Applied Biological Research 25(1): 71-82; (2023)
DOI: 10.5958/0974-4517.2023.00008.3

FTIR CHARACTERIZATION AND VALIDATION OF SECONDARY METABOLITES IN LEAF AND BARK EXTRACTS AND DIRECT POWDER OF *Erinocarpus nimmonii,* AN ENDEMIC PLANT

Anitha Puranik and Tarikere C. Taranath*

Environmental Biology and Green Nanotechnology Laboratory, P.G. Department of Studies in Botany, Karnataka University, Pavate Nagar, Dharwad – 580 003, Karnataka (India)
*****e-mail: *tctaranath@gmail.com*

(Received 21 September, 2022; accepted 22 January, 2023)

ABSTRACT

The present study focused on the screening of therapeutically valuable secondary metabolites and FTIR analysis of sequential leaf and bark extracts and direct powder of *Erinocarpus nimmonii* Grah. ex Dalz., an endemic medicinal species of Malvaceae. Secondary metabolites were screened by using standard methods. FTIR spectral profiling of all extracts and powder was done, and matched with a set of peak values specific for each secondary metabolite. The phytochemical screening showed the presence of flavonoids, phenolic compounds, tannin, saponin, terpenoids, steroids, triterpenoids, gum and mucilage, but not alkaloids. The FTIR profile showed coinciding peak values specific for all the biomolecules tested, except alkaloids. Further, the presence of bidesmosidic triterpenoid saponin in leaf extract, alkyne in bark extract, and absence of cyanide peak were disclosed. This simple, affordable, and novel approach of secondary metabolite validation directs further advancement towards fractionation, while the use of direct powder still expedites the procedure by skipping the laborious extraction process. It enables the revelation of therapeutic biomolecules capped on green metallic nanoparticles. To be concise, it is a straightforward way to bridge the gap between the spectral profile of plants with the actual secondary metabolites they own.

Keywords: *Erinocarpus nimmonii*, FTIR spectral profiling, green metallic nanoparticle, phytochemical screening, secondary metabolites

INTRODUCTION

Plants are known for an array of phytochemicals of two broad classes *viz.*, primary and secondary metabolites. The secondary metabolites (SMs), though not inevitable for survival, perform numerous functions, like assisting in defence against herbivory, competition, and species interactions and also represent economically valuable chemicals such as flavours, fragrances, insecticides, dyes, etc. Above all, the SMs decide the therapeutic efficacy of a plant. Hence, the profile of SMs furnishes a preliminary idea about the medicinal potential of a plant and its suitability in drug formulations used for specific ailments (Erb and Kliebenstein, 2020). The preliminary phytochemical screening reveals the presence of different SMs based on visual observations, whose reconfirmation using the advanced technology may be beneficial; whereas, Fourier-transform infrared (FTIR) spectroscopy has been a powerful technology for quantitative and qualitative analysis. It provides a unique molecular fingerprint of even complicated mixtures to investigate the surface and interfacial phenomena after significant developments (Thomas *et al.,* 2017). Hence FTIR facilitates the evaluation of functional groups of phytoextracts and extract-mediated green metallic nanoparticles, to learn about the functional groups contained in the extracts responsible for the reduction of metal ions thus giving stability to the

nanoparticles. However, it is impossible to disclose the profile of SMs without carrying out the regular solvent extraction process, and conventional phytochemical screening methods followed by their confirmation. This narrow gap between the functional groups (as analysed by FTIR spectra) and the assessment of actual therapeutically valuable SMs present in the phytoextract can be cracked by the modern techniques used in the validation of SMs of leaf and bark extracts and powders of plants like *Erinocarpus nimmonii*, which is based on the comparison of a specific set of FTIR peak values representing a single SM (identified using prior literature) with the profiled absorbance peak values of the samples tested. Thus, based on the occurrence of a certain set of peaks related to an individual SM, its presence might be identified. Here the use of powder samples has added advantages in the confirmation of SMs. This preliminary idea about phyto-constituents could decide the diverse therapeutic utilities of an extract or nanoparticle as individual SM is linked with a particular ailment.

Erinocarpus nimmonii Grah. ex Dalz. is an indigenous, monotypic, medicinal, evergreen tree of family Malvaceae. It is an endemic plant found in Western Ghats of Karnataka, Maharashtra, Kerala, Tamil Nadu and Goa. The leaf extracts of *E. nimmonii* exhibit significant amylase inhibitory, anti-inflammatory, anti-hypertensive, anti-mitotic, and antiviral properties; and they also exhibit high antioxidant and free radical scavenging abilities (Puranik *et al.*, 2015, 2016; Dhawan *et al.*, 2017; Puranik and Rajpurohit, 2018). So far, only a brief preliminary screening of phytochemicals of *E. nimmonii* leaves using its hydro-alcoholic extract has been reported (Ghadgi *et al.*, 2014). Therefore, the present study was aimed on the FTIR characterization and validation of secondary metabolites of sequential extracts and powdered *E. nimmonii* leaf and bark to disclose therapeutically active secondary metabolites, based on functional groups.

MATERIALS AND METHODS

Collection identification and processing of plant material

Fresh leaves and bark of *Erinocarpus nimmonii* were collected from the moist deciduous forest patch in Alnavar, Dharwad district, Karnataka (India). The plant sample was deposited in the Karnataka Science College, Dharwad (accession No. 19556). The leaves and barks were thoroughly cleaned, shade-dried, pulverized, and used for hot sequential extraction process, with undiluted AR grade solvents of petroleum ether, dichloromethane (DCM), acetone, methanol and deionized water, by using the Soxhlet apparatus (Harborne, 1973). The extracts were dried in a rotary evaporator, collected in sterile Eppendorf tubes, and refrigerated till further use. Analytical reagent grade chemicals and Borosil glassware were used in the experiment.

