Investigation of Antimicrobial Activity, Elemental Analysis and Isolation of organic compounds of *Coptisteeta* Wall. (Hkantauk)

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Abstract

This research work was focused on the investigation of antimicrobial activity, elemental analysis and isolation of organic compounds of *Coptisteeta* Wall. (Hkantauk). Literature review revealed its wide applicability for helping reduce the risk of heart disease, stroke, lower blood sugar, mental and physical effects of stress, stomach cramps, diarrhea, digestive disorders, parasite infections, antibacterial, antiviral and inflammatory properties. Preliminary phytochemical investigation of*Coptisteeta* Wall. Revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, organic acid, phenolic compounds, reducing sugars, saponins, starch, terpenoids, tannins and absence of α – amino acid, steroids. The elemental analysis of this sample was also determined by using EDXRF spectrometry. Pet-ether, ethyl acetate, ethanol and water crude extracts of *Coptisteeta*Wall.were subjected for screening antimicrobial activity against *Bacillus subtilis,Staphylococcus aureus, Pseudomonas aeroginosa, Bacillus pumilus, Escherichia coli* and *Candida albicans* by agar well diffusion method. Organic compounds from the methanolic extract of *Coptisteeta* Wall.were isolated.

Key words: *Coptisteeta* Wall. , EDXRF, Antimicrobial activity, Organic compounds

Aim

- To study the investigation of antimicrobial activity, elemental analysis and isolation of organic compounds of *Coptisteeta* Wall. (Hkantauk)
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Botanical Description of *Coptisteeta* Wall.

Family	Ranunculaceae
Genus	Coptis
Species	C.teeta
Botanical name	*Coptisteeta* Wall.
Myanmar name	Hkantauk
English name	Gold thread
Part used	Rhizomes

Figure1. Photographs of the Plant and the Rhizome of *Coptisteeta* Wall.

Coptisteeta Wall. (Hkantauk) is a perennial stemless herb belonging to the family Ranunculacene. The plant is small, stemless, perennial ever green herbs, which is 20-50 cm in height. Rhizome of *Coptisteeta* Wall.is 5-6 cm long,oblique to horizontal with persistent fibrous roots, bitter, yellowish brown externally, golden yellow internally. Leaves are 5-6 cm long, lamina 3- lobed, pinnatifid, glabrous, petioles very long. Flower is small, white or yellowish. Fruits usually contain many seeded and the seeds are black. The flowers and the fruits appear from February- April. If the soil is hard, rhizome will not develop well. Since the plant is generally cultivated in moist area, watering is not required. Collection of rhizomes is done from September onwards before snow fall. Well dried rhizomes can be stored and used

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for a maximum of twenty years (Selvam,2012).It is a rare species. It is more commonly found in India, Tibet, Nepal and China (Liu& et al., 2010). It can also be seen in Putao Township, Kachin State. *Coptisteeta* Wall.is bitter, cooling and a potent bacteriostatic herb. It is used for the treated of various types of fevers, dysentery, nausea, jaundice, flatulence and visceral obstruction, hemorrhages, conjunctivitis, nervous diseases, eye troubles, appetite, constipation, indigestion, dyspepsia, asthma, cough and toothache (Selvam, 2012). The rhizome of *Coptisteeta* Wall.contains 8-8.5% of berberine (Latif& Zuberi,2008). In Chinese pharmacopoeia, these compounds included magnoflorine, nor oxyhydrastinine, jatrorrhizine, columbamine, epiberberine, coptisine, berberubine, worenine, palmatine, berberine and oxyberberine which make up of the 11 major active alkaloids for this herb (Hung& et al.,2015).

Screening of Phytochemical Constituents on *Coptisteeta* Wall. (Hkantauk)

The different extracts from rhizomes of *Coptisteeta* Wall.were tested for the presence of phytochemical constituents by using standard procedures.The phytochemical tests revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, organic acid, phenolic compounds, reducing sugars, saponin, starch, terpenoids and tannins. However, α – amino acids and steroids were not found in *Coptisteeta* Wall.

Figure 2. Alkaloids, Carbohydrates and GlycosidesTests of *Coptisteeta* Wall.

Some Elemental Analysis of the Sample by Energy Dispersive X- Ray Fluorescence (EDXRF) Spectrometry

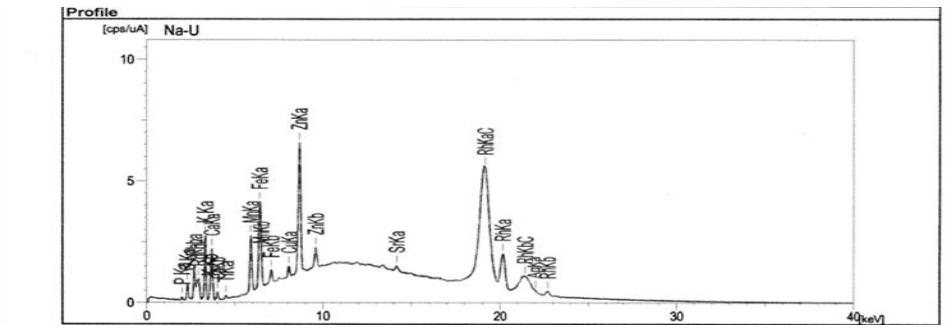


Figure 3. EDXRF spectrum of *Coptisteeta* Wall.

