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Identification And Detection of Functional Groups in *Deoni* Cow Urine Samples and *Acalypha Indica* Plant Extracts by Fourier Transform Infrared Spectroscopy

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ABSTRACT

In the current study, FTIR was employed to analyse the functional group profiles of *Deoni* raw cow urine, its distillate, concentrate, and *Acalypha indica* methanolic, ethanolic leaf extracts samples and to identify their major bioactive constituents for the first time using a mid-infrared (Mid-IR) Perkin Elmer FTIR spectrum 3 Spectrometer. Spectra were recorded in the range of 400–4000 cm^{-1}, generating transmission versus wavenumber profiles. The mid-IR spectrum was categorized into four distinct regions: (i) Single bond region (2500–4000 cm^{-1}), (ii) Triple bond region (2000–2500 cm^{-1}), (iii) Double bond region (1500–2000 cm^{-1}), and (iv) Fingerprint region (500–1500 cm^{-1}). The FTIR Spectrum results showed the presence of functionally significant compounds such as urea, creatinine, swarnkshar (aurum hydroxide), carbolic acid, phenols, calcium, and manganese present in urine samples. FTIR Profile of *Acalypha indica* leaf extracts identified the presence of organo halogens, disulfides, thioethers, alkynes, alkenes, aromatic ethers, and organic silicone, silicate and phosphate ions, primary and secondary amines, phenols, amides, quinones, and ammonium ions, azo and imino groups, methyl groups, and polymeric hydroxyl groups. The comparative FTIR profiling highlights the presence of diverse bio-functional moieties such as; epoxides (enhancing keratinocyte proliferation), imines (with antimicrobial and antifungal activity), phenols (antibacterial, anti-inflammatory and antioxidant), Urea, carbolic acid, phenols, creatinine (Antimicrobial activity) and peroxides (disinfectant ability). These functional groups are indicative of major biologically active compounds that may contribute to antimicrobial, anti-inflammatory, antioxidant and wound healing activities. These findings provide a chemical basis for the potential synergistic applications of *Deoni* cow urine and *Acalypha indica* extracts in pharmacological formulations and natural product research.

KEY WORDS: Cow urine, *Acalypha indica*, Phytochemicals, FTIR, Functional groups

1. INTRODUCTION

Fourier Transform Infrared (FTIR) Spectroscopy is a vibrational spectroscopic method that utilizes the infrared radiation to vibrate molecular bonds within the sample that absorbs it. Spectral bands in vibrational spectra are molecule specific and provide direct information about the biochemical composition [Zanyar Movasaghi *et al.*, 2008]. It is one of the analytical technique used for characterizing the test samples in the form of liquids, solutions, semi-solids, pastes, powders, films, fibers, and gases [Fan *et al.*, 2012]. FTIR is the most powerful tool for identifying the types of chemical bonds (functional groups) in active principles/compounds present in the urine samples and plant extracts. FTIR characterization analysis is quite rapid, good in accuracy, and relatively sensitive [Jaggi and Vij, 2006]. The identification of functional groups in phyto-constituents from the medicinal plants using FTIR technique [Grube *et al.*, 2008]. FTIR is one of the extensively used and more advanced method to categorize the chemical constituents/functional groups and has been used as a necessary method to identify the medicines for pharmacopeia in several countries [Subashini *et al.*, 2015]. It is a non-destructive analytical technique that provides structural

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information on molecular features of a large range of compounds [Agatonovic-Kustrin *et al.*, 2013]. FTIR Spectroscopy has been recognized as a dependable and sensitive method for finding the functional groups present in the plant extracts and they were determined with the aid of IR region in the range of 4000-400 cm^{-1} [Florence and Jeeva, 2015]. It is possibly the major, authoritative technique used for identifying the types of chemical groups (functional groups) present in compounds. The wavelength of light fascinated is a characteristic of the chemical bond which might be seen in the annotated spectrum and the chemical bonds in the molecules have been predicted using FTIR [Sahayaraja *et al.*, 2015]. FTIR Spectroscopy would allow accurate and precise assessment of the surface functional groups, bonding, types, and molecular confirmations.

Cow urine, particularly from indigenous breeds such as *Deoni*, is considered a bioenhancer and therapeutic agent in traditional Indian medicine (Ayurveda). It is a complex biological fluid containing volatile fatty acids, phenolic compounds, urea derivatives, and various minerals that contribute to its antioxidant, antimicrobial and wound healing properties. The composition of cow urine varies among breeds, feed, and physiological conditions, and hence profiling through FTIR spectroscopy can provide valuable insights into its biochemical complexity and pharmacological potential.

Acalypha indica L. is commonly known as *'Indian Copper leaf'*, is a medicinal herb traditionally used for its antimicrobial, anti-inflammatory and wound healing activities. It leaves contains several bioactive compounds, including phenolics, flavonoids, alkaloids, tannins, saponins and terpenoids. Characterising the functional groups present in its extracts using FTIR spectroscopy helps in understanding the nature of these phytochemicals and their role in therapeutic efficacy.

A comparative FTIR analysis of *Deoni* cow urine and *Acalypha indica* extracts is, therefore essential to elucidate the major functional groups and bio-molecular interactions that may contribute to their synergistic biological activities. Such profiling not only aids in the chemical standardization of natural formulations but also supports the development of cow urine-herbal based bio-products with improved therapeutic potential.

Scanty reports were available in indigenous cow breeds and *Acalypha indica* plant in diverse functional groups identification and detection by FTIR analysis but there was no study found till date in *Deoni* cow urine samples and *Acalypha indica* leaf extracts individually *in vitro.* Hence, in the current study we investigated, and explored the functional groups present in *Deoni* cow urine samples, *Acalypha indica* leaf extracts individually for the first time *in vitro*.

2. MATERIALS AND METHODS

2.1 Materials

Deoni cow urine samples were collected from the cattle yard of the Southern Regional Station, National Dairy Research Institute (SRS-NDRI), Bengaluru, India. *Acalypha indica L.* plants were obtained from the Gudiyatham region in Tamil Nadu, India. Whatman filter papers were purchased from Amazon Private Limited, Bengaluru. Medi-Test Combi 10^{R} VET strips were procured from MACHEREY-NAGEL, Germany.

