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# Investigation of Phytochemical, Chemical Composition and Antimicrobial Activities of Noni Leaf (*Morinda citrifolia* Linn)

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**Abstract:** *Morinda citrifolia* Linn (popularly known as "Noni") has been used in folk medicine by Polynesians for over 2,000 years. It is reported to have a broad range of therapeutic effects, including effects against headache, fever, arthritis, gingivitis, respiratory disorders, infections, tuberculosis, and diabetes. The aim of this study was to investigate the chemical constituents from leaves of *Morinda citrifolia* Linn (Noni). Preliminary phytochemical investigation on leaf of *Morinda citrifolia* Linn indicated the presence of steroids, terpenoids, flavonoids, glycosides, phenolic compounds, α-amino acids, reducing sugars, carbohydrates, saponins, tannins and alkaloids. The determination of elemental analysis by AAS method suggested that K (111.80 ppm), Ca (103.40 ppm), Fe (7.67 ppm), Mn (4.15 ppm) and Zn (1.15 ppm). By column chromatographic separation technique, isolated compound β-sitosterol (A, 0.029%) and ursolic acid (B, 0.01%) were isolated from PE crude extract of leaf of *Morinda citrifolia* Linn (Noni). The isolated compounds were identified by FT-IR spectroscopy. In vitro antimicrobial activity of some crude extracts (pet-ether, ethyl acetate, ethanol, and water) of Noni leaf was screened by agar-well diffusion method. $H_2O$ extract of Noni was found to exhibit potent of antimicrobial activity (25mm) against on *B. sub, S. sureous, P. seudomonus, B. pumalis, Candida* and *E. coli.*
**Keywords:** *Morinda citrifolia* Linn, Phytochemical, Antimicrobial activities, FT-IR spectroscopy.

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## Introduction

*Morinda citrifolia*, commonly named Noni, has been used as food and as a folk medicine throughout the tropics. Noni plant exhibits a remarkably high therapeutic and safety profile that makes it popular as a health enhancer and food supplement worldwide. The genus *Morinda* is present worldwide predominantly in tropical countries. It has been reported that different parts of Noni plant found to contain more than 160 phytoconstituents of which over 120 constituents have nutraceutical properties with proven biological activity [12]. It has been reported that *M. citrifolia* has wide range of therapeutic uses in ailments such as arthritis, burns, headache, wounds and skin infections [7]. Noni is pharmacologically active and is used in different forms of cancer, viz. colon, esophageal, breast, colorectal cancers; cardiovascular diseases, diabetes, arthritis, hypertension. Polynesian has been cultivated Noni

more than 1000 years where it is used as a coloring agent, medicine and food. The root has been used for dyeing agents by the Australians and Indians for various gloom of red, purple and yellow [9, 10].

Various parts of Noni plant such as stem, bark, root, leaf and fruits are used traditionally by Hawaiians and Tahitians as medicines for the treatment of ailments like cough, cold, pain and liver diseases, hypertension, blood pressure, tuberculosis, malaria, intestinal worms, diabetes, loss of appetite, hernias, urinary tract infection, menstrual disorder, cancer, cardiovascular diseases, arthritis etc [3]. Seeds and its oil applied topically on the scalp as insecticide and used for the treatment of arthritis. Flowers find in application in case of insect stings. In Hawaii, Noni has been reported for topical use in conditions such as swellings, sprains, bruises and wounds [2, 4]. This indigenous medicinal plant is of great importance to the health of individual and communities and has a long history of ancestors creating primitive medicines during their struggle against natural calamity and diseases [14].

