taken. Then one slide of suitable dimension was taken and the cream formulation was placed on that slide. Then other slide was placed on the top of the formulation. Then a weight or certain load was placed on the upper slide so that the cream between the two slides was pressed uniformly to form a thin layer. Then the weight was removed and excess of formulation adhering to the slides was scrapped off. The upper slide was allowed to slip off freely by the force of weight tied to it. The time taken by the upper slide to slip off was noted.

Spread ability= m × l/t

Where,

m= Standard weight which is tied to or placed over the upper slide (30g)

l= length of a glass slide (5 cm)

t= time taken in seconds.

RESULT

Phytochemical screening

The phytochemical screening of the organic extracts of Cinnamomum zeylanicum, Rosmarinus officinalis, and Tecoma stans revealed notable variations in the presence of secondary metabolites. The extract of Cinnamomum zeylanicum showed a high content of volatile oils, polyphenols, and flavonoids, with moderate levels of tannins and terpenoids, while alkaloids, saponins, and coumarins were absent. In the case of Rosmarinus officinalis, the organic extract was found to be rich in flavonoids, terpenoids, and polyphenols, along with moderate amounts of resins and volatile oils, but it lacked alkaloids, saponins, and mucilage. Similarly, the extract of Tecoma stans exhibited a moderate presence of glycosides, saponins, and resins, while flavonoids and terpenoids were present in smaller quantities, and alkaloids, tannins, and coumarins were not detected. Overall, across all three medicinal plants, the organic extracts consistently demonstrated higher concentrations of phytochemicals compared to their aqueous counterparts, indicating the efficiency of organic solvents in extracting bioactive constituents (as represented in Table 2).

Table:2 Phytochemical Screening of The Organic Extracts of Cinnamomum Zeylanicum, Rosmarinus Officinalis, and Tecoma Stans

Test Name	Cinnamon	Rosemary	Tecoma stans
Terpenoids	+	+	+
Glycosides	+	+	+
Reducing Sugars	+	+	+
Quinine	_	_	_
Saponins	+	+	+
Flavonoids (NH_3 Test)	+	+	+
Flavonoids (Shinoda)	+	+	+
Volatile Oils	+	_	_
Alkaloids (Dragendorff)	-	_	+
Alkaloids (Meyer's)	_	_	+
Steroids	_	_	+
Cardiac Glycosides	_	_	+

Antioxidant activity

According to the presented results in the table below (Table 3) the extracts of Tecoma stans, Rosmarinus officinalis, and Cinnamomum zeylanicum, as well as their combination, showed varying levels of antioxidant activity in DPPH, ABTS, and Nitric Oxide radical scavenging assays among other invitro models. The highest antioxidant potential was consistently shown by Cinnamomum zeylanicum across individual plant extracts, with IC_{50} values of 96.7 ± 2.1 µg/mL for Nitric Oxide, 98.2 ± 2.0 µg/mL for ABTS, and 92.4 ± 1.9 µg/mL for DPPH. Rosmarinus officinalis came next, with IC_{50} values of 114.5 ± 2.3 µg/mL (DPPH), 107.5 ± 2.4 µg/mL (ABTS), and 113.5 ± 2.5 µg/mL (NO). Tecoma stans has the lowest individual antioxidant capacity of the three, with IC_{50} values for DPPH, ABTS, and Nitric Oxide tests of 132.1 ± 2.6 µg/mL, 135.1 ± 2.7 µg/mL, and 129.6 ± 2.7 µg/mL, respectively.