In this work, relative abundance of elements present in *Coptisteeta* Wall.was determined by EDXRF spectrometer. It can be observed that P, Ca, Fe, Mn, Zn, Ti, Cu, Ag and Sr were present as trace elements in this sample. In addition, the relative abundance of S and K were observed to be highest.

Screening of Antimicrobial Activity of Various Crude Extracts by Agar Well Diffusion Method

The antimicrobial activities of different crude extracts such as pet-ether, ethyl acetate, 95% ethanol and watery extracts from the rhizomes of *Coptisteeta* Wall. 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 at Fermentation Department, Development Centre of Pharmaceutical Technology, Ministry of Industry I, Yangon, Myanmar.Ethanolic extract and watery extract of this sample were found to be significant antimicrobial activity against six microorganisms. In addition, the pet-ether extract and ethyl acetate extract showed a moderate antimicrobial activity against six strains but ethyl acetate extract did not show activity against Pseudomonas aeruginosa.

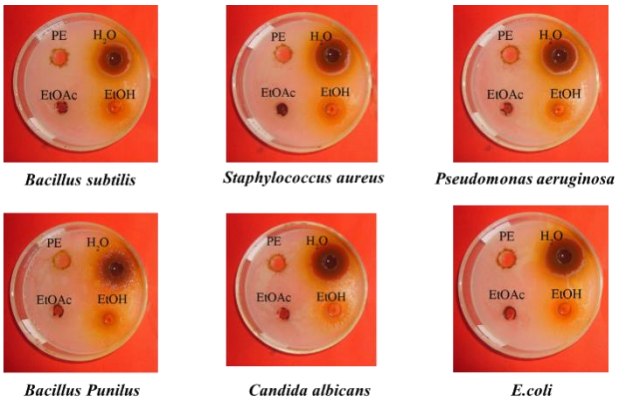


Figure 4. Effect of Different Extracts from *Coptisteeta* Wall. on*Bacillus substilis, Staphylococcus aureus, Pseudomonas aeruginosa,Bacillus pumalis,Escherichia coli* and *Candida albicans*

Isolation of organic compounds from rhizomes of *Coptisteeta* Wall.

Air-dried rhizomes powder of *Coptis teeta* wall. (150g)

↓ Extracted with methanol (1L)

Methanolic extract

↓ Concentrated

MeOH crude extract (5g)

↓ Dissolved in hot water

↓ Filtered

Filtrate

↓ Treating with excess 1% HCL

Berberine hydrochloride (precipitate)

↓ Dissolved in hot water

↓ Basified with 10% NaOH solution

↓ Addition of acetone, followed by dilution with equal volume of water

↓ Standing overnight

Berberine – acetone precipitate

↓ Washed with ice-cold water

↓ Evaporated to dryness at room temperature

Berberine-acetone (0.6891g)

Separated by column chromatography

$CHCl_3$:MeOH (19:1- 4:1)

$CHCl_3$:MeOH (19:1)

F1 (Compound I)

$CHCl_3$:MeOH (4:1)

F2 (Compound II)

Methanol crude extract CHCl3: MeOH (9:1)

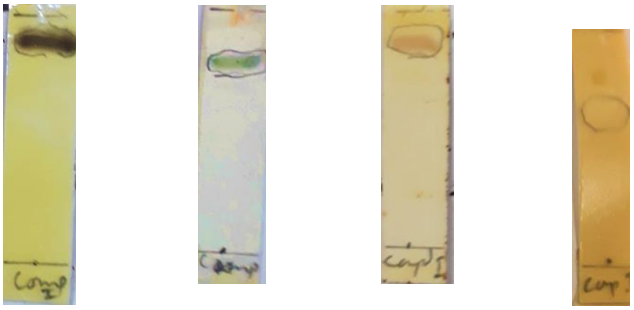
$CHCl_3$:MeOH (19:1)
R_f=0.6

yield percent (0.198%)

Ethyl acetate: MeOH (1:1)

R_f= 0.2

yield percent (0.4488%)



(A) (B) (C) (D)

A=Liebermann BurchardB=5%H_2SO_4 C=Iodine D= 5%$FeCL_3$

Figure 6. Phytochemical screening of isolated compound I with ethyl acetate: methanol (19:1) in methanol extract from *Coptisteeta* Wall.