2.2 Methods

2.2.1 Collection of cow urine, its distillation and concentration

Indigenous *Deoni cows* housed at the Livestock Research Centre of the National Dairy Research Institute in Bengaluru, Karnataka, India (latitude:12.972442, longitude:77.580643), were selected for this study. These cows were maintained under uniform farm conditions, stall-fed with seasonal green fodder (hybrid napier grass), dry

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fodder (ragi straw), and a mineral mixture concentrate from the Karnataka Milk Federation. Urine samples from ten healthy cows of the *Deoni* breed in their late lactation stage were collected randomly in a clean containers during the early morning hours. Samples were filtered with sterile Whatman filter paper and screened for health parameters using "Medi-Test Combi 10^{R} VET" dip sticks (examination of urine) and pooled in equal volume within 1-2 hours. The filtered cow urine was subjected to distillation to obtain cow urine distillate, the residue left after the reduction of volume to 1/10th by distillation procedure was designated as cow urine concentrate (Fig. 1).

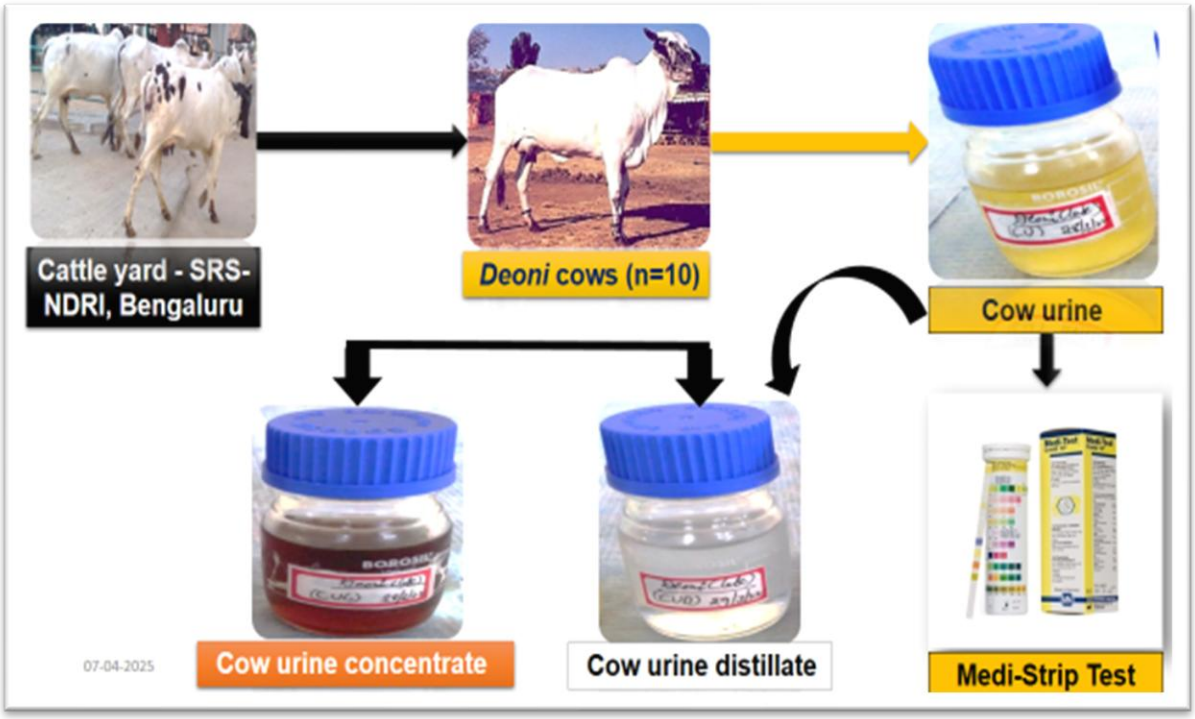


Fig. 1: Collection of cow urine samples and its cow urine distillate and concentrate preparation

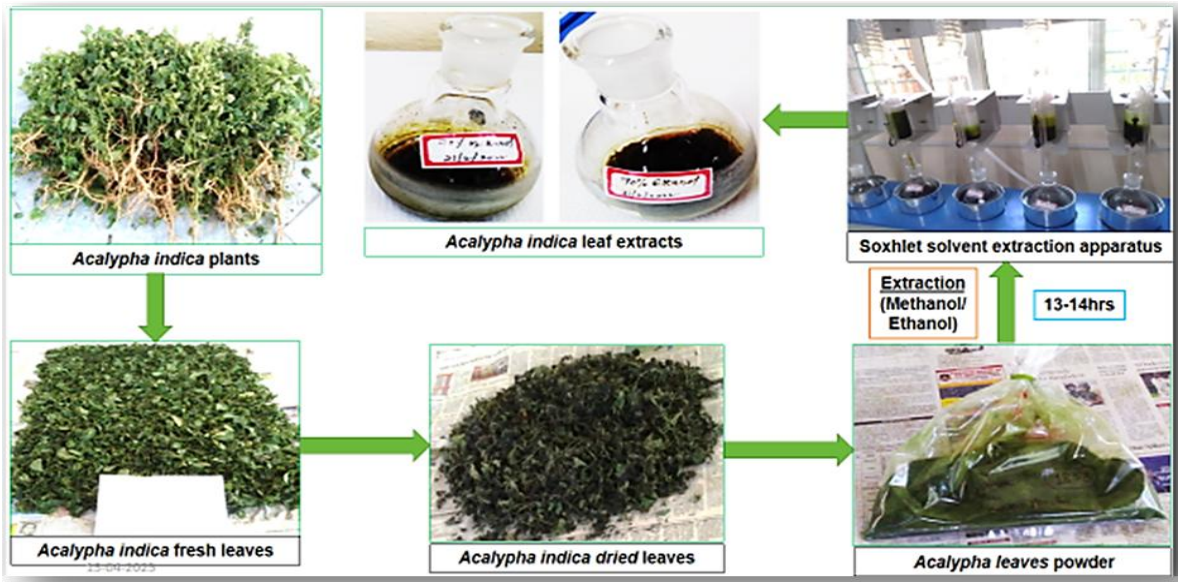


Fig. 2: Flow diagram of preparation of methanolic and ethanolic extracts of *Acalypha indica* leaves

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2.2.2 Acalypha indica plants leaf powder and its extracts

Acalypha indica L. plants were collected from the fields of Gudiyatham, Tamilnadu, India. The leaves were separated and thoroughly washed with tap water, followed by distilled water for the removal of dust and soil particles in a tray 2-3 times and shade dried for 3-5 days. Dried leaves were milled to obtain a fine powder. Extracts of *Acalypha indica* plant leaves were prepared by Soxhlet extraction method (Fig. 2). In a thimble, 10g of *Acalypha indica* leaves fine powder was extracted with 150 ml of 70% ethanol and 70% methanol solvents independently. The extraction was carried out for 13 hours continuously until the transparency of the extracts was obtained, and the obtained extracts were thereafter concentrated by evaporation in a rotary-vacuum. The obtained dry forms of extracts were used to prepare the samples. Finally, the leaf extraction yield was calculated as;

% Yield = Mass of total extracted compound/Mass of raw material × 100

2.2.3 FTIR Analysis

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in bioactive compounds. The FTIR analysis was performed as per the Standard protocol [Nautiyal and Dubey, 2021].

