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Physico-Chemical, Phytochemical and Pharmacological Attributes of *Caesalpinia crista* L. Leaves

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The present study aims to evaluate the antioxidant and antimicrobial activities of *Caesalpinia crista* L. (CCL) leaves extracts, along with conducts physico-chemical and phytochemical analysis. Herein, the Soxhlet and maceration methods were used to extract CCL leaves with solvents of varying polarity. The obtained extracts showed similar extractive content in alcohol and water with minimal ash content. FT-IR and GC-MS techniques were used to identify the nature and composition of phytochemicals revealing eight key compounds in the methanol extract and one in the water extract. These compounds were characterized by alcohol, alkane, carbonyl and ether functional groups. The antioxidant activity was evaluated by quantifying total phenolic and flavonoid content and assessing radical scavenging activity, with results indicating that both methanol and water extracts exhibited nearly identical antioxidant activity. Antimicrobial activity was evaluated using the zone of inhibition method along with MIC measurements. The chloroform and methanol extracts exhibited antibacterial efficacy against *E. coli*, *P. aeruginosa* and *S. aureus*, while the methanol extract showed antifungal activity specifically against *F. oxysporum*. The findings suggest that the key phytochemicals are responsible for the observed antioxidant and antimicrobial activities. Furthermore, isolating these significant phytochemicals could lead to the development of potent drugs for various diseases in the future.

Keywords: *Caesalpinia crista* L., Antimicrobial activity, Antioxidant activity, Phytochemical analysis.

INTRODUCTION

The WHO advocates the use of herbal medicine strategy due to their established quality, safety, efficacy, accessibility, affordability, cultural acceptance and effectiveness in managing various communicable and non-communicable diseases [1]. Meanwhile, the global adoption of herbal medicine is consistently increasing. Aboriginal communities across the globe has historically been primary users of herbal medicines and to this day they persistently rely on these traditional practices for their health needs [2-5]. The simplicity and effectiveness of herbal medicine practices in aboriginal communities captured the investigators attention leading them to focus on these specific practices. In the present study the authors selected to investigate *Caesalpinia crista* L. (CCL) leaves, a prominent plant species native to the hilly regions of the Satpura range, is spread over the Maharashtra, Gujarat and Madhya Pradesh states in India. The CCL is a member of the *Fabaceae* family holds a significant role in the field of human healthcare. The different parts of the plant offer a wide variety of medicinal applications in its native region [6].

Several researchers have studied the therapeutic effects of *C. crista* L. The anthelmintic activity of CCL leaves suggests their potential for addressing the parasitic infections [7]. Furthermore, Mishra [8] has explored their hepatoprotective effects indicating a role in safeguarding liver health. Their antioxidant and antibacterial properties contribute to overall well-being, while their antimicrobial attributes suggest the potential applications in fighting various pathogens [9,10]. The leaves also show potential antidiarrheal effects, which could be useful for managing the gastrointestinal issues [11]. Studies have also investigated their antimalarial properties and their potential in treating Alzheimer's disease highlighting the leaves as a topic of interest for addressing critical health challenges [12,13]. Moreover, the leaves also show promise in protecting against DNA and membrane damage and may have anti-amyloidogenic properties relevant for preventing neurodegenerative diseases such as aluminum-induced neurodegeneration in rats [14-16].

Previous research has highlighted the significance of CCL leaves in herbal medicines and pharmacology exhibit its diverse range of biological activities, for example, antiangiogenic [17], leishmanicidal potential, antibiotic and neuropharmacological [18], induced inflammatory effects and antioxidant [19], antimicrobial and cytotoxic [20], antifeedant and larvicidal [21], anti-angiogenic [22], antimycobacterial [23], antispasmodic [24], anti-insomnia effect [25,26], anti-quorum sensing activity [27], anti-herpetic [28], anti-asthmatic activity [29], *etc*. The phytochemical screening and the assessment of antimicrobial activity have also been performed, but only to a limited extent and not in a comprehensive manner. Consequently, the present work offers a thorough report with physico-chemical analysis and phytochemical screening conducted with FT-IR and GC-MS techniques. Moreover, it covers the quantification of antioxidants and testing against various bacterial strains, which have yet to be explored.

EXPERIMENTAL

Plant: Fresh leaves of *C. crista* L. were collected from the hilly area of *Satpura* Mountains near the Maharashtra-Gujarat state border (coordinates: 21.7469º N, 74.1240º E). The identification and authentication of the plant has done at the Botanical Survey of India, Pune (Cert. No. BSI/WRC//Idenser/2023/200623000881). The collected leaves were dried in the shade at room temperature and the dried leaves were powdered using a mixer and the resultant powder was stored in a cool and dry place in a sealed container for further analysis by weighing.