Screening of major secondary metabolites

The extracts were initially screened for alkaloids, flavonoids, glycosides-saponin, sterols, and phenols, including tannins using the standard phytochemical tests (Surendra *et al.*,2012). For FTIR spectral profile study and validation of the presence of secondary metabolites (SMs), the sequential extracts and direct fine powders of leaf and bark were ground with KBr crystals, dried under IR light, and compressed to form thin pellets. The measurements were carried out at 25-27°C and the spectrum was recorded from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} using a NICOLET NXR FT Raman Spectrometer. The functional groups were inferred from the spectral data of Stuart (2005) and Pavia (2009). Based on the information in FTIR spectral profile, namely peak values, and corresponding functional groups, the occurrence of different phytochemicals formed during normal metabolic processes in plants were identified (Kumar and Prasad, 2011). The validation of preliminary screening results was done by matching the set of spectral peak values pertinent to the individual SMs of relevant standard compounds or with well-established scientific evidence, with that of FTIR profile of test sample. Both preliminary phytochemical tests and FTIR analysis were performed in triplicates.

RESULTS AND DISCUSSION

Phytochemical screening

The fresh leaves and bark of *Erinocarpus nimmonii* (Fig. 1) were analysed for phytochemicals. The leaf and bark methanolic extracts showed the presence of saponin, phenolic, flavonoid, tannin, steroid, triterpenoid, gum and mucilage, except alkaloids. Though the acetone extract showed same results as those of methanolic and aqueous extracts, it lacked saponin. Steroids and triterpenoids were abundant in petroleum ether and dichloromethane extracts, while a few significant SMs, such as saponin, were not found in detectable amounts. As a result, nonpolar extracts contained lipophilic substances such as alkanes, fatty acids, sterols and certain terpenoids. Mid polar solvents (acetone) can intermediately solubilize polar compounds such as flavonoids; while more polar methanol and water extracts had flavonoids, glycosides, tannins and other terpenoids (Soon and Chiang, 2012). Methanolic extract emerged most potent owing to the multiple SMs. Various tannins, flavonoids, and other aromatic molecules help in defence mechanism against predation and pathogens; whereas flavonoids, tannins, phenolic compounds, saponins, and phytosterols are responsible for therapeutic powers, like analgesic, antiplasmodic, and bactericidal actions to the development of new drugs (Stary, 1998; Britto and Sebastian, 2012). Hence the preliminary screening of leaf and bark extracts of *E. nimmonii* revealed the presence of diverse bioactive principles, leading to hope for drug discovery and development.

Fig. 1: *Erinocarpus nimmonii*, A) tree, B) leaves and flowers, and C) stem showing bark

Table 1: Preliminary phytochemical screening of leaf & bark extracts of *Erinocarpus nimmonii*

Phytochemical	Tests employed	Petroleum ether extract		dichloromethane extract		Acetone extract		Methanol extract		Aqueous extract	
		Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark
Gums & mucilage	Gum and mucilage test	+	+	+	+	+	+	+	+	+	+
Steroids & triterpenoids	Salkowski's test	+	+	+	+	+	+	-	-	-	-
Alkaloids	Mayer's test	-	-	-	-	-	-	-	-	-	-
	Wagner's test	-	-	-	-	-	-	-	-	-	-
Flavonoids	Shinoda test	+	-	+	-	+	+	+	+	+	+
Glycosides & Saponin	Foam test	-	-	-	-	-	-	+	+	+	+
Tannin & phenolic compounds	Ferric chloride test	+	+	+	+	+	+	+	+	+	-
	Gelatin & lead acetate test	+	+	-	+	+	+	+	+	+	+

+ = present; - = absent

FTIR spectral analysis

The FTIR analysis was performed to ostensibly discover the key SMs, that could potentially contribute to the varied therapeutic actions of the extracts. The absorption band with vibrations, and the corresponding functional groups in 4000-1500 cm^{-1} represent the functional group region predominant in SMs (Hashimoto *et al.*, 2008; Hussain *et al.*, 2009), and are shown in Fig. 2 and 3, and Table 2 and 3. The FTIR spectra of methanolic leaf extract displayed broad peaks at 3446.97 cm^{-1} for O-H stretching, a moderate peak at 2938.36 cm^{-1} for C-H stretching, a band at 1687.13 cm^{-1} for C=O stretch (conjugated

Table 2: FTIR absorption peak values of *Erinocarpus nimmonii* leaves showing the types of vibration with assigned functional groups in the extracts of A) petroleum ether, B) dichloromethane, C) acetone, D) methanol, E) aqueous, and F) direct powder

Absorption peak (cm^{-1})	Types of vibrations	Assigned functional groups
A) Petroleum ether extract		
3431.63	O-H stretch-hydrogen bonded	OH compounds - alcohols, phenols, carboxylic acids
2915.41	Asymmetric/symmetric stretch of –	Alkanes - saturated aliphatic compounds-lipids, fatty
2850.37	$CH(CH_2)$ vibration	acids
2728.37	Aldehydic C-H stretch	Aldehyde doublet
1735.62	Strong C=O stretch	Carbonyl group - esters, aldehyde, carboxylic acids
1611.25	N-H bending	Primary amine compounds
	C=C stretch	Alkenes
1514.18	C=C stretch	Aromatic compound
1463.05	CH_2 bend	Methylene group
1377.93	CH_3 bend	Methyl group
B) Dichloromethane extract		
3403.78	O-H stretch- hydrogen bonded	OH compounds - alcohols, phenols, carboxylic acids
2918.59	Asymmetric/symmetric stretch of –	Alkanes - saturated aliphatic compounds-lipids, fatty
2849.84	$CH(CH_2)$ vibration	acids
1739.33	C=O stretch (keto C=O)	Carbonyl compounds - esters, aldehyde, carboxylic acids
1612.09	C=O stretch (enol C=O)/ N-H stretch	Carbonyl compounds/amines
1515.20	C=C stretch	Aromatic compound
1462.93	CH_2 bend	Methylene group
1377.85	CH_3 bend	Methyl group
C) Acetone extract		
3435.74	O-H stretch-hydrogen bonded	OH compounds - alcohols, phenols, carboxylic acids
2925.20	Asymmetric/symmetric stretch of	Alkanes - saturated aliphatic compounds-lipids, fatty
2854.95	$–CH(CH_2)$ vibrations	acids, protein
1739.33	C=O stretch (keto C=O)	Carbonyl compounds - esters, aldehyde, carboxylic acids
1689.40	C=C stretch/ N-H bend	Alkenes/primary amine compounds
1522.76	C=C stretch	Aromatic compound
1452.28	CH_2 bend	Methylene group
1384.25	CH_3 bend	Methyl group
D) Methanol extract		
3446.97	O-H stretch- hydrogen bonded	OH compounds - alcohols, phenols, carboxylic acids
2938.36	Asymmetric stretch of $–CH(CH_2)$ vibration	Alkanes - saturated aliphatic compounds-lipids
1687.13	C=O Stretch	Conjugated aldehyde
1635.13	C=C in conjugated aldehyde/N-H stretch	A, ß-unsaturated aldehyde/Amines
1515.62	C=C stretch	Aromatic compound
1451.23	CH_2 bend	Methylene group
1384.76	CH_3 bend	Methyl group