2.2.3.1 Sample Preparation

The test samples of *Deoni* cow urine, *Acalypha indica* leaf extracts individually were prepared using lyophilization process. The ample of each lyophilized test sample is used for FTIR analysis study.

2.2.3.2 FTIR Analysis Protocol

Fourier Transform Infrared Spectroscopy which is popular as FTIR analysis method is used to perform chemical identification of the components of any organic, inorganic or polymeric materials. The structural determination of different functional groups presents in lyophilized samples of *Deoni* cow urine, *Acalypha indica* leaf extracts and their combination was carried out by FTIR analysis (Perkin Elmer FTIR Spectrometer spectrum 3 equipped with ATR apparatus) within the spectral region of 4000-400 cm^{-1} (mid IR Region) wave number which reflects a molecular fingerprint of each sample at room temperature. The samples can be directly loaded onto the Diamond crystal and the samples were scanned 32 times to increase the signal to noise ratio at a resolution of 4.0 cm^{-1} to get proper infrared transmittance spectrum. The sample molecules convert the absorbed into rotational or vibrational energy. The detector of the resulting signal presents a spectrum generally from 4000 to 400 cm^{-1}, which reflects a molecular fingerprint of the sample. Each and every chemical or molecule form will generate a unique and inimitable spectral fingerprint. The spectral data were compared to reference in order to identify the chemical bond and functional groups existing in the sample [Coates, 2000; El-Naggar *et al.*, 2017]. Many techniques for explaining in detail regarding the FTIR analysis have been reported [Coates, 2000; Jaggi and Vij, 2006].

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3. RESULTS AND DISCUSSION

3.1 Results

In the current study, FTIR analysis was conducted on *Deoni* raw cow urine, its distillate, concentrate, and *Acalypha indica* methanolic and ethanolic leaf extracts using a mid-infrared (Mid-IR) Perkin Elmer FTIR spectrum 3 Spectrometer. Spectra were recorded in the range of 400–4000 cm^{-1}, generating transmission versus wavenumber profiles. The mid-IR spectrum was categorized into four distinct regions: (i) Single bond region (2500–4000 cm^{-1}), (ii) Triple bond region (2000–2500 cm^{-1}), (iii) Double bond region (1500–2000 cm^{-1}), and (iv) Fingerprint region (500–1500 cm^{-1}). Fingerprint region is the unique and specific region for each sample analyzed.