Extract preparation: The resulting powder (10 g) was successively extracted using a Soxhlet extractor with chloroform (99.8% LOBA Chemie, 100 mL) and methanol (99.8% LOBA Chemie, 100 mL) maintained at 40 and 50 ºC, respectively on a heating mantle for up to 6 h. The water extract was obtained by soaking the leaf powder (10 g) in double distilled water (100 mL) overnight with continues stirring on magnetic stirrer at room temperature. Then, the solvents were filtered and evaporated to dryness under reduced pressure using a rotary evaporator yielding crude chloroform (8.7 ± 0.32 % w/w), methanol (13.4 ± 0.47% w/w) and water (6.3 ± 0.55 % w/w) extract. This process was repeated three times to maximize the yield of the extracts [30].

Physico-chemical analysis: Physico-chemical values including ash and extraction values were determined using the quality control guidelines established by WHO for medicinal plant materials [31].

Alcohol soluble extractive value: Dried CCL leaves powder (5 g) was macerated into a sealed flask containing 50 mL ethanol and stirred continuously by using magnetic stirrer for 24 h. The resulting mixture was quickly filtered into a pre-weighted petri dish. The petri dish was then dried up to 50 ºC on heating mantle and weighed. A percentage of the ethanol-soluble extractive value was determined compared to the weighed dried plant.

Water soluble extractive value: Powdered CCL (5 g) material was macerated a sealed flask containing 50 mL of double distilled water for 24 h and stirred the mixture continuously on the magnetic stirrer. The mixture was then filtered into a weighed petri dish and evaporated to 110 ºC for drying. The percentage of extraction value dissolved in water was determined comp-ared to the air-dried powder.

Total ash value: Accurately weighed 3 g of dried powder of CCL leaves was placed in a silica crucible and then slowly heated to a gentle red heat to remove all the carbon. After cooling the crucible was weighed and the percentage of total ash for the drug compared to the dried powder was calculated.

Acid-soluble ash value: About 0.4 g of ash obtained from total ash determination was boiled with 25 mL of 2 N HCl for 15 min. The resulting insoluble material was collected on ash-less filter paper, washed thoroughly with hot water, ignited and weighed. By subtracting the weight of this insoluble matter from the weight of the reference ash, the percentage of acid-insoluble ash content was determined.

Water-soluble ash value: About 0.4 g of ash obtained from total ash determination was boiled in 25 mL of water for 15 min. The resulting insoluble material was collected on ashless filter paper washed with hot water and ignited for 15 min. By subtracting the weight of this insoluble material from the weight of the reference ash the percentage of water-soluble ash was determined.

Qualitative phytochemical screening: The obtained crude sticky extracts was subjected to qualitative phytochemical screening by using available testing protocols [32] (Table-1).

TABLE-1
DETAILS OF THE AVAILABLE QUALITATIVE PHYTOCHEMICAL SCREENING TESTS

Test	Observation	Inference
2 mL plant extract + 2 mL of 5% aqueous $FeCl_3$	Formation of blue colour	Phenols present
2 mL plant extract + few drops of 20% NaOH	Formation of intense yellow colour which is disappeared on the addition of 70% HCl	Flavonoids present
1 mL plant extract + 1 mL marquis reagent (mixture of 2 mL H_2SO_4 and few drops of 40% formaldehyde)	Formation of dark orange or purple colour	Alkaloids present
2 mL plant extract + 6 mL distilled water, shake vigorously	Formation of bubbles or persistent foam	Saponins present
2 mL plant extract + 10% alcoholic $FeCl_3$	Formation of brownish or black colour	Tannins present
2 mL plant extract + 1 mL NaOH + few drops of 1% $CuSO_4$	Formation of violet colour	Proteins present
1 mL plant extract + 0.5 mL glacial acetic acid + 3 drops of 1% aqueous $FeCl_3$	Formation of brown colour ring at the interface	Cardiac glycosides present
1 mL plant extract + 0.5 mL $CHCl_3$ + few drops of conc. H_2SO_4	Formation of reddish brown colour precipitate	Terpenoids present
1 mL plant extract + few drops of molish reagent + 1 mL of conc. H_2SO_4	Formation of red or dull violet colour	Carbohydrates present

These protocols are primarily targeted for the detection of alkaloids, flavonoids, tannins, phenols, terpenoids, proteins, carbohydrates and amino acids within the samples.