E) Aqueous extract		
3446.81	O-H stretch- hydrogen bonded	OH compounds - alcohols, phenols, carboxylic acids
2925.59	Asymmetric stretch of - $CH(CH_2)$ vibration	Alkanes - saturated aliphatic compounds-lipids
1609.49	C=C stretch	Alkenes
1530.34	C=C stretch	Aromatic compound
1451.23	CH_2 bend	Methylene
1384.31	CH_3 bend	Methyl group
F) Direct powder		
3327.66	O-H stretch- hydrogen bonded	OH compounds - alcohols, phenols, carboxylic acids
2926.20	Asymmetric/symmetric stretch of - $CH(CH_2)$ vibrations	Alkanes - saturated aliphatic compounds, lipids fatty acids
1739.33	C=O stretch (keto C=O)	Carbonyl compounds - esters, aldehyde, carboxylic acids
1652.62	C=C stretch /N-H bend	Alkenes/amines
1550.94	C=C stretch	Aromatic compound
1441.58	CH_3 bend	Methyl group

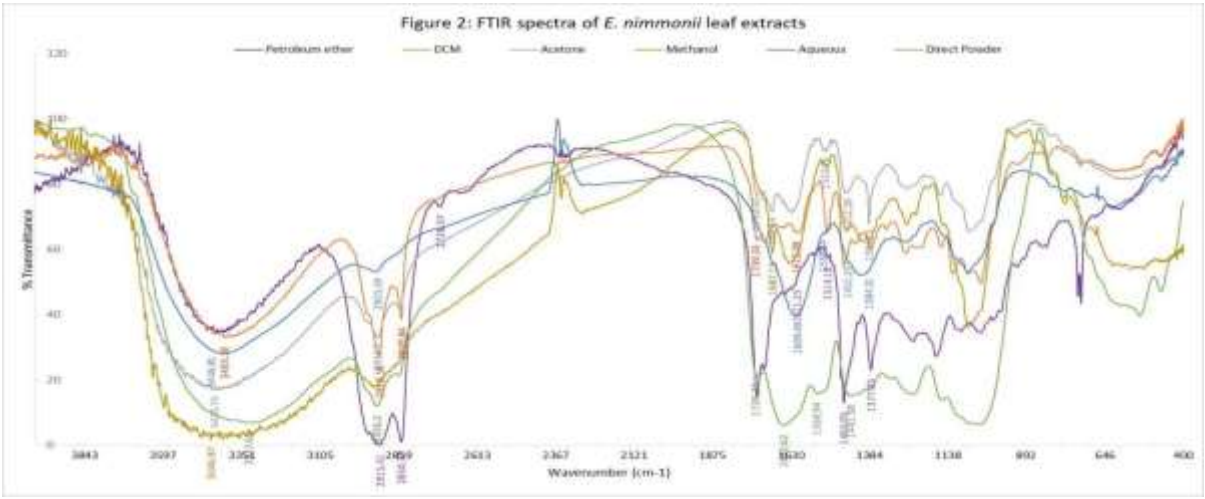

Fig. 2: FTIR spectra of *Erinocarpus nimmonii* leaf extracts using various solvents *viz*., petroleum ether, dichloromethane, acetone, methanol, aqueous, and direct powder

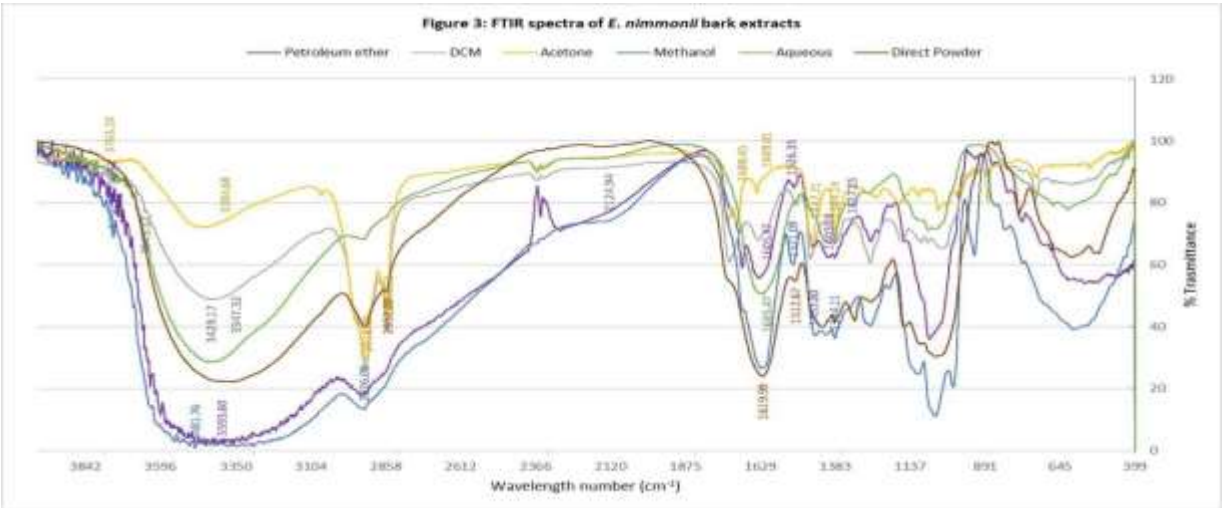

Fig. 3: FTIR spectra of *Erinocarpus. nimmonii* bark extracts using various solvents *viz*., petroleum ether, dichloromethane, acetone, methanol, aqueous, and direct powder