Basically, medicinal plants are potential sources of natural antioxidants. They absorb the sun's radiation and generate high levels of oxygen as secondary metabolites of photosynthesis. Oxygen is easily activated by ultra violet (UV) radiation and the heat from sunlight to produce toxic, reactive oxygen species (ROS). Plants produce various anti-oxidative compounds to counteract these ROS in order to survive [1]. Thus, antioxidants are vital inhibitors of lipid peroxidation, not only for food protection but also as a defense mechanism of living cells against oxidative damage [16, 17]. *M. citrifolia* L. native to Polynesia is one of the traditional folk medicinal plants that have been used for over 2000 years by polynesians for treating diabetes, high blood pressure, cancer, eye problems and many other illnesses. Many important modern drugs are plant-based or derived directly or indirectly from the plants. Noni leaf is used as treatment for malaria, general febrifuge and analgesic, laxative, hypertension, tuberculosis, sprains, deep bruising, rheumatism, fever, sites, stomachache, ghost medicine, fractures, diabetes, loss of appetite, urinary tract, ailments, abdominal swelling, hernias, sting from stonefish, and human vitamin A deficiency [15].

## Materials and Methods

### Collection of sample

Leaves of *Morinda citrifolia* Linn (Noni) were collected from Mingalar Taung Nyunt Township, Yangon Division, Myanmar. The leaves were separated from the stem and out into small pieces and air dried at room temperature. The collected sample was identified in Department of Botany, University of Yangon.

### Chemicals

All chemicals used in this work were from British Drug House Chemical Ltd., Poole, England. All standard solutions and other diluted solutions throughout the experimental runs were prepared by using distilled water. In all the investigations the recommended methods and standard procedures involving both conventional and modern techniques were employed [13]. Precoated aluminium (TLC) plate and silica gel (40–60 μm) reagent were obtained from Sigma-Aid-rich, USA. All other chemicals and reagents used were of analytical grade.

### Preparation of Noni (*Morinda citrifolia* Linn) Leaf extracts

The leaves were separated from the stem and out into small pieces and air dried at room temperature. The samples were powdered by grinding with motor. The powdered samples were stored in air-tight container to prevent moisture and other contaminations. The dried

powdered sample (100 g) was percolated with pet-ether (60–80˚C), ethanol, ethyl acetate and water (500 mL) at room temperature for one week and filtered separately. This process was repeated for three times. The combined extracts containing plan constituents were evaporated under reduced pressure at 45˚C using rotatory evaporator. Consequently, pet-ether soluble extract was obtained. Then filtrate were vacuum dried using rotary evaporator and concentrates were stored at 4°C. The residues were re-dissolved with the appropriate solvents from which they were prepared and used for further studies.

## Preliminary Phytochemical analysis

Qualitative phytochemical analyses were performed in filtrates of Noni (*Morinda citrifolia* Linn) leaf extracts. Preliminary phytochemical test were carried out according to determine the presence of phytochemicals the steroids, terpenoids, glycosides, flavonoids, carbohydrates, alkaloids, phenolic compounds, α-amino acids, reducing sugars, saponins and tannins as described by standard procedure.

## Test organism

Screening of antimicrobial activity of various crude extracts such as PE, EtOAc, 95% EtOH, and watery extract of Noni (*Morinda citrifolia* Linn) leaf sample was investigated by Agar Well Diffusion Methods. In the present work, the test microorganisms were *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli.*

## Isolation of Some Organic Constituents

A glass chromatographic column (45×2.5 cm) with a tap attached was clamped so that it was perfectly vertical. The column was packed with silica gel by the wet method, using the solvent system of PE: EtOAc, 40:1 (v/v). Gradient elution was performed successively with PE: EtOAc (40: 1, 9:1, 4:1 and 2:1 v/v) solvent systems and 120 fractions (25 $cm^3$/fraction) were collected.

The fractions were monitored by TLC. The fractions gave the similar appearance on TLC were combined and finally three main fractions F I to F III were collected. After removal of the solvents, fractions FI and F II provided solid substances. Both of these solid materials were washed with pet-ether and purified by recrystallization from EtOAc to give compound A and compound B as colourless crystals, respectively.

## Determination of $R_f$ values

The isolated compounds were subjected to TLC analysis and their $R_f$ values were determined. $GF_{254}$ silica gel percolated aluminium plate (Merck) was employed and the chromatogram was developed in the appropriate solvent system. After the plate was dried, the $R_f$ values of isolated compounds were measured. Localization of spot was made by viewing directly under UV 254 and 365 nm light or by treating with visualizing agents. The $R_f$ values observed for isolated compounds are then recorded.