TLC and FT-IR analysis: The chloroform, methanol and water crude extracts of CCL leaves were initially evaluated using TLC on an aluminium sheet coated with silica gel 60. Various solvent systems with different ratios were employed for the development of spot on TLC plate. These solvent system *e.g.* *n*-hexane-ethyl acetate, *n*-hexane-chloroform, *n*-hexane-methanol, *n*-hexane-dichloromethane, chloroform-ethyl acetate, chloroform-methanol and chloroform-dichloromethane. The developed TLC plates was observed into UV light chamber (LABLINE, India) including short (254 nm), long (365 nm) ultraviolet and visible lights. The FT-IR analysis of the extracts was performed using a Perkin-Elmer spectrophotometer with the stretching frequency between 4000 and 400 cm^{-1} and the IR spectra were generated with Origin 6.0 software.

GC-MS analysis: GC-MS analysis was conducted using a Shimadzu TQ 8030 spectrometer, which was equipped with a triple quadrupole and a capillary column [33]. The oven temperature was programmed to range from 50 to 250 ºC with a 5 min hold time, while helium was used as the carrier gas at a flow rate of 1.0 mL/min. At the same time, the injector temperature was set to 280 ºC using split injection mode with a split ratio of 1:10. To ensure stable operation, the interface and MS ion source were maintained at 320 and 200 ºC, respectively, while mass spectra were obtained by subjecting the sample to 70 eV of energy and scanning the mass range from *m/z* 40 to 800 amu. The phytochemicals were identified through real-time GC-MS analysis by comparing their mass spectra to the NIST 11 and/or NIST 14 libraries using post-run analysis software.

Antioxidant quantification

Determination of total phenolic content (TPC): The TPC quantification of the methanol extract of CCL leaves was performed using the Folin-Ciocalteau procedure with gallic acid as the standard reference [34]. In this procedure, 200 µL sample was mixed with 3.16 mL distilled water and then allowed to stand for 30 sec to 8 min before adding 600 µL 20% Na_2CO_3 solution. The resulting mixture was maintained at ambient temperature for 3 h during which the absorbance was measured at 765 nm using a UV-Vis spectrophotometer (LABINDIA, UV-3092). The TPC values were calculated using a gallic acid calibration curve in the range of 0 to 400 µg/mL exhibiting a strong correlation with an R^2 value of 0.9982. All the samples were subjected to triplicate analysis for accuracy and consistency. The TPC value was calculated using eqn. 1 and the results obtained were expressed as gallic acid equivalents per mg of extract weight.

$$\text{Total phenolic content (mg of GA/mg of sample)} = \frac{C \times V \times DF}{W} \quad (1)$$

where C is the concentration of sample in µg/mL; V is the volume of sample; DF is the dilution factor; W is the weight of sample.

Determination of total flavonoid content (TFC): The TFC of *C. crista* leaves water extract was evaluated by $AlCl_3$ spectrophotometric method using quercetin as standard reference compound [35]. Briefly, 0.5 mL of crude extract was diluted with 2 mL of double distilled water in a test tube. In each test tube, 0.15 mL of 5% $NaNO_3$ solution, 0.15 mL of 10% $AlCl_3$ and 1 mL of 1.0 M NaOH were added sequentially. The absorbance of the resulting mixture was then measured at 510 nm, with each sample analyzed in triplicate for accuracy. The TFC value was determined using eqn. 1 and the results were expressed in mg quercetin per extract weight.

Free radical scavenging activity: The radical scavenging activity of CCL leaves extract was evaluated using a modified method as described by Hajji *et al*. [36]. Briefly, 500 µL of each extract ranging in concentration from 40 to 200 µg/mL was combined with 375 µL of 99 % methanol and 125 µL of DPPH solution (0.2 M in methanol) serving as a free radical source in this process. After incubating the mixtures in dark at room temperature for 60 min the scavenging capacity was determined spectroscopically using UV-visible spectrometer (LABINDIA, UV-3092). The reduction in absorbance at 517 nm was monitored as DPPH, its radical form exhibits an absorption band at this wavelength that diminishes upon being reduced by an antiradical compound. After incubating the mixture in dark for 60 min at room temperature the scavenging capacity was determined spectroscopically using a UV-visible spectrometer (LABINDIA, UV-3092). A decrease in absorbance at 517 nm was observed as DPPH in its radical form exhibits an absorption band at this wavelength that decreases upon reduction with an antiradical compound. A lower absorbance in the reaction mixtures indicated a higher free radical-scavenging activity. Each sample underwent triplicate analysis for accuracy and consistency. The DPPH radical scavenging activity was quantified using eqn. 2 as:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (2)$$

where $A_{control}$ is the absorbance of control reaction (containing all reagents except the sample) and A_{sample} is the absorbance of the CCL leaves extract. Test was carried out in triplicate manner.