Table 3: FTIR absorption peak values of *Erinocarpus nimmonii* bark showing the types of vibration with assigned functional groups in the extracts of A) petroleum ether, B) dichloromethane, C) acetone, D) methanol, E) aqueous and F) direct powder

Absorption peak (cm^{-1})	Types of vibrations	Assigned functional groups
A) Petroleum ether extract		
3756.33	Non bonded/ free O-H stretch	Hydroxyl (OH) compound
3393.60	Hydrogen bonded O-H stretch	OH compounds - alcohols, phenols, carboxylic acids
2937.37	Asymmetric stretching of $CH(CH_2)$ vibrations	Alkanes - saturated aliphatic compounds
1605.47	C=C stretch	Alkenes
1526.33	C=C stretch	Aromatic compound
1403.18	CH_2 bend	Methylene group
1327.23	CH_3 bend	Methyl group
B) Dichloromethane extract		
3645.37	Non bonded/ free O-H stretch	OH compound
3429.17	Hydrogen bonded O-H stretch	OH compounds - alcohols, phenols, carboxylic acids
3347.32		
2920.14	Asymmetric stretching of $CH(CH_2)$ vibrations	Alkanes - saturated aliphatic compounds
2124.94	C≡C stretch	Alkyne
1611.50	C=C stretch	Aromatic compound
1453.80	CH_2 bend	Methylene group
1396.68	CH_3 bend	Methyl group
(C) Acetone extract		
3763.10	Non bonded/ free O-H Stretch	OH compound
3384.68	Hydrogen bonded O-H stretch	OH compounds - alcohols, phenols
2918.49	Asymmetric/symmetric stretch of –	Alkanes - saturated aliphatic compounds-lipids,
2849.53	$CH(CH_2)$ vibration	fatty acids, protein
1688.45	C=O stretch/ N-H bend	α, ß-unsaturated esters/amides
1609.01	N-H bend	Primary amine compounds
	C=C stretch	Alkenes
1521.33	C=C stretch	Aromatic compound
1447.71	CH_2 bend	Methylene group
1384.14	CH_3 bend	Methyl group
D) Methanol extract		
3750.23	Non bonded/ free O-H Stretch	OH compound
3481.76	Hydrogen bonded O-H stretch,	OH compounds - alcohols, phenols, carboxylic acids
2926.09	Asymmetric stretching of $CH(CH_2)$ vibrations	Alkanes - saturated aliphatic compounds
1624.71	N-H bend	Primary amine compounds
	C=C stretch	Alkenes
1521.09	C=C stretch	Aromatic compound
1453.80	CH_2 bend	Methylene group
1384.11	CH_3 bend	Methyl group
E) Aqueous extract		
3393.60	Hydrogen bonded O-H stretch	OH compounds - alcohols, phenols
2937.37	Asymmetric stretch of $CH(CH_2)$ vibrations.	Alkanes - saturated aliphatic compound-lipids
1605.47	C=C stretch /N-H stretch	Alkenes/amines
1521.44	C=C stretch	Aromatic compound
1403.18	CH_2 bend	Methylene group
1327.23	CH_3 bend	Methyl group

F) Direct powder		
3362.78	O-H stretch- hydrogen bonded	OH compounds - alcohols, phenols
2925.43	Asymmetric/symmetric stretch of –	Alkanes - saturated aliphatic compounds-lipids,
2852.00	$CH(CH_2)$ vibrations	fatty acids
1619.99	C=C stretch /N-H bend	Alkenes/amines
1512.67	CH_2 bend/ C=C stretch	Methylene/aromatic compound

aldehyde), at 1635 cm^{-1} for alkene C=C in a conjugated aldehyde or N-H stretch at 1073 cm^{-1} due to C-N stretch (aliphatic amines), and 1384.76 and 1453.46 cm^{-1} for CH_3 and CH_2 bend (methyl and methylene compounds). Methanolic and water extracts lacked ester group designated for C=O stretch (keto) as compared to the nonpolar solvents. The presence of same functional groups as that of methanolic leaf extract was confirmed by FTIR spectra of bark extracts and powders; but they lacked strong C=O stretch of carboxyl esters (representing oleanolic acid) in 1750-1730 cm^{-1} range. Both plant parts did not show absorbance in 2220-2260 cm^{-1} range to represent the harmful cyanide. Additionally, dichloromethane extract of bark contains C≡C functional group corresponding to the alkyne compounds like propyne, butyne, and pentyne, which are used in the organic synthesis of compounds, as an ingredient for petrol fuel, as a solvent, etc., along with alcohols, aldehydes, and ketones derivatives.

The intensity of peaks and ratios discovered in the IR spectra were connected to their quantitative characteristics even though the principal functional groups present in all the sequential extracts were nearly identical. In FTIR, an increase in peak intensity often indicates the existence of more functional groups connected to the molecular bond (per unit volume) (Li, 2014). Although FTIR analysis is frequently employed as a qualitative technique for material identification, it can be utilized as a quantitative tool for counting specific functional groups when the chemistry is understood and standard reference materials are available. Infrared spectroscopy is well suited for quantitative studies. The Quant Calibration program in Resolutions Pro software (*https://rtilab.com/techniques/ftir-analysis/*) makes the determination of sample concentrations simple (Wojciechowski *et al*., 1998). As a result, the variation in peak ratios reveals the quantitative variations among SMs present in various solvent extracts as well as between leaf and bark extracts.

The current experiment on FTIR spectroscopic studies of leaves and bark of *E. nimmonii* upholds the usefulness of direct powder in the characterization of functional groups representing all the major SMs examined. The comparison of functional groups of powder and extraction in *Cleome gynandra* led to significant changes in chemical constituents of leaf sample (Lingegowda *et al.,* 2012). The aqueous extracts of well-known anti-diabetic plants such as *Nigella sativa, Eugenia jambolana, Andrographis paniculata,* and *Gymnema Sylvestre* were found to have a substantial amount of aliphatic and aromatic amines as per the FTIR study reports of Sangeetha *et al.* (2014). These plants were later concluded to have the highest antioxidant activity. Ashok Kumar and Ramaswamy (2014) applied the same approach to several extracts of four medicinal plants and identified potential -OH groups in the methanolic extract showing strong antibacterial activity.