Figure 7. TLC plate show spot of isolated compound II with ethyl acetate: methanol (1:1) solvent system spraying with Dragendorff's reagent

Compound I was isolated as yellow needle shspe crystal in 0.198% of yield from methanolic extract of *Coptisteeta* Wall. It has the melting point 210 ^{o} C. Its R_f value was found to be 0. 93 in $CHCl_3$: MeOH (9:1) solvent system. It also showed a dark brown coloration with Liebermann Burchardand and green coloration with 5%H_2SO_4 .Isolated compound I was added to chloroform and 3 drops of acetic anhydride. Then a few drops of concentrated sulphuric acid were carefully added and shaken. Formation of brown color indicates the presence of terpenoid.

Figure 9. Observation of Terpenoid

(ii) **Spectroscopy study of isolated compound II in methanolic extract from *Coptis*teeta Wall.**

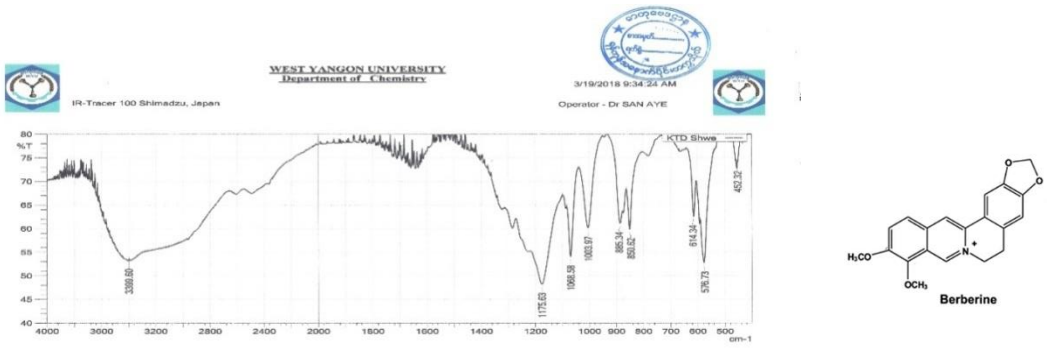


Figure 8. FT IR spectrum of isolated compound II from *Coptisteeta*Wall.

Compound II was isolated as yellow crystal in 0.4488% of yield from methanolic extract of *Coptic teeta* Wall. It has the melting point of 147^{o}C. Its R_f value was found to be 0.34 in $CHCl_3$: MeOH (9:1) solvent system. It also showed a brown sport on TLC chromatogram with Dragendorff's reagent, indicating that it may be alkaloid compound. The melting point of isolated compound II(145-147)^{o}C was identical with that of berberine (Lit. 145^{o}c)(Blaska*et al*., 1988).The functional groups present in isolated compound were studied by FT IR spectrum (Figure 8). The broad band at (3600- 3000) cm^{-1} may be assigned to O-H stretching band. The band at 3399 cm^{-1} represents N-H stretching band of amine. Ether may be identified by a strong C-O stretching band near 1100cm^{-1} due to the C-O-C linkage. Cyclic ether show a C-O stretching band over a broad 1250- 900 cm^{-1} range. Therefore, the band appeared at 1176 cm^{-1} is due to the C-O-C stretching for cyclic ether. The band appeared at 1068 cm^{-1} and 1003 cm^{-1} also represent the aliphatic C-N stretching bands respectively. The bands at 884 cm^{-1}, 849 cm^{-1}, 614cm^{-1} and 577 cm^{-1} showed C-H stretching of cyclic ether. According to the information such as physicochemical property, melting point, and FT IR, it may structurally be berberine, alkaloid.

Conclusion

From overall assessment of the present work concerning, alkaloids, carbohydrates, flavonoids, glycosides, organic acid phenolic compounds, reducing sugars, saponin, starch, terpenoids and tannina were present. However, α – amino acids and steroids were not found in *Coptisteeta*wall.According to the EDXRF elemental analysis, it can be observed that P, Ca, Fe, Mn, Zn, Ti, Cu, Ag and Sr were present as trace elements in this sample. In addition, the relative abundance of S and K were observed to be highest.From the results of antimicrobial activity by agar well diffusion method, polar extract was found to be more significiant activity than nonpolar extract. The two compounds were isolated from methanolic extract of *Coptisteeta* Wall. by column chromatographic technique. The isolated compounds were characterized by physiochemical properties and spectroscopic study. According to melting point and FT IR spectra data, compound I may be terpenoid and compound II may be berberine alkaloid.

Outcome

Coptisteeta Wall. is used as a medicinal herb in China and India where it is used as a bitter tonic for treating malarial fever and dyspepsia .Therefore, the use of Myanmar herbal medicines for diabetes treatment or for the prevention of diabetes complications might be

generally considered good for the patient's general well- being, apart from their effectiveness and safety.

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