Antimicrobial activity

Microbial strains: The antibacterial potency of CCL leaves extracts was assessed against *Bacillus subtilis* (MTCC 1133), *Escherichia coli* (MTCC 452), *Pseudomonas aeruginosa* (MTCC 3541) and *Staphylococcus aureus* (MTCC 96). Futhermore, the antifungal efficacy of the extracts was evaluated against *Aspergillus niger* (MTCC 281), *Candida albicans* (MTCC 854), *Fusarium oxysporium* (MTCC 4353) and *Trichoderma asperellum* (MTCC 4347). These strains were kindly provided by the Aakaar Biotechnologies Pvt. Ltd., Lucknow (India).

Agar diffusion method: The antimicrobial activity was evaluated by following zone inhibition method [37]. In this method, the SDA plates were inoculated by spreading with 100 µL of microbial culture (adjusted to 0.5 McFarland unit - approximately cell density (1.5×10^8 CFU/mL) and followed by placing the discs containing 10 µL of test extracts at different concentration (0 to 100 mg/mL). One disc in each plate was loaded with solvent alone which served as vehicle control and amphotericin B disc (50 µg) was taken as positive control. The plates of microbes were incubated at 37 ºC for 24 h. The

clear zones created around the disc were measured and recorded. The measurements of inhibition zones were done in triplicate and values are the averages of three measurement.

Determination of MIC: To determine the MIC, adjusted to 0.5 McFarland standard (composition: 0.05 mL of 1.175% $BaCl_2 \cdot 2H_2O$ with 9.95 mL of 1% H_2SO_4) dilution of the micro-organism was used. The diluted microbial culture (100 µL) were added to eight micro-centrifuge tube, followed by the addition of 5 µL of test extracts at concentrations of 1000, 500, 100, 10, 1 and 0.1 µg/mL. During this process, the control tubes contained only the test strain and did not include the test extract. After incubation, all content was transferred to the 96-well plate and turbidity was taken by using Elisa Plate Reader (iMark Biorad) at 630 nm. Ciprofloxacin (10 µg) was used as the positive control for antibacterial activity, while amphotericin B (10 µg) served as the positive control for antifungal activity. The lowest concentration at which no turbidity was observed was considered the MIC of the test extract [38].

Statistical analysis: The antibacterial activity of the CCL leaves extracts was evaluated by measuring the zone of inhibition for the test organisms and calculating the mean and standard deviation (± SEM) of three parallel measurement. The correlation and regression analysis were conducted using the EXCEL program developed by Microsoft Corporation, USA.

RESULTS AND DISCUSSION

Herbal medicine is a valuable gift from nature, playing a crucial role in the prevention and treatment of various health ailments. Such medicines are composed of plant extract or ash obtained from various parts of the plant. The utilization of natural plant extracts for medical and therapeutic applications gained considerable attention due to the adverse effects of synthetic medications on the infectious diseases. Therefore, the global adaptation of this ancient treatment method is rapidly increasing [39]. Meanwhile, the various researchers have been conducting research on herbal medicines and identify the numerous effective natural remedies [40]. The extraction process and solvent polarity are crucial factors in the analysis of herbal medicine. Therefore in present study, the CCL leaves extract was extracted using cold maceration and Soxhlet extraction techniques. The primary purpose of using these methods is that it does not require special experimental conditions and provides a higher yield of extracts compared to other methods. The solvents of increasing polarity were used in the extraction process to ensure that less polar phytochemicals dissolve in non-polar solvents, medium polarity compounds in medium polarity solvents and more polar compounds in polar solvents. However at the end of the extraction process, it was observed that chloroform and methanol extracts had similar phytochemical profiles, while aqueous extracts had different profiles. The crude extracts may contain substances that are either naturally present or intentionally added as a form of adulteration. Therefore, it is essential to quantify this adherence by measuring ash and extractive values. Ash values are useful indicators for assessing the quality and purity of drugs. If the ash values fall outside the acceptable limits, the crude drug can be deemed unsuitable and rejected.

Table-2 shows the results of the physico-chemical analysis including the measurement of extractive and ash values of CCL leaves. The water soluble (13.69 ± 0.17% w/w) and alcohol soluble (14.71 ± 0.26% w/w) extractive values are closely matched indicating a similar efficiency in extracting phytochemicals from the dried plant material by both solvents. Additionally, the total ash value (73.24 ± 0.09% w/w), acid soluble ash value (70.26 ± 0.13% w/w) and water soluble ash value (62.00 ± 0.18% w/w) suggest a minimal presence of inorganic salts or natural adulterants in the CCL leaves extract. In this context, the negligible ash content in the extracts suggests that they can be directly used for medicinal purposes. Extractive values reflect the quantity and nature of the phytochemicals present in crude drugs.