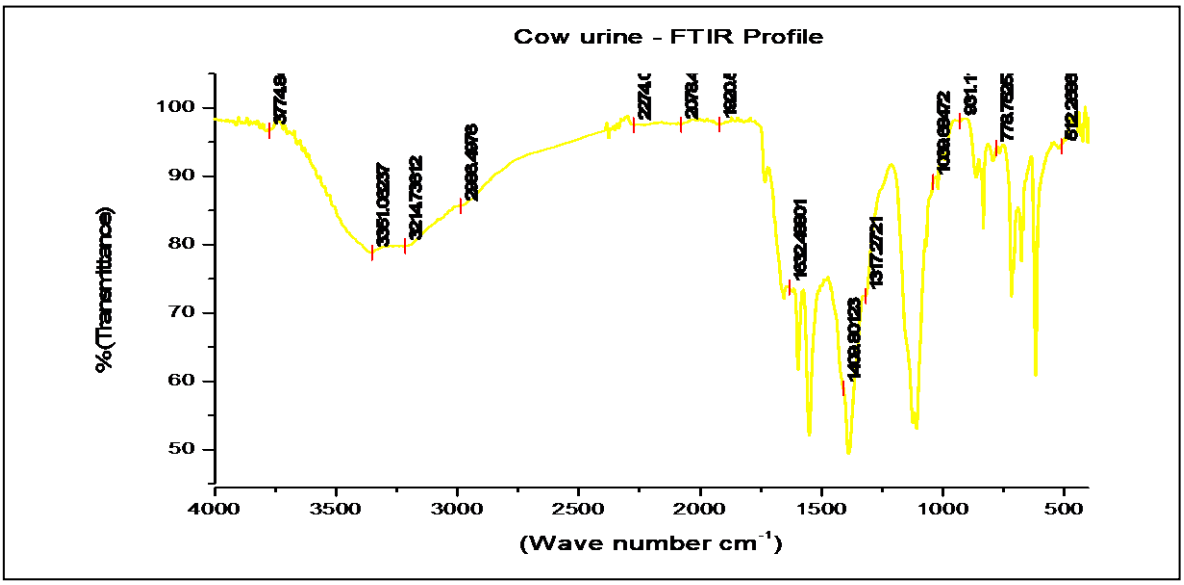


Fig. 3a: Fourier transform infrared spectra of *Deoni* cow urine sample

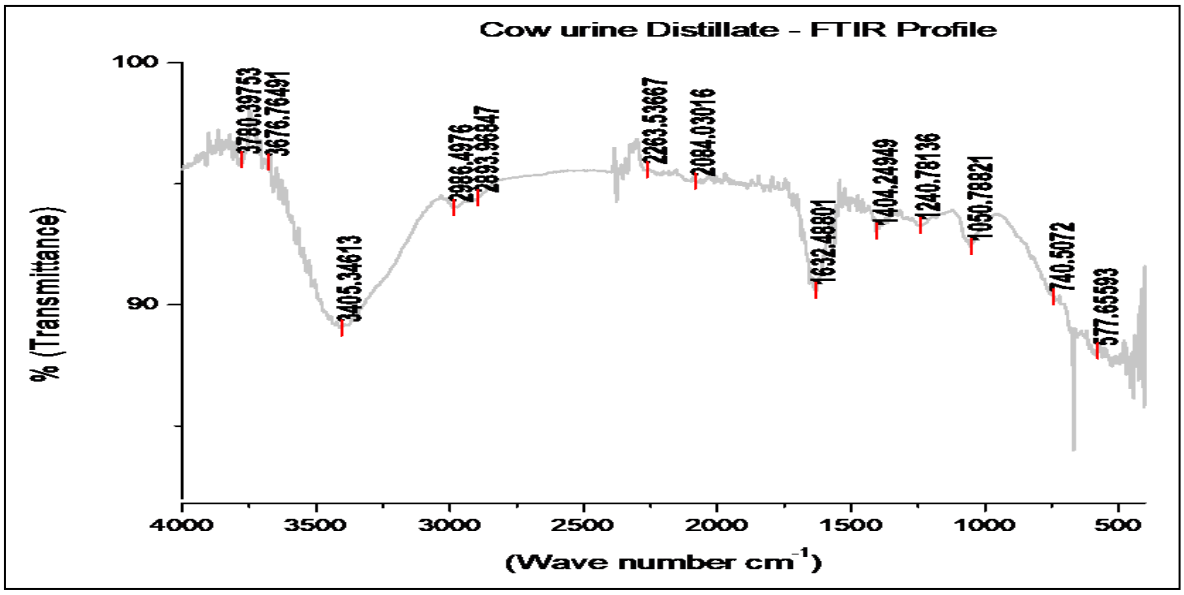


Fig. 3b: Fourier transform infrared spectra of cow urine distillate

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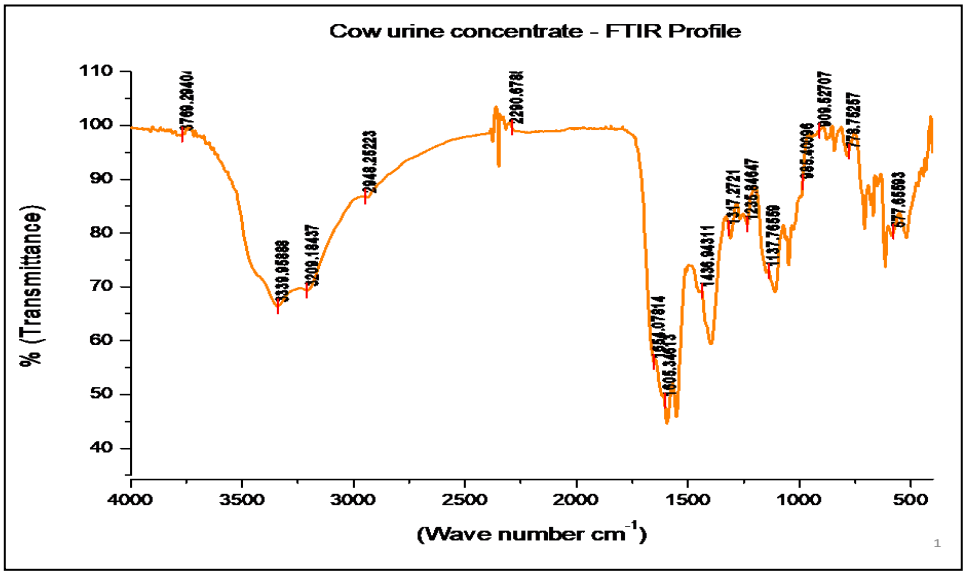


Fig. 3c: Fourier transform infrared spectra of cow urine concentrate

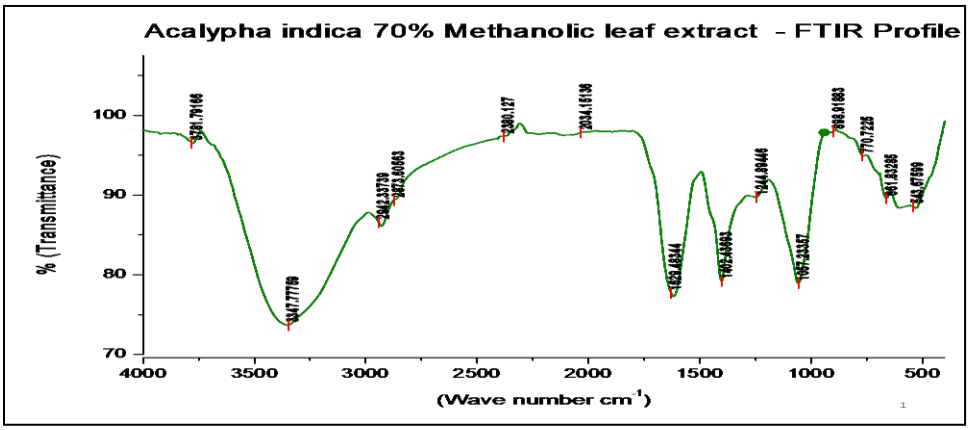


Fig. 4a: Fourier transform infrared spectra of *Acalypha indica* methanolic leaf extract sample

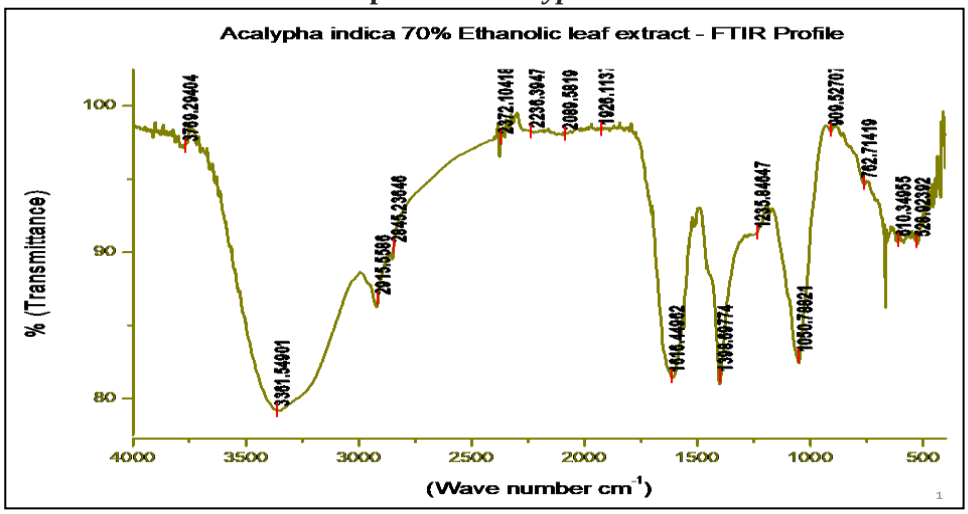


Fig. 4b: Fourier transform infrared spectra of *Acalypha indica* ethanolic leaf extract

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FTIR spectra of *Deoni* raw cow urine samples revealed a complex composition with diverse functional groups, confirming the presence of halogens, silicate ions, aromatic phosphates, and aromatic amines, primary and secondary amines, ammonium ions, and nitrogenous compounds, carboxylate groups, phenols, quinones/conjugated ketones, and amides, poly hydroxyl groups, water, alcohols, alkenes, methyl groups, and transition metal carbonyls. These functional groups are indicative of biologically active compounds that may contribute to antimicrobial, anti-inflammatory, and antioxidant activities. The FTIR spectrum of cow urine sample is presented in Fig. 3a. FTIR spectra of the cow urine distillate sample showed epoxy and oxirane rings, organic nitrates, organo halogens, and phosphate/silicate ions, cyclohexane groups, primary alcohols, and organic sulfates, ammonium and carboxylate ions, phenols, amides, quinones, and aromatic ethers. These functional groups suggest potential antiseptic, antioxidant, and wound healing properties. The corresponding FTIR spectrum of distillate is shown in Fig. 3b. The concentrate sample exhibited an even broader array of functional groups sulfonates, methyne groups, carbonate ions, tertiary alcohols, and aromatic nitro compounds, open-chain azo and imino groups, aromatic phosphates, and methyl groups, polymeric hydroxyl groups, amides, and phenols. These findings support the concentrate's potent bioactivity and pharmacological potential. The FTIR spectrum of concentrate is displayed in Fig. 3c. Functional groups corresponding to peaks of *Deoni* cow urine samples in each region are identified and are summarized in Table 2.