The profile of leaf and bark sequential extracts and direct powders of present FTIR study provides the fundamental knowledge needed to identify the kind and quantity (approximate ratio) of various primary and SMs, and to guide the selection of the best extract for treating illnesses or serving as a source of raw materials for industry. Similar FTIR profiling work of various solvent extracts has been reported in *Piper sarmentosum* by Hussain *et al.* (2009), *Urtica dioica* by Maobe and Nyarango (2013), *Eclipta alba* by Natesan *et al.* (2018), and *Grewia tilifolia* by Ramadevi and Battu (2019). The various functional groups identified using IR analysis in *E. nimmonii* leaf and bark extracts confirmed the possession of diverse therapeutically potent components in various extracts and direct powders, which corroborate their appropriateness for further phytochemical analysis and pharmacological study.

Validation of major therapeutic secondary metabolites

For the validation of preliminary phytochemical screening results, the assistance of FTIR spectral profiled data of *E. nimmonii* leaf and bark were sought (Fig. 2 and 3, Table 2 and 3) to match these

peak values with those of empirically proved data of individual SMs as per the literature.

Polyphenols - phenols, flavonoids, and tannin

The extensive FTIR spectroscopic study reports revealed that polyphenol chemicals such as phenols, flavonoids, and tannins were responsible for broad and strong absorption peak around 3400 cm^{-1}. All extracts and direct powders of *E. nimmonii* leaf and bark had O-H stretch at 3400 cm^{-1}, followed by C-C bands of aromatic compounds at 1612, 1521–1514, 1420, and 1050 cm^{-1}. These constant features were consistent with previous reports (Pantoja-Castro and González-Rodríguez, 2011; Wahyonol *et al.*, 2019), and confirmed the presence of phenols, and demonstrated the abundance of polyphenol.

All leaf and bark extracts and direct powders in the FTIR profile showed strong O-H absorption between 3403.78 and 3446.97 cm^{-1}, 3384.68 and 3481.76 cm^{-1}, a C-H peak between 2938.36 and 2825.30 cm^{-1}, and 2937.37 to 2918.49 cm^{-1}, a C=O bend between (1609.49-1635.00 cm^{-1}) and (1605.47-1624.71 cm^{-1}) (1327.23-1384.14 cm^{-1}) respectively. The precise matching of these with the flavonoid-specific peak values of *Alium cepa* (Divya *et al.,* 2017), which were centred at 3265 cm^{-1} (O-H hydroxyl), 2926 cm^{-1} (C-H aromatic compounds), 1619 cm^{-1} (C=O carbonyl), and 1395 cm^{-1} (O-H carboxylic acid), the presence of flavonoid in *E. nimmonii* leaf and bark extracts and direct powders was detected, endorsing the outcome of phytochemical tests. Principal component analysis and FTIR spectroscopy were used to separate different classes of flavonoids and their derivatives (Noh *et al.,* 2017). The wavelength range of flavonoids was 3500-3200 cm^{-1}, 3200-2800 cm^{-1}, and 1800-1600 cm^{-1}. The wide O-H stretching and C-H bend in *Grewia tilifolia* served as indicators for the presence of phenols and flavonoids, respectively (Ramadevi and Battu, 2019).

The peak values specific for tannins in FTIR profile of leaf extracts of leaves and bark of *E. nimmonii* is given in Table 4. A strong OH absorption peak was observed around 3700 and 3000 cm^{-1} with a broad band located between 3403.78 and 3446.97 cm^{-1}; and 3384.68 and 3481.76 cm^{-1} in leaf and bark extracts, respectively (Pantoja-Castro and González-Rodríguez, 2011). A sharp peak with approach frequency centred at 2920 cm^{-1} extending from 2938.36 to 2825.30 cm^{-1} and from 2937.37 to 2918.49 cm^{-1} and a tiny shoulder at 2854.95 to 2849.84 cm^{-1} and 2849.53 cm^{-1} in acetone extracts of *E. nimmonii* leaf and bark, respectively, were assigned to symmetric and asymmetric C-H stretching vibrations of alkane groups. Further, CH_2 bonds in phenolic groups show distorted vibration and exhibit absorption in the region of 1500-1400 cm^{-1} with approach frequency centred at 1420 cm^{-1}, ranging from 1462.28 to 1451.23 cm^{-1} in leaf and 1453.80 to 1403.18 cm^{-1} in bark extracts and direct powders. The approach frequency of C-O positioned at 1050 cm^{-1} was observed in 1073.52 to 1031.90 cm^{-1}; and 1056.4 to 1061.2 cm^{-1}, and for other aromatic C-O band with an approach frequency at 800 cm^{-1} showed peaks at 868.03 to 823.88 cm^{-1} and 862.14 to 820.93 cm^{-1} in leaf and bark extracts and direct powders,

Table 4: Infrared positions of tannic acid and different solvent extracts and direct powder of leaf and bark samples of *Erinocarpus nimmonii*

Absorption peak (cm^{-1}) and types of vibrations								
Aromatic group	Vibrations	Approach frequency tannic acid	Petroleum ether	Dichloromethane	Acetone	Methanol	Aqueous	Direct powder
Erinocarpus nimmonii leaf; experimental values								
-CH_2-OH	OH	3400	3431.63	3403.78	3435.74	3446.97	3446.81	3327.66
-CH_2-OH	CH_2	2920	2915.41	2918.59	2925.20	2938.36	2925.59	2926.20
-CH_2-OH	CH_2	1420	1463.05	1462.93	1452.28	1451.23	1451.23	1441.58
-O-	C-O	1050	1035.36	1034.90	1071.05	1073.52	1073.85	1037.33
-O-P	C-O	800	826.82	823.88	821.11	868.03	820.93	-
Erinocarpus nimmonii bark; experimental values								
-CH_2-OH	OH	3400	3393.60	3429.17	3384.68	3481.76	3393.60	3362.78
-CH_2-OH	CH_2	2920	2937.37	2 920.14	2918.49	2926.09	2937.37	2925.43
-CH_2-OH	CH_2	1420	1403.18	1453.80	1447.71	1453.80	1403.18	1425.42
-O-	C-O	1050	1056.40	1076.91	1061.23	1053.21	1056.40	1053.04
-O-	C-O	800	826.82	862.14	820.93	829.76	-	-

respectively. Similarly, around 1452 cm^{-1} stretching vibrations of -CH_2OH aromatic groups appeared in both leaf and bark spectra. Since tannin in direct extracts is relatively impure in comparison to tannic acid, some variation from the approach frequency was obvious. Thus, the matching of spectral values of both leaf and bark of *E. nimmonii* with the standards confirmed the presence of tannin. The FTIR spectral details of samples of *Acacia mangium* and *Sorghum* panicle were used to confirm the presence of tannin by Bharudin *et al.* (2013) and Wahyonol *et al.* (2019), respectively.