TABLE-2
PHYSICO-CHEMICAL ANALYSIS
RESULT OF CCL LEAF EXTRACT

Parameter	Result (% w/w)
Alcohol soluble extractive value	14.71 ± 0.26
Water soluble extractive value	13.69 ± 0.17
Total ash content	73.24 ± 0.09
Acid-soluble ash content	70.26 ± 0.13
Water soluble ash content	62.00 ± 0.18

Result values are expressed as mean value of triplicate determinations ± SEM

Phytochemical studies: The TLC analysis revealed that the chloroform and methanol extracts of CCL leaves displayed similar phytochemical profiles, while the aqueous extract showed distinct profiles. Subsequent qualitative tests confirmed the presence of phenolic compounds and flavonoids. The FT-IR spectroscopy and GC-MS are well known methods for identifying and quantifying phytochemicals. FT-IR spectra (Fig. 1a-c) further indicated the presence of organic compounds in the extracts including functional groups such as alcohol, alkane, carbonyl and ether as shown in Table-3. Additionally, GC-MS analysis identified eight major phytochemicals in the methanol extract *e.g*. tetremethyl silane, 2,2-dimethoxypropionamide, 2-ethyl-1,3-dioxalane-4-yl)methanol, neophytadiene, (2,6,6-trimethyl-2-hydroxycyclohexylidene)acetic acid lactone, phytol, 6,10,14-trimethylpentadecan-2-one, *bis*(2-ethylhexyl) ester and hexanedioic acid. In contrast, only 4-O-methylmannose was found in the water extract (Fig. 2). The similar prominent phytochemicals have been identified by other researchers in their extracts and they have confirmed various pharmacological activities associated with these compounds (Table-4). Therefore, this information suggests that CCL leaves extracts may also be useful for the pharmacological activities reported by others.

Antioxidant activity: The determination of TPC and TFC in relation to extraction time highlights the potential of the plant extracts as a natural antioxidant. Table-5 provides a detailed summary of the total phenolic and flavonoid content along with the DPPH radical scavenging activity. The highest TPC recorded was 85.15 ± 0.44 based on the gallic acid standard curve (Fig. 3a). The correlation between TPC of CCL leaves extract and extraction time (Fig. 3b) indicates that TPC remained stable for the first 5 h of extraction after which it declined. This

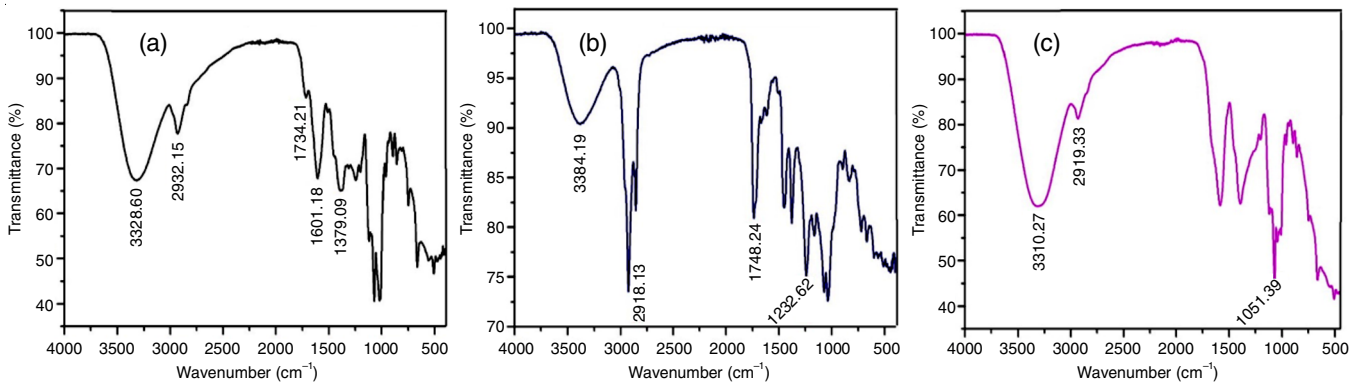

Fig. 1. FT-IR spectra of (a) chloroform extract, (b) methanol extract and (c) water extract

TABLE-3
KEY FUNCTIONAL GROUPS INTERPRETATION BY FT-IR SPECTROSCOPIC ANALYSIS

Extract	Frequency (cm^{-1})	Absorption intensity	Functional group	Bond assignment
Chloroform	3328.60	Broad, strong	Alcohol or amine	–O-H or -N-H
	2932.15	Medium, weak	Aliphatic	-C-H
	1734.21	Sharp, strong	Carbonyl	-C=O
	1601.18	Medium, medium	Alkene	-C=C-
	1329.09	Medium, medium	Ether	-C-O-C-
Methanol	3384.19	Broad, strong	Alcohol or amine	–O-H or -N-H
	2918.13	Medium, weak	Aliphatic	-C-H
	1784.24	Sharp, strong	Carbonyl	-C=O
	1232.62	Medium, medium	Ether	-C-O-C-
Water	3310.27	Broad, strong	Alcohol or amine	–O-H or -N-H
	2919.33	Weak, medium	Aliphatic	-C-H
	1106.23	Medium, medium	Ether	-C-O-C-

