Microscopical Studies and Antimicrobial Activities of *Crepis japonica* (L.) Benth.

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ABSTRACT

Crepis japonica (L.) Benth.is a medicinal plant belongs to the family Asteraceae which is widely grown in central Myanmar. The plants are used in Myanmar traditional medicine as in cough, dyspepsia and febrifuge. The plant is used to serve as herbal tea and in cuisine as salad in central Myanmar. It is known as Dauk-khwa in central Myanmar and Oriental hawk's beard in English. The plant was collected from Magway township, Magway Region. The microscopical characters of leaves, stems, roots in this plant has been undertaken by freehand section. In the microscopical study, the vascular bundles of these plant parts were collateral and closed type. Bundle sheathes of vascular bundles were made up of parenchymatous cells distinctly and anomocytic types of stomata were found on both surface of leaves. The transverse section of stem and root of this plant, secondary growth was found in nature. The Antimicrobial activities, the plant powdered were extracted by using Pet-ether, chloroform, acetone, methanol, ethyl-acetate and ethanol. This test was conducted at Development Centre for Pharmaceutical Technology (DCPT). Ethanolic extracts of the whole plant showed the highest activity on six Pathogenic microorganisms. These results indicate that the plant has appreciable antimicrobial activities and microscopical characters were accurate for plant identification.

Keywords: *Crepis japonica* (L.) Benth., febrifuge, Antimicrobial activities, Ethanolic extracts, Pathogenic microorganism.

INTRODUCTION

The wild plant *Crepis japonica* (L.) Benth. belongs to the family Asteraceae according to Hundley and Chit Ko Ko (1987). There are about five species in Myanmar. In Heywood (1993) reported the family Asteraceae comprises about 1,100 genera and 25,000 species. In Trease and Evans (2002) stated that have 900 genera and 13,000 species. It is native of Japan and Korea to Western China, Malay Peninsula to North Western India, Phillipines and Malay Archipelago (Dassanyake 1980). It is also found in India and Ceylon, China, Affghanistans and Mauritius (Hooker, 1880). The plant is also the native of throughout India, ascending to 10,000 ft and East Asia. (Collett, 1971).

Crepis japonica (L.) Benth. is commonly known as Dauk-khwa in Central Myanmar and Oriental hawk's beard in English. It is traditionally used as carminative, purgative, asthma, diabetes and dyspepsia. Accordingly it is also used to serve as herbal tea and in cuisine as salad in Central Myanmar. In the literature, hand book of natural foods by San Hla (1960), it was said to be used in asthma, carminative and purgative.

MATERIALS AND METHODS

Histological Study of *Crepis japonica* (L.) Benth.

Histological characters of leaves, stems, roots and powdered sample were presented by using available literature in the Department of Botany, University of Yangon. The fresh specimens were examined by preparing freehand section and studied under microscope. Temporary mounts were prepared with glycerin.

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Antimicrobial activities of various solvent extracts from the whole plant *Crepis japonica* (L.) Benth. by using agar-well diffusion method

For the determination of antimicrobial activity of the whole plant extracts agar well diffusion method. (Cruickshank, 1975).

The powdered leaves were extracted with chloroform, petroleum-ether (60°-80°C), acetone, ethyl acetate, 95% ethanol, methanol and distilled water by percolation method. The filtrate solvents were evaporated by using water bath.

Crude extracts of various solvents were tested on six pathogenic microorganisms, such as *Bacillus pumalis, Bacillus subtilis, Candida albican, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus*. The test organisms used in this research were kindly supplied from the Development Centre for Pharmacutical Technology for determination of the antimicrobial activities.

RESULTS

Outstanding Characters of *Crepis japonica* Benth.

Habit	-	annual or perennial glabrous herbs
Leaves	-	simple, radical, petiole very short, lyrate, reticulate venation
Infloresence	-	Terminal head, small, oblong
Flower	-	Pale yellow, strap-shape. Multiseriate bracteate, zygomorphic, pentamerous, epigynous
Calyx	-	Pappus, fine slivery hairs
Corolla	-	Petals (5), strap-shape
Androecium	-	Stamens 5, syngenesious, longitudinal dehiscences
Gynoecium	-	carpels 2, style long and slender, bifid stigma, inferior
Fruit	-	achene, flattened, many pappus
Seed	-	erect, embryo straight

Habit

Inflorescence

Histological Characters of leaves, stem, root and powdered of *Crepis japonica* (L.) Benth.