The combined extract of the three plants exhibited noticeably higher antioxidant activity in every assay that was evaluated, suggesting that the phytoconstituents may interact synergistically. In comparison to the individual extracts, the combined extract had the lowest IC_{50} values, which were 59.2 ± 1.7 µg/mL (DPPH), 63.7 ± 1.7 µg/mL (ABTS), and 60.4 ± 1.6 µg/mL (NO)

Table:3 Inhibitory concentration 50 (IC50) values for Antioxidant Assay

Antioxidant Assay	IC50 value of Extract			
	Cinamon	Rosemary	Tecoma	Combination of all extract
DPPH Assay	98.2 ± 2	107.5 ± 2.4	135.1 ± 2.7	63.7± 1.7
ABTS Assay	92.4 ± 1.9	114.5 ± 2.3	132.1 ± 2.6	59.2 ± 1.7
Scavenging of nitric oxide radical	96.7 ± 2.1	113.5 ± 2.5	129.6 ± 2.7	60.4 ± 1.6

Phytochemical analysis by the FTIR technique

To determine distinctive functional groups based on their absorption bands, the FTIR spectra of the separate plant extracts (Cinnamomum zeylanicum, Rosmarinus officinalis, and Tecoma stans) were examined. Tables 4, 5, and 6 display the main peaks and their respective functional groups, and Figures 1, 2, and 3 display the spectra. According to previously published phytochemical FTIR studies, these spectral interpretations are consistent [40].

A. Extract from Cinnamomum zeylanicum Cinnamomum zeylanicum's methanolic extract showed noticeable absorption bands around 3320–3300 cm^{-1}, which corresponded to N–H or O–H stretching vibrations and were suggestive of primary amines or phenols. A prominent band close to 1634 cm^{-1} indicated the existence of C=C stretching (aromatic compounds) or an amide I group, whereas peaks around 2915–2849 cm^{-1} were ascribed to aliphatic C–H stretching. C–N stretching vibrations of aromatic amines and amide III regions were indicated by additional peaks in the 1380–1240 cm^{-1} area.

B. Extract from Rosmarinus officinalis A large O–H stretching band at about 3302 cm^{-1} was visible in the Rosmarinus officinalis methanolic extract's FTIR spectrum, indicating the presence of alcohols and polyphenols. Aromatic C=C or conjugated C=O vibrations were represented by a strong peak at 1641 cm^{-1}. CH_2 bending and C–N vibrations were identified as the causes of the medium to strong bands seen at 1454 cm^{-1} and 1371 cm^{-1}, respectively. The C–O–C and C–O stretching from ethers and esters was suggested by vibrations in the 1248–1047 cm^{-1} range.

C. Extract from Tecoma stans According to Tecoma Stans' FTIR profile, hydroxyl and N-H groups exhibit strong absorption between 3400 and 3305 cm^{-1}. C-H stretching vibrations of -CH_2 groups, both symmetric and asymmetric, were identified as the cause of the peak about 2920 cm^{-1}. C=O stretching or aromatic C=C bonding were suggested by the band at 1635 cm^{-1}. Bands ranging from 1456 cm^{-1} to 1030 cm^{-1} suggested the presence of esters, aliphatic amines, and maybe glycosidic linkage.