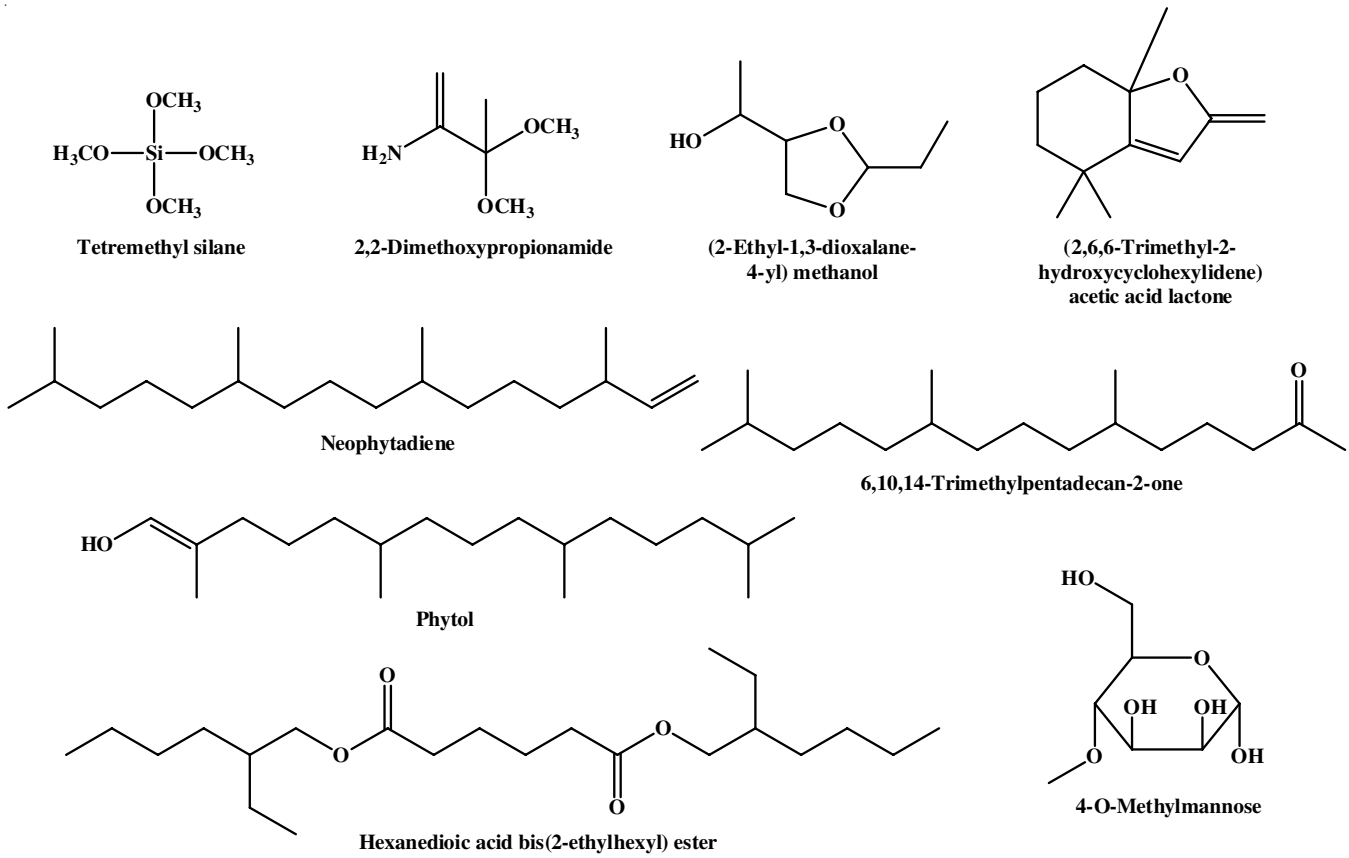

Fig. 2. Probable structure of major phytochemicals identified by GC-MS

TABLE-4
PROMINENT PHYTOCHEMICALS PRESENT IN CCL LEAF IDENTIFIED BY GC-MS ANALYSIS

Name of compound	Retention time (min)	m.f.	m.w.	Peak area (%)
Tetremethyl silane	3.253	$C_4H_{12}O_4Si$	152	3.22
2,2-Dimethoxypropionamide	4.272	$C_5H_{11}NO_3$	133	2.38
(2-Ethyl-1,3-dioxalane-4-yl)methanol	4.400	$C_6H_{12}O_3$	132	1.71
(2,6,6-Trimethyl-2-hydroxycyclohexylidene) acetic acid lactone	21.969	$C_{11}H_{16}O_2$	180	3.00
Neophytadiene	28.465	$C_{20}H_{38}$	278	37.59
6,10,14-Trimethylpentadecan-2-one	28.619	$C_{18}H_{36}O$	268	9.98
Phytol	33.789	$C_{20}H_{40}O$	296	5.95
Hexanedioic acid, *bis*(2-ethylhexyl) ester	38.615	$C_{22}H_{42}O_4$	370	2.49
4-O-methylmannose (Water extract)	25.828	$C_7H_{14}O_6$	194	98.70