FTIR Profile of *Acalypha indica* methanolic leaf extract identified the presence of organo halogens, thioethers, alkynes, alkenes, aromatic ethers, and organic silicone, silicate and phosphate ions, primary and secondary amines, phenols, amides, quinones, and ammonium ions, azo and imino groups, methyl groups, and polymeric hydroxyl groups. These constituents may account for the extract's antimicrobial and antioxidant activity. The FTIR spectrum of methanolic leaf extract is shown in Fig. 4a. The FTIR profile of ethanolic leaf extract similarly revealed the presence of disulfides, aromatic phosphates, cyclohexane rings, carboxylates, and carbon dioxide, phenols, quinones, primary/secondary amines, transition metal carbonyls, azo/imino groups, water, alcohols, methyl/methylene groups, and nitrogenous compounds. The ethanolic extract, like the methanolic one, demonstrated a rich profile of bioactive functional groups, as depicted in Fig. 4b and Table 3.

3.2 DISCUSSION

In the present study; we evaluated the FTIR profiles of *Deoni* cow urine and *Acalypha indica* leaf extracts samples and their functional groups identification and detection for the first time.

Urine samples from ten healthy cows of the *Deoni* breed in their late lactation stage were collected randomly in a clean container and were filtered with sterile Whatman filter paper and the filtered cow urine was subjected to distillation, the residue left after the reduction of volume to $1/10^{th}$ by distillation procedure was designated as 'Cow urine concentrate' [Wate *et al.*, 2011] (Fig. 1).

Raw cow urine is highly dilute and complex biological fluid is mostly water (about 95-98%), with only 2-5% solids which include urea, creatinine, phenols, volatile fatty acids, enzymes, various minerals and with small amounts of organic and inorganic solutes are present in trace amounts. Early morning first voided cow urine is more sterile and has more macro and micronutrients along with other enzyme/urea content could be more effective. The main of cow urine is phenols. Phenols are bactericidal to *gram positive* and *gram negative* bacteria. Therefore, the presence of phenols in cow urine has a potent anti-microbial activity. The fresh cow urine contains more amount of phenol and hence has a better anti-microbial activity [Jarald *et al.*, 2008]. Fresh cow urine decomposes quickly due to microbial growth and enzymatic reactions (like urease breaking down Urea to Ammonia and CO_2). Cow urine has different fractions like anti-microbial activity due to the presence of certain components like volatile and non-volatile ones [Jarald *et al.*, 2008]. Presence of creatinine, urea, swarnakshar (aurum hydroxide), phenols, carbolic acid, calcium and manganese has strongly explained the germicidal and anti-microbial properties of cow urine. Presence of amino acids and urinary peptides may enhance the bactericidal effect by increasing the bacterial cell surface hydrophobicity.

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Fresh cow urine contains higher amounts of phenols makes it more effective against microbes. After photo-activation, few biogenic volatile inorganic and organic compounds such as; CO_2, NH_3, CH_4, methanol, propanol, acetone and some metabolic secondary nitrogenous products are also formed [Singh *et al.*, 2012]. Cow urine contains phenolic acids (gallic, caffeic, ferulic, o-coumaric, cinnamic and salicylic acids) which have anti-fungal characteristics [Upadhyay *et al.*, 2010]. When we want to analyse active compounds, direct analysis of raw urine is not effective. Hence, distillation and concentration are essential preparatory steps for separating organic compounds of interest.

Distillation helps separate volatile (phenols, aldehydes, short chain acids) and non-volatile substances, removing unwanted interfering substances before testing for analysis. Distillation and concentration of urine samples are crucial to remove water and impurities, enrich the organic constituents, and improve the accuracy and sensitivity of bioactive compound analysis. Distillation (evaporation) removes the bulk water, leaving behind a concentrated residue containing the. This concentration step improves the detection sensitivity of various analysis. Without concentration, the extraction efficiency is poor. After concentration, FTIR signals become stronger and clearer, making it possible to detect specific active compounds.

By concentrating urine (through evaporation or distillation; the bioactive compounds (urea, uric acid, volatile fatty acids, phenols and hippuric acid) become more potent per unit volume. This improves the biological activity and therapeutic efficacy in formulations. The concentrated urine reduces the volume needed for storage and transport and allows easier incorporation into the formulations. Concentration helps to standardize the active fractions and allow for quantitative estimation of key biomarkers for batch-batch consistency. Concentrated urine acts as a bio-enhancer (increasing absorption or activity of metabolites). Concentration-especially after mild heating or distillation – inactivates enzymes and reduces microbial load, adjusting P^H improves stability. Concentration removes volatile ammonia and offensive odour (partially), microbial contaminants and organic debris. Thus making it more acceptable and safer for pharmaceutical or veterinary formulations.

Acalypha indica L. plants were collected from the fields of Gudiyatham, Tamilnadu, India. The leaves were separated and dried leaves were milled to obtain a fine powder. Extracts of *Acalypha indica* plant leaves was prepared by Soxhlet extraction method using 70% methanol and 70% ethanol as solvents and obtained a good yield of extracts (Fig. 2).

Soxhlet extraction is preferred in our present study because it provides a reproducible, exhaustive, and solvent-efficient sequential extraction way to isolate active compounds allowing precise identification and characterization of functional groups. It also allows continuous washing of the sample with fresh solvent, ensuring that all extractable compounds are completely removed from the matrix. This exhaustive extraction is essential for accurate functional group analysis. The solvent collected after Soxhlet extraction is relatively pure and concentrated, containing only the soluble active molecules. This facilitates accurate analysis of compounds. It also offers high reproducibility compared to other methods.