Alkaloids

The FTIR spectra of all the extracts and direct powder of *E. nimmonii* lacked strong N-H stretching vibrations at 3400 cm^{-1}. Singh *et al.* (2017) and Fachriyah *et al.* (2018) in their FTIR study reports on alkaloid isolates from *Peperomia pellucida* and (Borreverine-alkaloid) from *Borreria verticillate*, respectively, found that the specific sets of bands associated with each alkaloid were N-H (at 3432.13 and 3375 cm^{-1}, respectively), C-H, C=C, C=O, C-N, C-O-C group and aromatic rings. Since there were no alkaloids found in our samples, the results of preliminary phytochemical screening were spectrally verified.

Gum and mucilage

The FTIR spectral profile of *E. nimmonii* leaf and bark sequential extracts, as well as direct powder revealed the presence of characteristic peaks for crude mucilage, including OH (3435 cm^{-1}), CH (2925 cm^{-1}), CH (2854 cm^{-1}), C=O (1642 cm^{-1}), C=C (1578 cm^{-1}), and C-O-C (1175 cm^{-1}), matching that of *Lepidium sativum* mucilage (Bhatia *et al.,* 2014), formulation at 3198 cm^{-1}, CH stretching aromatic at 3034 cm^{-1}, CH stretching aliphatic at 2956 cm^{-1}, C=O at 1642 cm^{-1}, C=C at 1578 cm^{-1}, and C-O-C ether linkage at 1189 cm^{-1}. This is in agreement with Singh and Bothara (2014), Ellerbrock *et al*. (2019) and Soni *et al.* (2019) who worked on *Diospyros melonoxylon,* Chia seeds and *Plantago ovata,* respectively. The presence of mucilage in the extracts and direct powder of *E. nimmonii* leaf and bark was thus confirmed and corroborated the results of phytochemical screening, by close coincidence of all absorption bands.

Saponins

The FTIR spectra of all the extracts and direct powder of *E. nimmonii* leaf and bark showed saponins specific IR values of O-H group ranging from 3446 to 3347 cm^{-1}, C-H extending from 2938 to 2918 cm^{-1} and oligosaccharide linkage to sapogenins (C-O-C) at 1074 cm^{-1} and between 1071 to 1034 cm^{-1} (Table 5). The absorbance peak values ranging from 1632.6 to1636.3 cm^{-1} related to C=C for a stretch have been reported in standard Quillaja saponin (Almutairi and Ali, 2015). Our findings outcomes are comparable to *Entada laptostachya* extract's typical saponin values (Kirmizigul *et al.,* 2002; Kareru *et al.,* 2008; Rawat *et al*., 2021). Thus, the presence of saponin in the FTIR profiles was confirmed in all the extracts and direct powder of *E. nimmonii* leaf and bark. Further, the petroleum ether, dichloro-methane, and acetone extracts of leaf are characterized by C=O infrared absorbance similar to the oleanolic acid or ester (a standard of triterpenoid saponins) as described by Li *et al.* (2013) in *Sapindus* extract. Such triterpenoids are likely to be bidesmosides as they are linked to two glycones (namely glycosidic and ester groups) to sapogenin (Kirmizigul *et al.,* 2002). Interestingly, neither bark extracts nor powder demonstrated any vibration for ester bond of oleanolic acid and did not match to oleanane triterpenoid saponin and were monodesmosidic due to the differential synthesis of triterpenoid saponins in leaves and bark. The isolation and identification of the types of saponins may be beneficial to understand their practical significance, as each class of saponin has diverse medicinal values. Triterpenoid saponins of bark of *Grewia tilifolia* exhibited cytotoxic activity against LEUK-L1210 cells (Badami *et al*., 2003), while oleanolic acid and its derivatives have anti-inflammatory, antioxidant, anticancer, and hepatoprotective properties. The molecule appears essential for numerous studies given the recent understanding of its biosynthesis and the impending commercialization of the first oleanolic acid-derived medication (Pollier and Goossens, 2012). Bupleurum, ginseng, platycodon, and polygala are widely used Chinese herbs with oleanolic acid saponins as the main active constituents (Long *et al.,* 1989). This economical, fast and convenient technique of saponin detection using direct

Table 5: Characteristic peaks and functional groups of saponins in *Erinocarpus nimmonii* leaf/ bark extracts and direct powders

Erinocarpus nimmonii		Absorption peak (cm^{-1}) and types of bonds					
Part	Extract	3500 O-H	2942 CH_3	1750-30 C=O	1628 C=C	1261 C-O-H	1076 C-O-C
Leaf	Petroleum ether	3435.74	2925.20	1735.62	1611.25	1267.00	1071.05
	DCM	3403.78	2918.59	1739.33	1612.09	1270.44	1034.90
	Acetone	3435.74	2925.20	1739.33	1689.40	1267.00	1071.05
	Methanol	3446.97	2938.36	-	1635.00	1266.90	1073.52
	Aqueous	3446.81	2925.59	-	1609.49	1265.21	1073.85
	Direct powder	3327.66	2926.20	-	1652.62	1248.75	1037.33
Bark	Petroleum ether	3393.60	2937.37	-	1605.47	1243.75	1056.40
	DCM	3461.64	2920.14	-	1611.50	1247.73	1056.40
	Acetone	3384.68	2918.49	-	1609.01	1284.14	1061.23
	Methanol	3481.76	2926.09	-	1624.71	1267.26	1053.21
	Aqueous	3393.60	2937.37	-	1605.47	1247.75	1056.40
	Direct powder	3362.78	2925.43	-	1619.99	1268.36	1053.04

powdered samples of *E. nimmonii* leaf or bark by FTIR spectroscopy is highly recommended.