## Identification of Isolated Compounds

The infrared spectra of the isolated compounds were recorded on a Shimadzu Perkin Elmer Spectrum GX FT-IR spectrophotometer at Universities' Research Centre, Yangon University.

## Determination of Element with Atomic Absorption Spectrophotometry (AAS)

Atomic Absorption Spectrophotometer (Perkin Elmer A Analyst 880) was used for analyzing the elements of sample. The method involves measuring the absorption of radiation by the

atomic vapour produce from the sample solution, at a wavelength that is characteristic of element being determined.

## Screening of Antimicrobial Activity

The antimicrobial activities different crude extracts such as pet-ether, chloroform, 95% ethanol, ethyl acetate and watery extracts from leaves sample were determined against six strains of microorganisms such as *Bacillus substilis*, *Bacillus pumalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Escherichia coli* by employing agar well diffusion method [4] in Department Centre of Pharmaceutical Technology, Yangon.

## Culture of Bacteria

The bacteria species used were obtained from the Fermentation Department, Department Centre of Pharmaceutical Technology (DCPT), Ministry of Industry I, Yangon. A few colonies of the organism to be tested were incubated into the triple sugar iron agar and incubated at 37˚C for 24 hours in an incubator. A few colonies of the organism from triple sugar iron agar introduced into the tripticase soy broth (TSB) and incubated for 3 hours at 37˚C to obtain the bacteria suspension of moderate cloudiness. This contained approximately $10^6$ to $10^7$ organisms per mL.

## Screening of Antimicrobial Activity

In this method, PE, 95%EtOH, EtOAc extracts of Noni leaves were used as the samples. The extract (1 g each) was introduced into sterilized petri-dish and dissolved in 1 mL or with least amount of its respective solvent (PE or 95% EtOH or EtOAc or distilled water) till it was dissolved. The bacterial suspension from tripticase soy broth was done evenly onto the surface of the tripticase soy agar plates immediately after hardening of the agar well were made with a 10 mm sterile cork bore from each seeded agar. After inoculum has dried for 5 minutes, the agar disks were removed and the wells plates were incubated at 37˚C. After overnight incubation at 37˚C, the diameter of inhibition zone including 10 mm wells was measured. The well plate dilution method was used to test antimicrobial action of the extracts on 24 hours broth culture of the organism used.

## Results and Discussion

Phytochemicals detected in Noni leaf extracts According to the experiments steroids, terpenoids, glycosides, flavonoids, carbohydrates, alkaloids, phenolic compounds, α-amino acids, reducing sugars, saponins and tannins. Alkaloids, phenolics, terpenoids and cardiac glycosides detected in the extracts are compounds that have been documented to possess medicinal properties and health–promoting effects. Phenolics are the largest group of phytochemicals and have been said to account for most of the antioxidant activity of plant extracts. The results obtained by AAS are reported in Table 1. From these results, it was found that Potassium (K, 111.80 ppm), Calcium (Ca, 103.40 ppm), Iron (Fe, 7.67 ppm), Manganese (Mn, 4.15 ppm) and Zinc (Zn, 1.15 ppm). Among them, K and Ca were more predominant than other elements. Antimicrobial activities of various crude extracts such as PE, EtOAc, 95% EtOH and $H_2O$ extracts from Noni leaves were investigated against six species of microorganisms by employing agar well diffusion method.