We used 70% methanol as a solvent for Soxhlet extraction, because it provides balanced polarity, capable of extracting a wider spectrum of plant bioactive compounds both polar and moderately non-polar. This methanol penetrates through plant cell wall more effectively and dissolve intracellular metabolites. This synergy enhances overall extraction efficiency. The boiling point of 70% methanol is slightly higher (70ºC) than pure methanol (65ºC), which provides a milder extraction temperature and helps preserve heat-sensitive compounds (flavonoids and vitamins). 70% methanol gives high phenolic and flavonoid contents and shows better antioxidant and antimicrobial activities. In phytochemical analysis alkaloids and phenols are more in methanol, saponins are present in only methanol. Methanol has a good affinity towards alkaloids, phenols and flavonoids and low affinity towards saponins and amino acids. Methanol extract has the more active compounds. Phytochemical analysis shows that most of the phytochemicals got dissolved in methanol solvent [Sudhakar Chekuri *et al.*, 2016].

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The phytochemical constituents commonly present in both the leaf extracts include saponins, flavonoids, terpenoids and cardiac glycosides. Overall, the 70% methanol is used in Soxhlet extraction because the water-methanol combination provides optimal polarity, enhances cell wall swelling, improves solubility of diverse phytochemicals, and preserves thermo-labile bioactive compounds resulting in maximum yield, high reproducibility and functional bioactivities of extracted compounds.

We selected 70% ethanol as a solvent for Soxhlet extraction, because it provides balanced polarity, capable of extracting a wider and broad spectrum of plant bioactive compounds both polar (phenolics, flavonoids, some alkaloids) and moderately non-polar (essential oils and terpenoids). This ethanol penetrates through plant cell wall more efficiently and dissolve intracellular metabolites. This synergy enhances overall extraction efficiency. Many bioactive compounds (especially polyphenols, flavonoids and saponins) are more soluble in aqueous ethanol than absolute ethanol. The boiling point of 70% ethanol is slightly higher (78-83ºC) than pure ethanol (78ºC), which provides a milder extraction temperature and helps preserve heat-sensitive compounds (flavonoids and vitamins). 70% ethanol gives high phenolic and flavonoid contents and shows better antioxidant and antimicrobial activities. Overall, the 70% ethanol is used in Soxhlet extraction because the water-ethanol combination provides optimal polarity, enhances cell wall swelling, improves solubility of diverse phytochemicals, and preserves thermo-labile bioactive compounds resulting in maximum yield, high reproducibility and functional bioactivities of extracted compounds.

Qualitative phytochemical analysis were conducted to confirm the presence of active principles in *Deoni* cow urine samples and *Acalypha indica* leaf extracts exhibited a broader range of phytochemicals, including phenols, alkaloids, flavonoids, saponins, terpenoids, and tannins (Table 1). These phyto-constituents are known for their significant antioxidant, antibacterial, and anti-inflammatory properties, indicating the potential therapeutic efficacy strongly supports in the plant based novel formulation in veterinary applications. Phenolic compounds are more effective, inhibits lipid peroxidation (by inactivating lipoxygenase), scavenges free radicals and actives oxygen species by propagating a reaction cycle and chelates heavy metal ions. Phenolic compounds are responsible for the antimicrobial, anti-allergic, anti-diabetic, antioxidant, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties. Alkaloids were known to be possessing analgesic as well as antibacterial properties. Flavonoids are water-soluble antioxidants present in all vascular plants and have been reported to exert multiple biological effects including anti-inflammatory, anti-ulcerogenic, anti-allergic, anti-viral and cancer activities. Saponins are glycosides of steroids, steroid alkaloids found in plants, especially in the plant skins where they form a waxy protective coating. They are useful in lowering cholesterol, as antioxidants and anti-inflammatory agents. In addition to this, saponins also very important because of use in the treatment of hypo cholesterols and hyperglycaemia due to anticancer, antifungal, antioxidant, and antibacterial. Terpenoids are large and diverse class of naturally occurring organic chemicals found in all classes of living organisms. The presence of terpenoids in the leaf extract of *Acalypha indica* were well known due to the antibacterial, anti-inflammatory, anticancer, and antiviral properties. Tannins, another class of poly hydroxyl compounds are effective in scavenging free radicals as antioxidant, anti-cancerous and antibacterial activities. Tannins also has the ability to inter-fare the protein synthesis are implicated in protein, peptide, and amino acid binding which is important in wound healing and inflammation control.

Fourier transform infrared (FTIR) spectroscopy is a vibration spectroscopic method that utilizes the infrared radiation to vibrate molecular bonds within the sample that absorbs it. Most of the examples have diverse sub-atomic bonds or distinctive setups of sub-atomic bonds, FTIR enables to get compound data on particles inside the specimen [Baker *et al.*, 2008]. The FTIR spectrum is used to recognize the functional groups of the active components present in the cow urine and extract samples based on the strong instance peak values in the region of IR radiation.

The FTIR analysis of *Deoni* raw cow urine, distillate and concentrate samples results showed the various peaks in each region wise are identified. At fingerprint region (500-1500 cm^{-1}) the active peaks identified in the range of - 512-578, 740-780, 909-985, 1039-1138, 1235-1245, 1315-1350, and 1404-1437. At double bond region (1500-2000 cm^{-1}) - 1605-1660, and 1920. At triple bond region (2000-2500 cm^{-1}) - 2000-2300. Similarly at single bond (2500-

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4000 cm^{-1}) - 2800-3000, 3000-3500, and 3500-3800 respectively and these peaks can represent the active functional groups which was mentioned in Table 2.

Qualitative test	Active principles	Cow urine	Cow urine Concentrate	Methanolic leaf extract	Ethanolic leaf extract
Wagner's	Alkaloids	+	+	+	+
Ferric Chloride	Tannins	-	+	+	+
Foam test	Saponins	+	+	+	+
Ferric Chloride	Phenolics	+	+	+	+
Shinoda/Lead acetate	Flavonoids	+	+	+	+
Liberman Burchard	Terpenoids	-	+	+	+

Table 1: Phytochemical screening of cow urine samples and *Acalypha indica* leaf extracts
(+) indicates presence; (-) indicates absence of phyto-chemicals

Table 2: Functional groups in *Deoni* cow urine samples analysed by Fourier Transform Infrared Spectroscopy