Conclusion: The study showed that in FTIR spectral signature of each peak corresponds to a functional group, which in consortium with other peaks represents one or more secondary metabolites produced by leaf and bark extracts and direct powder of *Erinocarpus nimmonii*. Thus, the presence of diverse SMs could be validated based on the details of FTIR profile, without any immediate fractionation, purification, or elucidation of their structural features using a range of advanced analytical techniques. Though FTIR studies of saponin direct plant powder have been used in many cases, the current study spectrally confirmed the presence of phenols, flavonoids, tannins, gum and mucilage, terpenoids, monodesmosidic and bidesmosidic triperpenoid saponins, their nontoxic nature (without cyanide) as well as the lack of alkaloid in *E. nimmonii* leaf and bark disclosing the therapeutic potentialities. Likewise, the current simple approach of validation may be employed to unveil the details of capped SMs on the surface of green metallic nanoparticles, to assess their probable therapeutic utility. Nevertheless, the FTIR profile direct powder of *E. nimmonii* may serve as a taxonomic tool to help in resolving the conflicts at the genus and species levels as well as serve as a benchmark to maintain the efficacy and purity of herbal medicines by tracing the adulterant.

Conflict of interest statement: The authors declare no conflict of interest.

Acknowledgments: The authors are thankful to the Chairman, Department of PG Studies in Botany, Karnatak University, Dharwad; the University Scientific Instrumentation Centre (USIC), Karnatak University, Dharwad (for providing the FTIR facility); and one of the authors Anitha Puranik is thankful to the Management and Principal of JSS Banashankari Arts, Commerce, and S.K. Gubbi Science College, Vidyagiri, Dharwad, for extending their help in conducting the experiments.

REFERENCES

Almutairi, M.S. and Ali, M. 2015. Direct detection of saponins in crude extracts of soap nuts by FTIR. *Natural Product Research*, **29**: 1271-1275.

Ashok Kumar, R. and Ramaswamy, M. 2014. Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian medicinal plants. *International Journal of Current Microbiology and Applied Sciences*, **3**(1): 395-406.

Badami, S., Vijayan, P., Mathew, N., Chandrashekhar, R., Godavarathi, A. and Dhanarajan, S.A. 2003. *In vitro* cytotoxic properties of *Grewia tiliaefolia* bark and lupeol. *Indian Journal of*

Pharmacology, **35**: 205-251.

Bharudin, M.A., Zakaria, S. and Chia, C.H. 2013. Condensed tannins from *Acacia mangium* bark: Characterization by spot tests and FTIR. *AIP Conference Proceedings,* 1571: 153. [*doi: 10.1063/1.4858646*].

Britto, J.D. and Sebastian, S.R. 2012. Biosynthesis silver nanoparticles and their antibacterial activity against human pathogens. *International Journal of Pharmaceutical Sciences,* **5**: 257-259.

Dhawan, B.N. 2012. Anti-viral activity of Indian plants. *Proceedings of the National Academy of Sciences, India. Section B,* **82**(1): 209-224.

Divya, B.J., Suman, B., Venkataswamy, M. and Thyagaraju. A. 2017. A study on phytochemicals, functional groups, and mineral composition of *Allium sativum* (garlic) cloves. *International Journal of Current Pharmaceutical Research,* **9**(3): 42-45.

Ellerbrock, R.H., Ahamed, M.A. and Gerke, H.H. 2019. Spectroscopic characterization of mucilage (Chia seed) and polygalactouronic acid. *Journal of Plant Nutrition and Soil Science,* **182**: 888-895.

Erb, M. and Kliebenstein, D.J. 2020. Plant secondary metabolites as defenses, regulators and primary metabolites: The blurred functional trichotomy. *Plant Physiology,* **184**(1): 39-52.

Fachriyah, E., Ghifari, M.A., and Anam, K. 2018. Isolation, identification, and xanthine oxidase inhibition activity of alkaloid compound from *Peperomia pellucida*. *IOP Conference Series: Materials Science and Engineering,* **349**(1): 012017.

Ghadge, T.A., Tare, H.L., Gharge, H.L. and Shinde, M.B. 2014. Preliminary anticancer activity of *Erinocarpus nimmonii*. *International Journal of Institutional Pharmacy and Life Sciences*, **4**: 1-6.

Harborne, J.B. 1973. *Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis.* Chapman & Hall, London, UK.

Hashimoto, A. and Kameoka, T. 2008. Applications of infrared spectroscopy to biochemical, food, and agricultural processes. *Applied Spectroscopy Reviews,* **43**: 416-451.

Hussain, K., Ismail, Z., Sadikun, A. and Ibrahim, A. 2009. Evaluation of metabolic changes in fruit of *Piper sarmentosum* in various seasons by metabolomics using Fourier transform infrared (FTIR) spectroscopy. *International Journal of Pharmaceutical and Clinical Research,* **1**(2): 68-71.

Kareru, P., Keriko, J., Gachanja, A. and Kenji, G. 2008. Direct detection of triterpenoid saponins in medicinal plants. *African Journal of Traditional Complementary Alternative Medicine,* **5**: 56-60.

Kirmizigul, S., Anil, H. and Rose, M.E. 2002. New triterpenic saponins from *Celphalaria transsylvanica. Turkish Journal of Chemisty,* **26**: 947-995.

Kumar, K.J. and Devi Prasad, A.G. 2011. Identification and comparison of biomolecules in medicinal plants of *Tephrosia tinctoria* and *Atylosia albicans* by using FTIR. *Romanian Journal of Biophysics,* **21**(1): 63-71.

Li, Jerry. 2014. Re: What is the meaning of the size of the peak in FTIR spectra? [Retrieved from: *https://www.researchgate.net/post/What-is-the-meaning-of-the-size-of-the-peak-in-FTIR-spectra/5479698fd3df3e45668b464d/citation/download*].