In this study, the sample were tested on six species of microorganisms such as *Bacillus substilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albicans* (a fungus) and *E. coli*. The inhibition zone diameters of all crude extracts against all six microorganisms are summarized in Table 2. According to this results, watery extract showed inhibition zone diameters between 19~23 mm against all species of microorganisms,

whereas its pet-ether, 95% ethanol and EtOAc extracts exhibited lower antimicrobial activity inhibition zone diameters ranged between 14 ~ 18 mm against all of test microorganisms. By column chromatographic separation technique, four main fractions (F I, F II,F III and F IV) were obtained. F II and F III respectively gave the colourless solid materials A and B after removal of the solvents. Both compounds were purified by washing with PE followed by crystallization from PE and EtOAc to give compound A as a colourless needle shaped crystal and compound B as a colourless crystal. Compound A isolated as colourless needle shaped crystal from PE extract of Noni leaves, soluble in ethylacetate, ethanol, methanol and chloroform but insoluble in PE and water. According to reaction of 'A' with Libermann Burchard solution giving greenish blue colouration in $CHCl_3$ confirmed that 'A' was a sterodial compound [11].

The $R_f$ value of 'A' was found to be identical with that of β-sitosterol in any solvent system and they also gave the same behavior on TLC. The compound 'A' was inactive in UV light. According to the FT-IR spectrum Figure 4 and spectral data Table 3, the bands appeared at 3430 $cm^{-1}$, 1627 $cm^{-1}$, 1053 $cm^{-1}$ confirmed the presence of respective functional groups such as OH, C=C and C–O group in 'A'. The peaks at 2959, 2934, 2868 and 1459 $cm^{-1}$ showed the presence of $–CH_3$ and $–CH_2$ groups and the doublet peaks appeared at 1382 and 1370 $cm^{-1}$ revealed that 'A' had the $–CH(CH_3)_2$ isopropyl skeleton. The FT-IR spectral data of compound 'A' were found to be similar that of β-sitosterol [8].Consequently, from all of the information such as $R_f$ value, chemical tests and FT-IR spectral data of 'A', it can be inferred that compound 'A' was assigned as β-sitosterol and the structure of 'A'. Compound 'B', a colourless crystal isolated from PE extract of Noni leaves. It is soluble in chloroform, ethylacetate, ethanol and methanol, but insoluble in pet-ether. According to 1% $FeCl_3$ solution test, no colouration was observed, indicating that phenolic-OH group was absent in compound 'B'. Reaction with Libermann Burchard solution giving pink colouration ($CHCl_3$) confirmed that compound 'B' was a terpenoid [5].

It also gave a purple colour spot on TLC by spraying with vanillin-$H_2SO_4$ reagent after heating showed that it was a terpenoid compound. The FT-IR spectrum of compound 'B' depicted in Figure 5 and the corresponding spectral data assignment is also listed in Table 4. These results revealed the presence of –COOH group due to a broad band ranged between 2620–3442 $cm^{-1}$ and a sharp peak and 1690 $cm^{-1}$ appeared by the O–H and C=O stretching vibration. The board absorption was found to be overlapped with that of –COOH group at 3442 $cm^{-1}$. The presence of olefin (C=C group) was confirmed by the corresponding characteristic band at 1368 $cm^{-1}$. The band at 2928 $cm^{-1}$ confirmed that 'B' possess the $CH_3$ and $CH_2$ groups, it appeared due to asymmetric and symmetric C–H stretching vibration of alkyl groups.

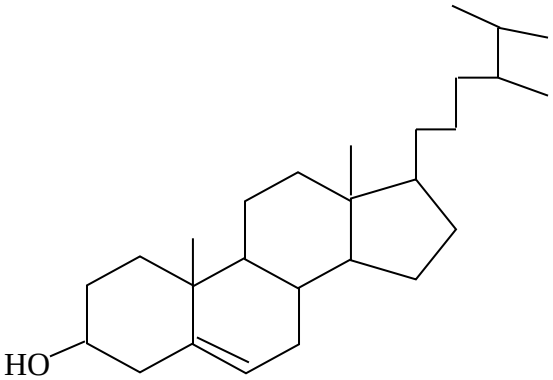
The absorption bands appeared as a doublet at 1385 and 1370 $cm^{-1}$ are probably due to isopropyl skeleton. All of these IR spectral data were observed to be similar to that of ursolic acid [6] (Table 4) One steroidal compound: β-sitosterol (A, 0.029%,$R_f$ = 0.52; PE : EtOAc = 9 : 1 v/v) and one triterpenoid compound: ursolic acid (B, 0.01%, $R_f$ = 0.55; PE : EtOAc = 1 : 1 v/v) were isolated from PE crude extract of Noni leaves sample by employing silica gel column and thin layer chromatographic methods .These isolated compound identified by FT-IR spectroscopy.