Spectral Peaks	Cow urine	Distillate	Concentrate	Possible biomolecules
(1) Fingerprint Region (500 - 1500 cm^{-1})				
512 – 578	Aliphatic iodo compounds (C-I stretch) (organo halogen)	Aliphatic iodo compounds (C-I stretch) (organo halogen)	Aliphatic iodo compounds (C-I stretch) (organo halogen), Disufides (C-S stretch) (Thiols)	Organo-halogens, Disulfides
740 – 780	Aliphatic chloro compounds (C-Cl stretch) C-H 1,3-Disubstitution (meta)	Aliphatic chloro compounds (C-Cl stretch), C-H Mono-substitution (Phenyl), Methylene-CH2	Aliphatic chloro compounds (C-Cl stretch), C-H 1,3-Disubstitution (meta)	Organo-halogens
909 – 985	Cyclohexane ring (Methylene-CH2), Silicate ion, Aromatic phosphates (P-O-C stretch)	-	Silicate ion, Aromatic phosphates (P-O-C stretch), Vinyl C-H out of plane bend, skeletal C-C vibrations	Inorganic ions, aromatic phosphates
1039 - 1138	Aliphatic fluoro compounds (C-F stretch), Phosphate ion, C-OH stretch, Primary amine (C-N stretch)	Aliphatic phosphates (P-O-C stretch), Silicate ion, Phosphate ion, Organic silicone (Si-O-Si), Aliphatic fluoro compounds (C-F stretch), Primary amine (C-N stretch), Primary alcohol, C-O stretch, Aromatic C-H in plane bend	Sulfonates, Secondary amine (C-N stretch), Cyclic ethers (C-O stretch), Aliphatic fluoro compounds (C-F stretch)	Sulfonates, Amines, Organo halogens, Inorganic ions, Cyclic ethers, Carbohydrate, residues of cellular protein

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1235 - 1245	-	Aromatic phosphates (P-O-C stretch), Aromatic ethers, Aryl-O stretch, C-O-C stretch, Cyclohexane ring vibrations	Aromatic phosphates (P-O-C stretch), Aromatic ethers, Aryl-O stretch	Aromatic phosphates
1315 - 1350	Aromatic tertiary amine (C-N stretch), Aromatic amino acids (NO2 stretch), Phenol or Tertiary alcohol, OH bend	-	Sulfonates, Aromatic nitro compounds, Methyne C-H bend, Aromatic secondary amine (C-N stretch), Phenol or Tertiary alcohol, OH bend	Sulfonates, Aromatic nitro compounds, Amines, Phenol
1404 - 1437	Carboxylate COO- (Carboxylic acid salt), Ammonium ion (NH4+)	Organic-sulfates, Ammonium ion (NH4+), Epoxy and Oxirane rings	Carbonate ion (H2CO3-), Methyl C-H, Aromatic nitro compound (NO2)	Carboxylate, carbonate (precipitates proteins),Organic sulfates, Inorganic ion
(2) Double bond region (1500 - 2000 cm^{-1})				
1605 - 1660	Quinone/Conjugated ketone (Carbonyl compound C=O), Amide, Alkenyl C=C stretch	Organic nitrates, Amide, Carboxylate, Phenol or Tertiary alcohol, OH bend, Alkenyl C=C stretch, Secondary amine (NH stretch)	Organic nitrates, Amide, Phenol or Tertiary alcohol, OH bend, Alkenyl C=C stretch, Secondary amine (NH stretch), Quinone/Conjugated ketone (Carbonyl compound C=O), Open chain Azo (-N=N-) & Open chain Imino (-C=N-) Groups	Quinone, protein (amide), Phenol, amines, Proline amino acid, organic nitrate
1920	Transition metal carbonyls (Carbonyl compounds)	-	-	Transition metal carbonyls
(3) Triple bond region (2000 – 2500 cm^{-1})				
2000 - 2300	Isothiocyanate (-NCS), Aliphatic cyanide/nitrile (OCN, C≡N), Carbonyl compounds (C=O)	Thiocyanate ion, cyanide ion, Aliphatic cyanide/nitrile (OCN, C≡N)	Aliphatic cyanide/nitrile (OCN, C≡N), C-N (Inorganic compounds), Primary amine (NH stretch)	Nitrogen compounds
(4) Single bond region (2500 – 4000 cm^{-1})				
2800 - 3000	Methyl C-H stretch	Methyl C-H stretch, Methylene C-H stretch, -C-H aldehydic	Methyl C-H stretch	Alkane, fat
3000 - 3500	Polymeric OH stretch (Alcohol & hydroxyl compounds), Aliphatic primary amine (NH	Hydroxy group (H bonded to OH stretch), N-H stretch	Polymeric OH stretch (Alcohol & hydroxyl compounds), Ammonium ion (NH4+)	Carbohydrates, water

	stretch), Alcohol (OH stretch)			
3500 - 3800	Alcohol (OH stretch), Water (OH stretch)	Alcohol (OH stretch), Water (OH stretch), Non-bonded OH group (Hydroxy radicals)	Alcohol (OH stretch), Water (OH stretch)	Alcohol, Water

(-) = Absence of functional groups

The FTIR Spectrum results showed the presence of functionally significant compounds such as urea, creatinine, swarnkshar (aurum hydroxide), carbolic acid, phenols, calcium, and manganese present in urine samples are well known for their antimicrobial and germicidal properties. Notably, phenols identified in both plant and urine samples are poly hydroxyl compounds with strong bactericidal effects against both *Gram-positive* and *Gram-negative* bacteria. The presence of hydroxyl (-OH) groups, known for their toxicity to microbial cells, underscores the antibacterial potency of these samples. Similarly, the role of urinary peptides and amino acids in enhancing bactericidal activity through increased bacterial cell surface hydrophobicity. Tannins, another class of poly hydroxyl compounds detected in cow urine concentrate but absent in raw urine, are effective in scavenging free radicals and are implicated in protein, peptide, and amino acid binding which is important in wound healing and inflammation control.

The FTIR analysis of *Acalypha indica* leaf extracts samples results showed the various peaks in each region wise are identified. At fingerprint region (500-1500 cm^{-1}) the active peaks identified in the range of - 520-545, 610-662, 760-771, 1050-1060, 1235-1245, and 1398-1405. At double bond region (1500-2000 cm^{-1}) - 1615-1630, and 1926. At triple bond region (2000-2500 cm^{-1}) - 2000-2375. Similarly at single bond (2500-4000 cm^{-1}) - 2845-2945, 3347-3361 and 3769-3780 respectively and these peaks can represent the active functional groups (Table 3).