FTIR spectra of the Cinamon Extract

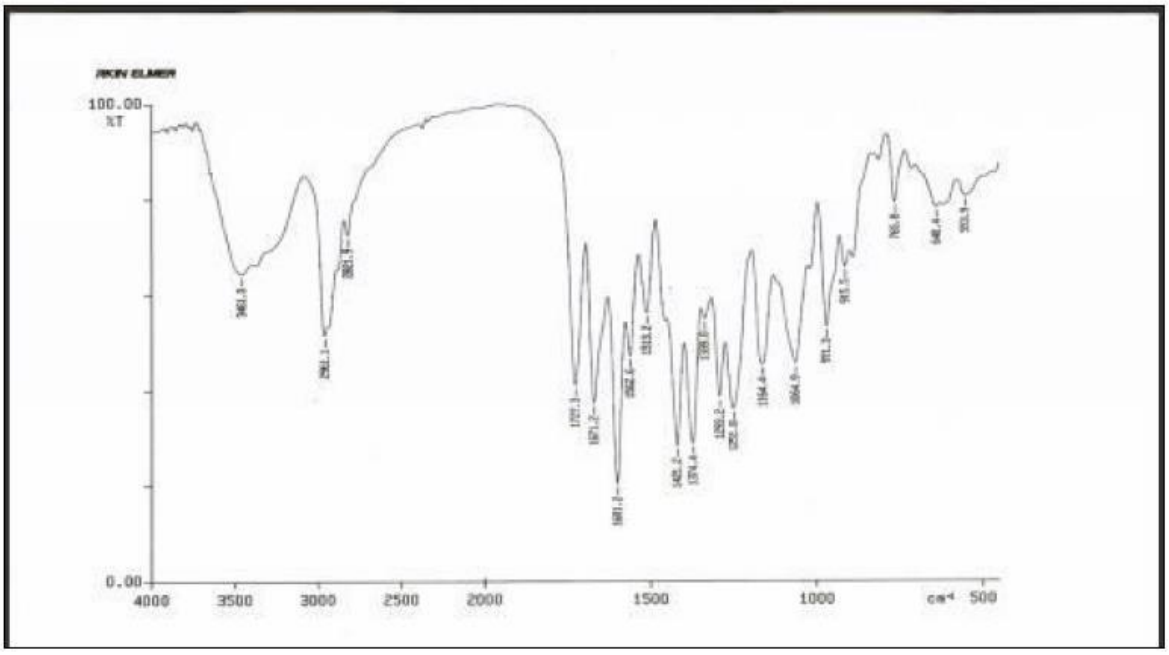

Fig:1 Spectra of cinnamon extract

S.No.	IR Absorption band cm	Assignments
1	3461.0	O-H stretching (Alcohol, ROH)
2	2961.1	Aliphatic C-H stretch
3	2821.9	C-H stretch (Carboxylic acid)

4	1727.3	C=O stretch of aldehyde
5	1671.2	C=O stretch (cyclic amide)
6	1601.2	C=C stretch Benzene (aromatic)
7	1526.6	Asymmetric (ArNO2) stretch
8	1421.2	O-H bending (Carboxylic acid)
9	1064.9	C-N stretch (Amine)
10	1164	C-O stretch (3 ROH)

Table:4 General band assignments of the FTIR spectra of the Cinamon Extract

FTIR spectra of the Rosemary Extract

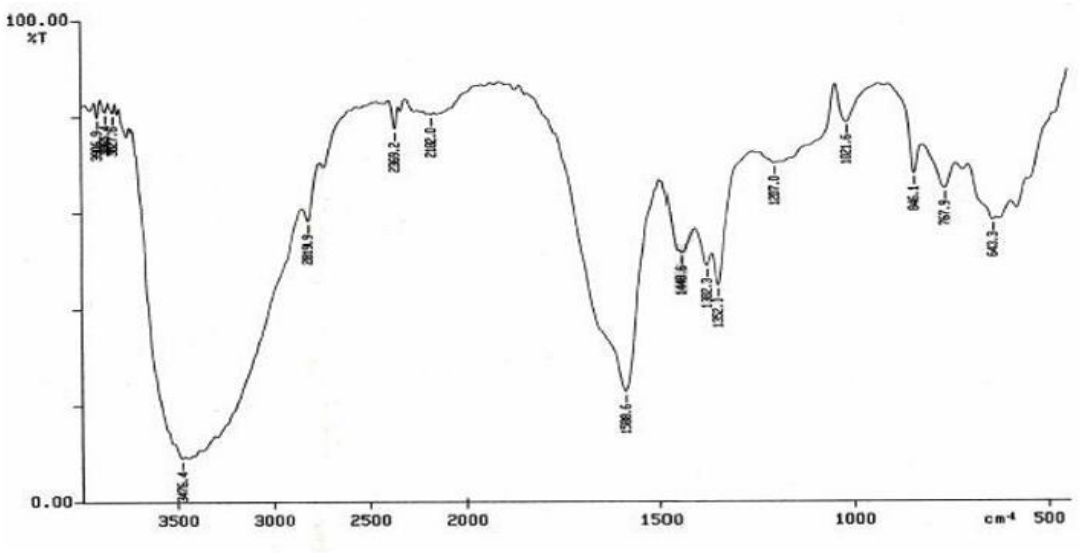

Fig2: Spectra of Rosemary extract

S.No.	IR Absorption band cm	Assignments
1	3610	O–H stretch O–H stretch of mannose
2	3610	C=N stretch Amide group
3	21575	N-H bending Secondary amine
4	2841.9	C-H bending (in plane) Methyl and methylene group
5	1382.31	C-H bending (out plane) Methyl and methylene group
6	1075821.9	C–O stretch of carbohydrate around