## Conclusion

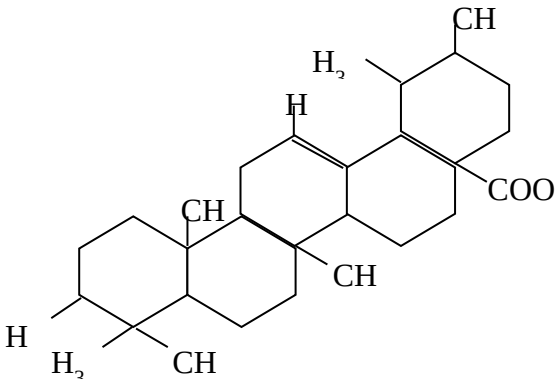
From overall assessment of the present work concerning with the chemical investigation and biological investigation of leaves of Noni, the following inferences could be drawn.

According to the phytochemical investigation, steroids, terpenoids, flavonoids, glycosides, phenolic compounds, α-amino acids, reducing sugar, carbohydrates, saponins, tannins and alkaloids were found to be present in these leaves. Moreover, Potassium and calcium were present in high content. These are extremely important elements in the human body. Screening of antimicrobial activities of crude extracts by agar disc diffusion method showed that the crude extract from water had highest activities on the tested organisms than the other extracts.

Many important modern drugs are plant-based or derived directly or indirectly from the plants. The popularity of Noni as a dietary supplement, a food functional ingredient, or as a natural health enhancer is increasing throughout the world. Noni contains phytochemicals that own antibacterial, antiviral, antifungal, antitumor, anthelminthic, analgesic, hypotensive, anti-inflammatory and immune enhancing effects. Moreover, the increasing vogue of Noni has attracted industries to employ it as a part of various products and for wide applications such as a natural source of medicines and chemical reagents as well as a green insecticidal.




**Structure of Compound 'A'**




**Structure of Compound 'B'**

**Table 1. Results of Elements in Ash Sample of Noni Leaf by Atomic Absorption Spectrometry**

| No. | Elements | Content (ppm) |
|---|---|---|
| 1 | Potassium | 111.80 |
| 2 | Calcium | 103.40 |
| 3 | Iron | 7.67 |
| 4 | Manganese | 4.15 |
| 5 | Zinc | 1.15 |

**Table 2. Inhibition Zone Diameters of Various Extracts Against six Microorganisms by Agar well Diffusion Method**

| Microorganism | Inhibition Zone Diameters (mm) | | | |
|---|---|---|---|---|
| | PE | EtOAc | EtOH | $H_2O$ |
| *Bacillus substilis* | - | 15 (++) | 17 (++) | 22 (+++) |
| *Staphylococcus aureus* | 18 (++) | 14 (++) | 18 (++) | 20 (+++) |
| *Pseudomonas aeruginosa* | 16 (++) | 15 (++) | 16 (++) | 23 (+++) |
| *Bacillus pumalis* | 17 (++) | 15 (++) | 16 (++) | 25 (+++) |
| *Candida albicans* | 16 (++) | 16 (++) | 18 (++) | 19 (+++) |
| *E-Coli* | 18 (++) | 15 (++) | 18 (++) | 20 (+++) |
| Agar well-10 mm, 10mm ~ 14 mm (+), 15 mm ~ 19 mm (++), 20 mm above (+++) | | | | |