TABLE-5
ANTIOXIDANT QUANTIFICATION OF
CCL LEAVES CRUDE EXTRACT

Antioxidant properties	Result value
Total phenol (mg of GA/g of plant extract)	83.35 ± 0.25
Total flavonoids (mg of QA/g of plant extract)	10.20 ± 0.10
RSA of methanol & water extract (%)	94.01 ± 0.57 & 94.34 ± 0.23

Result values are expressed as mean value of triplicate determinations ± standard mean deviation

decline suggests susceptibility to antioxidant degradation when exposed to ambient conditions for prolonged periods. The maximum TFC was 112.22 ± 1.11 as determined using quercetin as the standard (Fig. 3c). Again, analysis of TFC of CCL leaves extract in relation to extraction time (Fig. 3d) reveals a decrease in flavonoid content with longer extraction times. The DPPH radical scavenging activity demonstrated a concentration-dependent response (Fig. 3e). The results of antioxidant quantification underline the critical importance of controlling extraction time to maintain antioxidant quality particularly in the context of commercially available foods and pharmaceutical industries.

Antimicrobial activity: The antimicrobial susceptibility of CCL leaves extracts can also be evaluated by assessing the MIC. However, the MIC depends on the methods and mediums used, the duration of bacterial incubation and the inoculum size; therefore, it can vary when these experimental conditions are altered. Table-6 reports the varying degrees of efficacy of methanol and water extracts of CCL leaves extracts against the tested bacteria with respective MIC. Both methanol and water extracts exhibited notable antibacterial activity with *E. coli* and *S. aureus* being the most susceptible showing MIC values of 0.1, 5, 1000 and 100, respectively. The highest MIC value was observed for *S. aureus* at 1000 µg/mL. Interestingly, the methanol extract displayed susceptibility against *P. aeruginosa*