Li, R., Wu, Z.L., Wang, Y.J. and Li, L.L. 2013. Separation of total saponins from the pericarp of *Sapindus mukorossi* Gaerten. by foam fractionation. *Industrial Crops and Products,* **51**: 163-170.

Lingegowda, D.C., Komal, k.J., Devi Prasad, A.G., Zarei, M. and Gopal, S. 2012. FTIR spectroscopic studies on *Cleome gynandra* – Comparative analysis of functional group before and after extraction. *Romanian Journal of Biophysics, **22***(3-4): 137-143.

Long, Q.Q. 1989. Total saponin contents of the root of wild and cultivated *Platycodon grandiflorum*. *Journal of Chinese Medicinal Materials,* **12**(3): 37-38.

Maobe, M.A.G. and Nyarango, R.M. 2013. FTIR Spectrophotometer analysis of *Warburgia ugandensis* medicinal herb used for the treatment of diabetes, malaria, and pneumonia in Kisii region, southwest Kenya. *Global Journal of Pharmacology, **7***: 61-68.

Natesan, G., Hansiya, V.S. and Uma, M.P. 2018. Application of phytochemical screening and a combined FTIR spectroscopy and principal component analysis for effective discrimination of two varieties of *Eclipta alba* (L.) Hassk. *International Journal of ChemTech Research,* 11(11): 337J-3347.

Noh, C.H., Azmin, N.F., Amid, A. and Asnawi, A.L. 2017. Algorithm for rapid identification of flavonoids classes. *International Food Research Journal,* **24**: S410-S415.

Pantoja-Castro, M.A. and González-Rodríguez, H. 2011. Study by infrared spectroscopy and thermos-gravimetric analysis of tannins and tannic acid. *Revista Latinoamericana de Química,* **39**: 107-112.

Pavia, D. L., Lampman, G.M., Kriz, G.S. and Vyvyan, J.R. 2009. *Introduction to Spectroscopy* (4th edn.) Brooks/Cole, Cengage Learning, Washington, USA.

Pollier, J. and Goossens, A. 2012. Oleanolic acid. *Phytochemistry*, **77**: 10-15.

Puranik, A, Parimala, R. and Rajpurohit, A.G. 2016. Amylase inhibitory activity of rare endangered and threatened species *Erinocarpus nimmonii* leaf extracts. *World Journal of Pharmacy and Pharmaceutical Sciences.* **5**(1): 856-863.

Puranik, A., Parimala, R. and Rajpurohit, A.G. 2015. *In-vitro* antioxidant activity and free radical scavenging potential of a rare endangered and threatened species *Erinocarpus nimmonii* leaf extracts. *Asian Journal of Plant Science Research*. 5(8): 11-15.

Puranik, A. and Rajpurohit, A.G. 2018. *In vitro* anti-inflammatory and anti-hypertensive activity of *Erinocarpus nimmonii* methanol leaf extract. *International Journal of Green and Herbal Chemistry,* **7**(2): 180-187.

Ramadevi, D. and Battu, R.G. 2019. Qualitative phytochemical screening and FTIR spectroscopic analysis of *Grewia tilifolia* (Vahl) leaf extracts, *International Journal of Current Pharmaceutical Research,* **11**(4): 100-107.

Rawat, B. and Garg, A. 2021. Characterization of phytochemicals isolated from *Cucurbita pepo* seeds using UV-Vis and FTIR spectroscopy. *Plant Archives,* **21**(1): 892-899.

Sangeetha, S., Archit, R. and Sathiavelu, A. 2014. Phytochemical testing, antioxidant activity, HPTLC and FTIR analysis of antidiabetic plants *Nigella sativa*, *Eugenia jambolana*, *Andrographis paniculate* and *Gymnema sylvestre*. *Research Journal of Biotechnology,* **9**(9): 64-72.

Singh, S. and Bothara, S.B. 2014. Physicochemical and structural characterization of mucilage isolated from seeds of *Diospyros melonoxylon* Roxb. *Brazilian Journal of Pharmaceutical Sciences,* **50**(4): 713-725.

Singh, S., Singh, H., Karthick, T., Tandon, T., Dethe, D.H. and Erande, R.D. 2017. Conformational study and vibrational spectroscopic (FTIR and FT-Raman) analysis of an alkaloid – borreverine derivative. *Analytical Sciences,* **33**(1): 99-104.

Socrates, G. 2004. *Infrared and Raman Characteristic Group Frequencies: Tables and Charts* (3rd edn.). Madison Avenue, New York, USA.

Soni, A., Hitesh, T., Sachin, G. and Singla, S. 2017. Isolation and characterization of mucilage from *Plantago ovata*. *World Journal of Pharmaceutical and Life Sciences, 3*(7): 285-291.

Soon, L.K. and Chiang, L.K. 2012. Influence of different extraction solvents on lipophilic extractives of *Acacia hybrid* in different wood portions. *Asian Journal of Applied Sciences,* **5**: 107-116.

Stary, F. 1998. *The Natural Guide to Medicinal Herb and Plants*. Tiger Books International, London, UK.

Stuart, B. 2005. *Infrared Spectroscopy*. Wiley Online Library *[https://onlinelibrary.wiley.com/doi/abs/10.1002/0471238961.0914061810151405.a01.pub2*].

Surendra, R., Bhatt, S., Dhyani, S. and Jain, A. 2012. Preliminary phytochemical screening of medicinal plant *Ziziphus mauritiana* Lam fruits. *International Journal of Current Pharmaceutical Research,* **4**(3): 160-162.

Thomas, S., Thomas, R., Zachariah A.K. and Mishra, K.R. 2017. *Spectroscopic Methods for Nanomaterials Characterization*. Elsevier, New York, USA.

Wahyonol, T., Astuti, D.A., Wiryawan, K.G., Sugoro, I. and Jayanegara, A. 2019. Fourier transform mid-infrared (FTIR) spectroscopy to identify tannin compounds in the panicle of *Sorghum* mutant lines. Materials Science and Engineering 546.042045. IOP Publishing; 1-7. [*doi:10.1088/1757-899X/546/4/042045*].

Wojciechowski, C., Dupuy, N.T., Huvenneb, O. and Legrandb, P. 1998. Quantitative analysis of ATR-FTIR. *Food Chemistry,* **63**: 13-40.