**Figure 1. Leaves of *Morinda angustifolia* (Noni)**

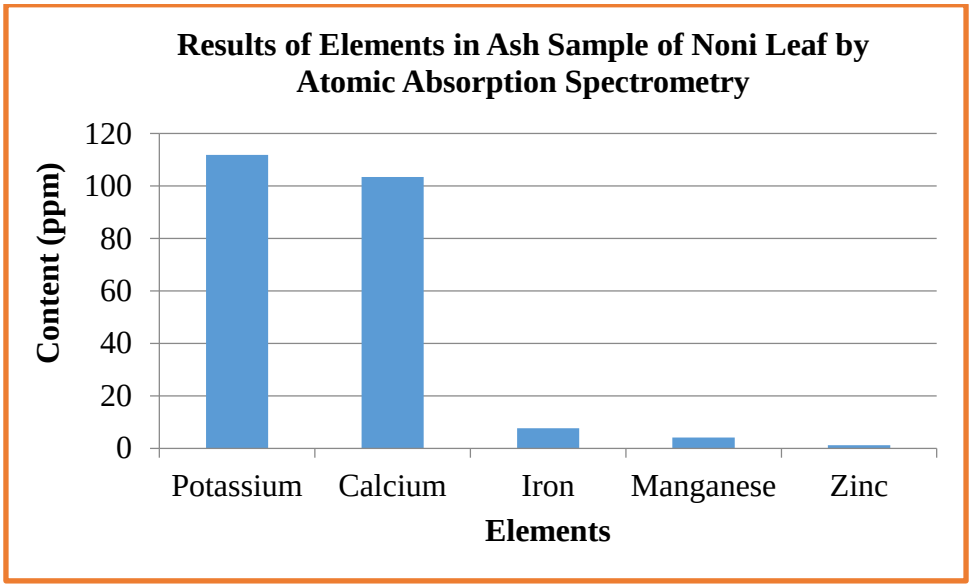



**Figure 2. Quantitative result of some Element in *Morinda angustifolia* (Noni)**

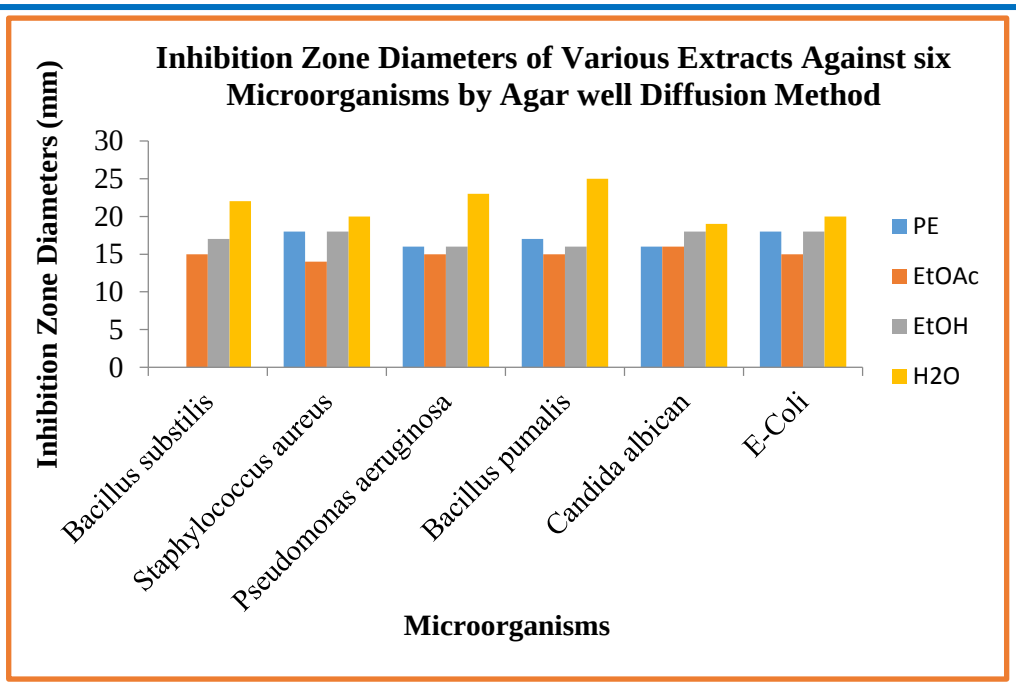



**Figure 3. Evaluation on Antimicrobial Activities of *Morinda angustifolia* (Noni)**

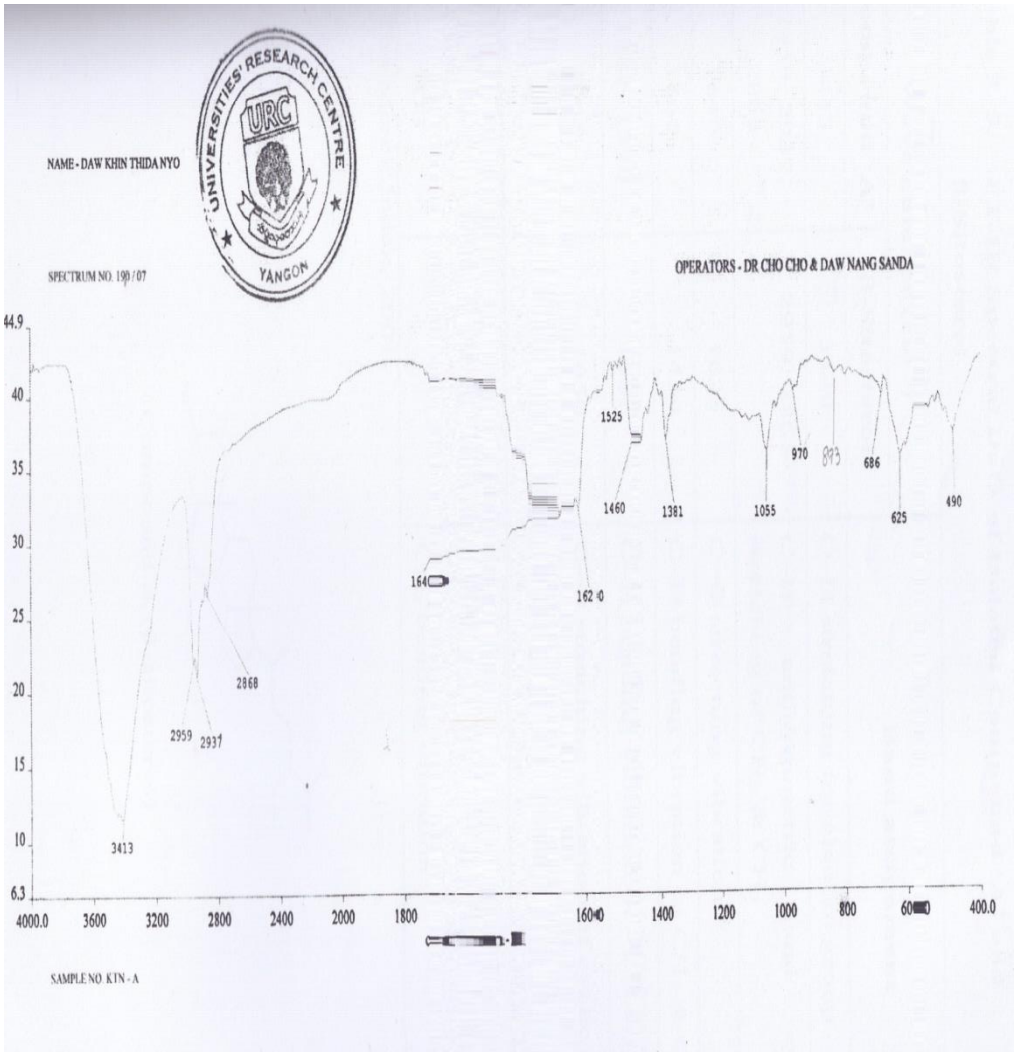



**Figure 4. FT-IR spectral data of isolated compound 'A'**

**Table 3. FT-IR Spectral Data of Isolated Compound 'A' and β-Sitosterol**

| Wave number ($cm^{-1}$) | | Band assignment |
|---|---|---|
| **Compound 'A'** | **B-Sitosterol** | |
| 3413 | 3430 | O–H stretching for alcohol group |
| 2959, 2937, 2868 | 2959, 2865 | C–H antisymmetric and symmetric stretching of $CH_2$ & $CH_3$ |
| 1640 | 1625 | C=C stretching vibration |
| 1460 | 1450 | C–H bending vibration of $CH_2$& $CH_3$ |
| 1381, 1370 | 1388 | C–H bending vibration of cylic alcohol |
| 1055 | 1050 | C–O stretching vibration of cylic alcohol |
| 873 | | $CH_2$ bending vibration |