Table 3: Functional groups in *Acalypha indica* leaf extracts analysed by Fourier Transform Infrared Spectroscopy

Spectral Peaks	*Acalypha indica* Methanolic leaf extract	*Acalypha indica* Ethanolic leaf extract	Possible biomolecules
(1) Fingerprint Region (500 - 1500 cm^{-1})			
520 - 545	Aliphatic iodo compounds (C-I stretch)	Aliphatic iodo compounds (C-I stretch)	Organo halogens
610 - 662	Aliphatic bromo compounds (C-Br stretch), Sulfate, Thioethers (CH3-S), Alkyne C-H bend	Aliphatic bromo compounds (C-Br stretch), Sulfate, Disulfides (S-S stretch, Alkyne C-H bend	Organo halogens, Sulfate, Disulfides, Thioethers
760 - 771	Aliphatic chloro compounds (C-Cl stretch), C-H Mono substitution (Phenyl), C-H 1,2 Di-substitution (ortho)	Aliphatic chloro compounds (C-Cl stretch), Methyne-CH, C-H Mono substitution (para),C-H 1,2 Di-substitution (ortho)	Organo halogens
1050 -1060	Primary amine (C-N stretch), C-H 1,3 Di-substitution (meta), Aromatic C-H stretch out of plane bend, Alkene, C-O-C stretch	Aliphatic fluoro compounds (C-F stretch), Phosphate ion, primary alcohol (C-O stretch), cyclohexane ring/Alkyl substituted ether (C-O stretch)	Amine, alcohol, Inorganic ion, Organo halogens
1235 -1245	Aromatic ethers, Aryl-O stretch, Oranic silicone (Si-O-Si stretch), Silicate, Phosphate ion	Aromatic phosphates (P-O-C stretch), Aromatic ethers, Aryl-O stretch	Inorganic ions, Aromatic compounds, aromatic ethers

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1398 -1405	Carboxylate (COO-), Ammonium ion (NH4+), Organic sulfates, Phenol or Tertiary alcohol, OH bend)	Carboxylate (COO-), Ammonium ion (NH4+), Organic sulfates	Carboxylate, Organic sulfates, Phenol, Inorganic ion
(2) Double bond region (1500 - 2000 cm^{-1})			
1615 -1630	Quinone/Conjugated ketone (Carbonyl compound C=O), Secondary amine N-H, Open chain Azo (-N=N-), Amide, Primary amine N-H, Alkene	Quinone/Conjugated ketone (Carbonyl compound C=O), Secondary amine N-H, Primary amine N-H bend, Aromatic ring stretch C=C-C (aryl), Open chain Azo (-N=N-), Open chain imino (-C=N-)	Quinone, Phenol, Proline amino acid, Amine, Amide
1926	-	Isothicyanate (-NCS), Transition metal carbonyls	Transition metal carbonyls
(3) Triple bond region (2000 – 2500 cm^{-1})			
2000 - 2375	Isothicyanate (-NCS), Transition metal carbonyls, cyanide ion, thiocyanate ion, Aromatic cyanide/Nitrile	Isothicyanate (-NCS), cyanide ion, thiocyanate ion, C≡C Alkyne, Carbon dioxide (O=C=O)	Nitrogen compounds, Carbon dioxide
(4) Single bond region (2500 – 4000 cm^{-1})			
2845 - 2945	Methyl C-H stretch, -C-H Aldehydic	Methylene CH2 stretch	Fat
3347 – 3361	Polymeric OH stretch (Alcohol & hydroxyl compounds)	Polymeric OH stretch (Alcohol & hydroxyl compounds)	Carbohydrates, water
3769 – 3780	Alcohol (OH stretch), Water (OH stretch)	Alcohol (OH stretch), Water (OH stretch)	Alcohol, Water

(-) = Absence of functional groups

Additionally, recent studies highlight the therapeutic significance of other functional groups. For instance, epoxides, known for their high reactivity, contribute to keratinocyte proliferation and accelerate wound re-epithelialization [Lydia *et al.*, 2022]. Imine groups, recognized for their antibacterial and antifungal activities, enhance structural stability and act as barriers against pathogenic invasion, promoting wound closure [Yunlong Yang *et al.*, 2016]. The detection of peroxides (used in disinfectants), carbonate/carboxylic acid salts (which precipitate proteins), amines (from urea and amino acids), and amides (from urinary peptides and other low molecular weight compounds) further supports the multifunctional therapeutic potential of cow urine. The presence of these bioactive groups across different lactation stages also aligns with findings from recent studies, which underline the efficacy of epoxy- and imine-containing compounds in wound repair and antimicrobial defense [Cleiton *et al.*, 2011; Salomatina *et al.*, 2022]. These results clearly showcased the efficacy of individual samples as a comparison in a synergistic approach *in vitro*. These phyto-constituents are likely responsible for the observed *in vitro* antioxidant, antibacterial, anti-inflammatory and wound healing activities.

Previous literature studies conducted already on *Acalypha indica* plant leaves by FTIR Analysis confirmed the main active functional groups such as; C=O carbonyl group, C-O group, C=C group, C-H stretching, -OH group, CHO group with associated group like alkyl halides, aromatic compounds, alkaloids, aldehydes, phenols, carboxylic acids, amines in it [Umate *et al.*, 2020]. Similarly, the FTIR spectrum of *Acalypha indica* plant analysed clearly reveals the functional groups such as; sulfoxide, alkene, carbon dioxide, alkane, alcohol etc. present in it [Manna and Sharma, 2023]. These studies were also strongly supported the results what we obtained in our current study.

4. CONCLUSION

In the current investigation; we selected *Deoni* cow urine samples as animal-based as well as *Acalypha indica L.* plants as plant-based rich source of bioactive components and to identify the active functional groups using FTIR spectroscopy. The FTIR spectral analysis of *Deoni* cow urine and *Acalypha indica* leaf extracts revealed the presence of diverse functional groups representing various bioactive constituents. The prominent spectral bands corresponding to hydroxyl, carbonyl, amine, epoxides (enhancing keratinocyte proliferation), imines (with antimicrobial and antifungal activity), phenols (antibacterial, anti-inflammatory and antioxidant), urea, carbolic acid, phenols, creatinine (Antimicrobial activity) and peroxides (disinfectant ability). These active functional groups are indicative of major biologically active compounds are known to play significant roles in antimicrobial, anti-inflammatory, antioxidant and wound healing activities. The results provide a chemical basis for their traditional use in indigenous medicine and support their further exploration in developing natural formulations and bio-enhancers. Overall, the FTIR study establishes a molecular fingerprint that can be utilized for quality control, standardization, and authentication of cow urine-herbal based preparations. In future advanced spectroscopic investigations are needed for the identification and structural elucidation of compounds present in the cow urine samples and *Acalypha indica* leaf extracts in a promising way.

5. DECLARATION

Conflict of interest statement

The author (s) clearly certify that there is no conflict of interest in any manner with regard to the content/research work presented in the manuscript briefly as an original research article for submission to Journal of Chemical and Pharmaceutical Sciences for publication.

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Author contribution declaration

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