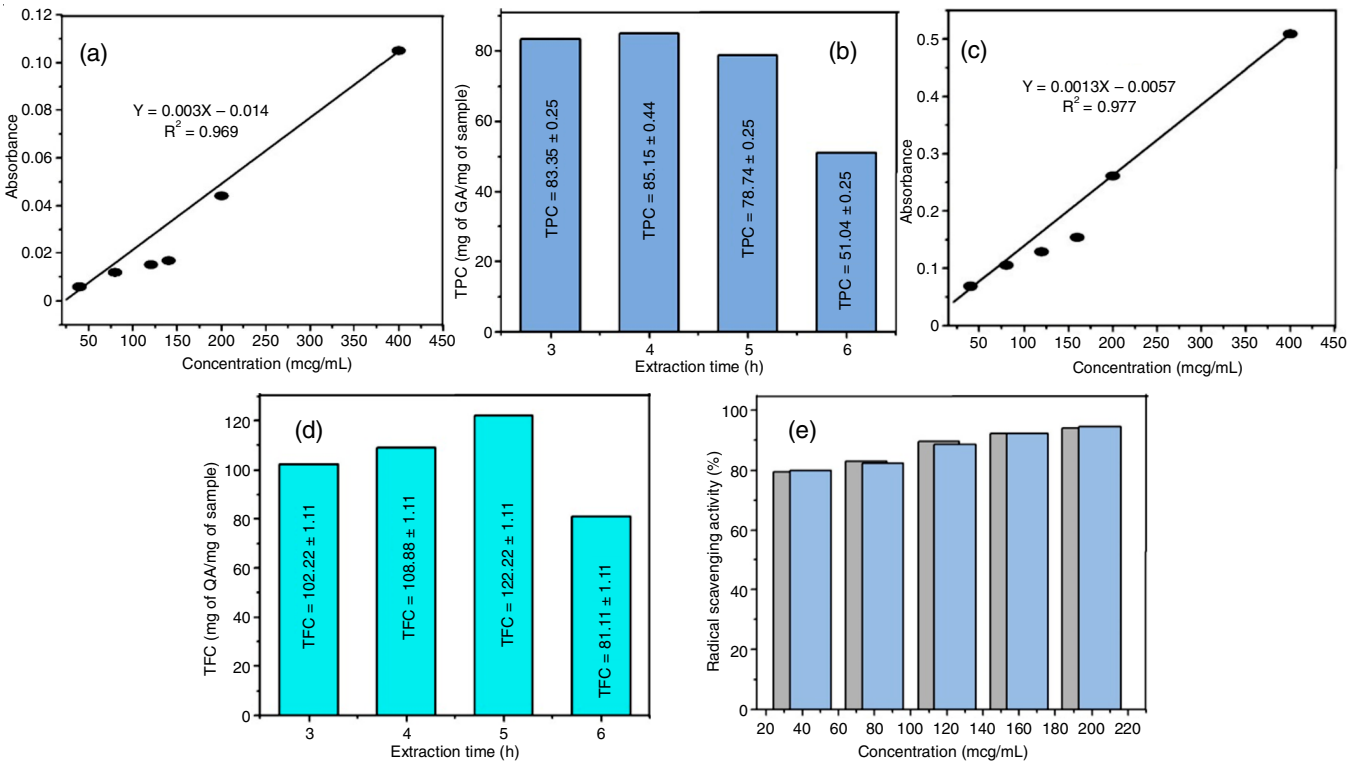

Fig. 3. (a) Gallic acid standard curve used for TPC determination, (b) TPC determination of plant extract with extraction time, (c) quercetin standard curve used for TFC determination, (d) TFC determination of plant extract with extraction time and (e) radical scavenging activity determination of plant extract with concentration

TABLE-6
EFFECT OF METHANOL AND WATER CRUDE EXTRACTS ON ANTIMICROBIAL ACTIVITY (ZONE INHIBITION DIAMETER (mm) AND MIC (µg/mL)

	Zone inhibition diameter (mm)						MIC (µg/mL)
	Sample loaded (µg/mL)					[a]Positive control	
	50	125	250	500	1000	10	
Solvents: Methanol							
Bacterial strains							
B. subtilis	–	–	–	–	–	32.6 ± 0.3	5
E. coli	6 ± 0.5	6 ± 0.5	5.6 ± 0.6	6 ± 0.5	6 ± 0.5	29.3 ± 0.3	0.1
P. aeruginosa	6 ± 0	6 ± 0	–	–	–	30 ± 1.1	5
S. aureus	8 ± 0	8 ± 0	8 ± 0	8.3 ± 0.3	8.3 ± 0.3	22.3 ± 0.6	1000
Fungal strains							
A. niger	–	–	–	–	–	24.6 ± 0.4	100
C. albicans	–	–	–	–	–	24.3 ± 0.4	500
F. oxysporum	–	–	–	4 ± 2.4	6.6 ± 0.4	14.3 ± 0	1
T. asperellum	–	–	–	–	–	12 ± 0.8	100
Solvents: Water							
Bacterial strains							
B. subtilis	–	–	–	–	–	33.3 ± 0.3	5
E. coli	–	–	6 ± 0	6.3 ± 0.3	6.3 ± 0.3	31 ± 0	5
P. aeruginosa	–	–	-	3.6 ± 2	4.6 ± 2.3	31 ± 0.5	10
S. aureus	7 ± 0	7 ± 0	7 ± 0	7.6 ± 0.3	7.6 ± 0.3	21.6 ± 0.3	100
Fungal strains							
A. niger	–	–	–	–	–	23.3 ± 0.4	100
C. albicans	–	–	–	–	–	25.6 ± 0.5	1000
F. oxysporum	–	–	–	–	–	13.6 ± 0	100
T. asperellum	–	–	–	–	–	13 ± 0.5	100

– Indicates no inhibition and [a]Ciprofloxacin was used as a standard for antibacterial activity and amphotericin B was used for antifungal activity.

at lower concentrations, whereas water extracts showed efficacy at higher concentrations. However, no significant effect was observed against *B. subtilis* in both methanol and water extracts. Regarding the antifungal activity only *F. oxysporum* showed susceptibility in the methanol extract with a single MIC, while water extracts exhibited no effect across all concentrations. The presence of a wide spectrum of bioactive phytochemicals with various reported potent therapeutic activities suggests that CCL leaves have potential medicinal value. However, the antimicrobial study of CCL leaves showed lower susceptibility against the tested strains of bacteria and fungi; nonetheless, they can still be used as an antibiotic.

Conclusion

The presence of potent phytochemicals combined with the documented therapeutic effects indicates that *Caesalpinia crista* L. (CCL) leaves extracts is a promising source for isolating molecules responsible for these benefits. Furthermore, the physicochemical analysis confirmed that the extracts of CCL leaves are suitable for use as a crude drug. A FT-IR and GC-MS analysis study revealed that the CCL leaves contained seven major phytochemicals with alcohol, alkane, carbonyl and ether functional groups which could be considered as sources of natural antioxidants and antibacterial activities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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