Table:5 General band assignments of the FTIR spectra of the Rosemay Extract

FTIR spectra of the Tecoma Extract

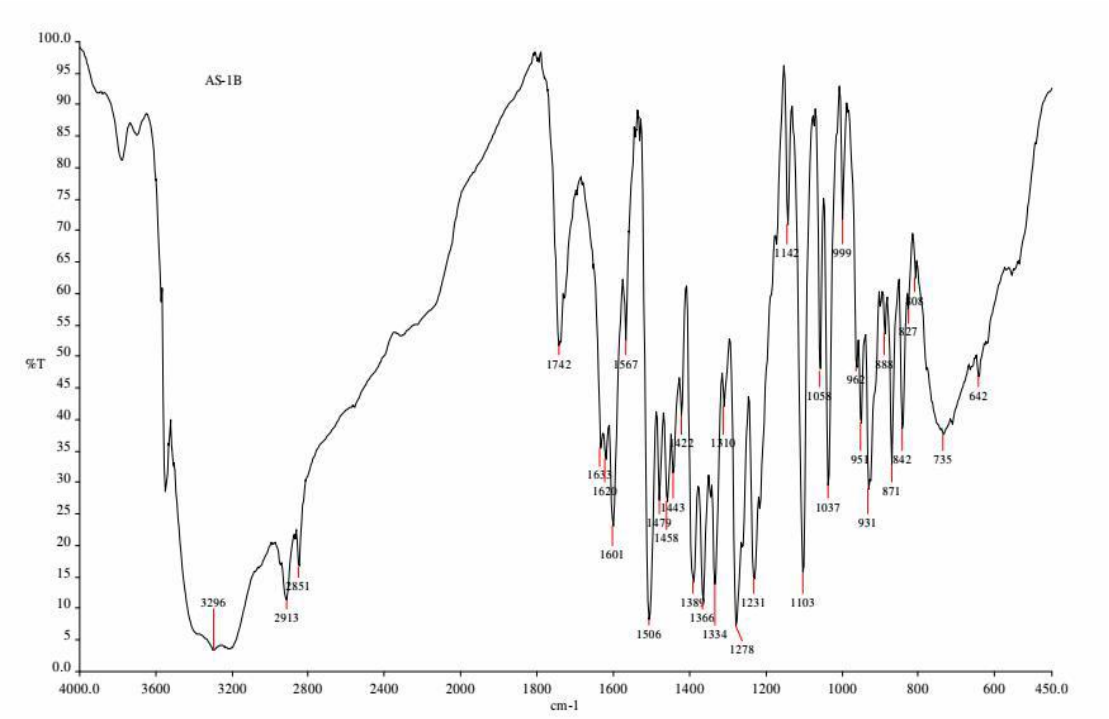

Fig:3 Spectra of Tecoma extract

Table:6 General band assignments of the FTIR spectra of the Tecoma Extract

S.No.	IR Absorption band cm	Assignments
1	3520	O–H stretch O–H stretch of mannose
2	1310	C=N stretch Amide group
3	1565	N-H bending Secondary amine
4	2851	C-H bending (in plane) Methyl and methylene group
5	1334	C-H bending (out plane)
6	1058	C–O stretch of carbohydrate Around

Evaluation of herbal cream

The formulated cream was evaluated for its physicochemical properties. It exhibited good organoleptic characteristics, smooth texture, appropriate pH, optimum viscosity, easy washability, no phase

separation, and good spread ability, indicating stability and suitability for topical application. (Shown in table 7)

Table:7 Evaluation of herbal cream

Parameter	Observation
Colour	Pale Yellow
Odor	Pleasant
texture	Smooth
State	Semi solid
Washability	Eazy Washability
pH	6.7
Viscosity	18820
Phase Separation	No separation
Spread ability	32.8

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