The Merck Index, 2001 [8]

**Table 4. FT-IR Spectral Data of Isolated Compound 'B' and Ursolic Acid**

| Wave number ($cm^{-1}$) | | Band assignment |
|---|---|---|
| **Compound 'B'** | **Ursolic Acid** | |
| 2620 – 3442 | 3448 | OH stretching vibration of carboxylic acid overlapped with alcoholic OH stretching vibration |
| 2928 | 2943 | symmetric and asymmetric C–H stretching vibration of $–CH_3$ & $–CH_2$ group |
| 1690 | 1715 | C=O stretching of carboxylic acid group |
| 1638 | 1638 | C=C stretching vibration |
| 1385, 1370 | 1386, 1355 | C–H bending vibration of–$CH(CH_3)_2$group |
| 1313 | - | C–O symmetric stretching vibration |
| 1045 | - | Broad C–OH asymmetric stretching vibration |
| 998 | 997 | C–H out of plane bending |

Harbone, 1973 [6]

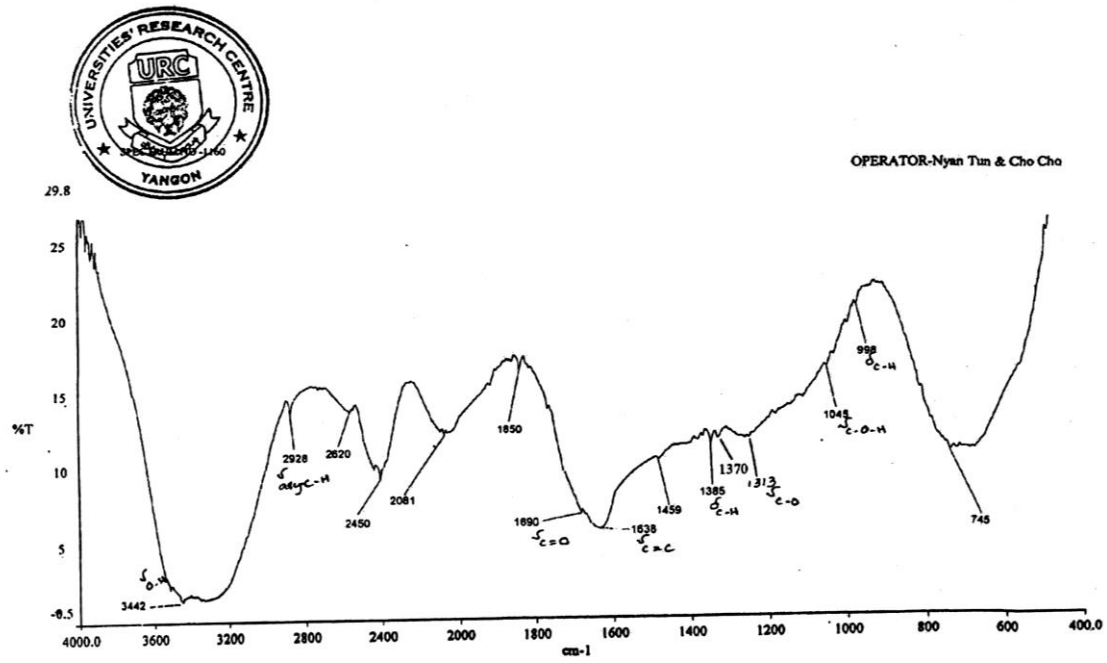



**Figure 5. FT-IR spectral data of isolated compound 'B'**

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