Microscopical Characters of leaves

Lamina

In surface view, the epidermal cells of both surfaces were parenchymatous and thin walled. The cell walls of upper surface were wavy. The cell walls of the lower surface were more than wavy of the upper epidermis (Fig. 1.1-1.3). The cells were polygonal in shape. Stomata were present on both surfaces and more abundant in lower epidermis. They were anomocytic, oval. The guard cells were cresent shape and

contain abundant chloroplasts. Trichome absent on both surface. In transverse section, the arrangement of lamina tissue is dorsiventral. The upper epidermal cells were rectangular or barrel-shaped. The lower epidermal cells were different in size.

The mesophyll consisted of palisade and spongy parenchyma. The palisade mesophyll was made up of two layers of vertically elongated cylindrical cells, which are closed packed with one another. They contains numerous chloroplasts and oil drops. The spongy mesophyll consisted of 2 to 3 layers of cells, which were irregular to isodiametric in shape and compactly arranged. They contained numerous chloroplast. The vascular bundle of lateral veins consisted of xylem between the upper and lower of the phloem rounded in shape. These arrangement were collateral type. Each vascular bundle was surrounded by a compact layers of thin-walled parenchymatous cells with numerous starch which was known as a bundle sheath (Fig. 1.1).

Midrib

In surface view, the epidermal cells of both surfaces were parenchymatous and elongated along the length of the midrib and rectangular. The upper epidermal cells were comparative smaller than the lower epidermal cells. Trichome and stoma were absent in both surfaces. In transverse section, the upper surface of midrib was slightly convex and moderately concave in lower surface. The cuticle layer of the lower surface was thicker than the upper. The upper epidermal cells and lower epidermal cell were similar in size and shape. They were polygonal to barrel shaped.

The cortex was made up of angular collenchymatous and thin walled parenchyma cells. The collenchyma cells were 2 to 3 layers in thickness towards the upper surface. They were rounded to isodiametric in shape. The parenchyma cells were 2 to 3 layers above the vascular bundle and 3 to 4 layers beneath the vascular bundle. Sandy Calcium Oxalate Crystal and intercellular spaces were numerous among them. The vascular bundles were more or less rounded in outline, collateral type. One main vascular bundle and two accessory bundles were occur at the base and middle regions of midrib. Only one vascular bundle was found at the tip of mid-vein (Fig. 1.2).

Petiole

In surface view, the epidermal cell of both surfaces were rectangular shaped and elongated; Stoma were absent. In transverse section of petioles, the petiole were cresent shaped with two lateral processes. The upper and lower epidermal cells were barrel to polygonal shaped and single layered (Fig. 1.3-1.4).

The cortex made up of single layer of collenchymatous and thin wall parenchyma. The parenchyma cells were four to five layers above the vascular bundle and five to six layer below bundles. They were polygonal shaped.Below the vascular bundle, the parenchyma cells includes 5 to 6 layer in thickness.The vascular bundles were surrounded by bundle sheath. They were distinct and made up of parenchymatous cells, polygonal shaped and vascular bundles were collateral type. Xylem cells were towards the upper surface. Phloem cells were towards the lower surface.

Stem

In surface view, epidermal cells were compactly packed, rectangular to polygonal shaped. Stomata were present and anomocytic type. Trichomes were absent (Fig. 1.9). In transverse section, the stems were oval in outline. Epidermal cells were one layered, rectangular shaped. The cortex were made up of collenchymas and parenchyma. Collenchyma cells were adjacent to the epidermal cell 2 to 3 layers, oval

or polygonal. Parenchyma cell were placed beneath the collenchyma cells, 3 to 4 layers, rounded or polygonal. Intercellular spaces were numerous among them. Endodermis were made up of parenchymatous cell and one layered. They were polygonal shaped (Fig. 1.5-1.6).

Pericycyclic layer were lying in between the endodermis and the vascular bundles. They were semi-lunar patches of sclerenchyma and the interveing masses of parenchyma. Each patch lying associated with the phloem and vascular bundle. Pith was extends from below the vascular bundles to the centre. They were made up of 15 layers parenchymatous cells with conspicuous intercellular spaces between them. Vascular bundles were about 12 to 14 in number and collaterally closed types.

Root

In surface view, epiblema cells were longitudinally elongated to rectangular. The epiblema cells were obliterated and filled with dark-brown pigment. In transverse section, the roots were more or less circular in outline, periderm consisted of three regions. They were phellem (or) cork, phellogen (or) cork cambium and phelloderm (or) secondary cortex. The phellem 2 to 3 layered of cells rectangular to nearly polygonal shaped. Phellogen were 2 to 3 layered, cell thin walled radially flatterned rectangular. Phelloderm were 4 to 5 layered, oval to polygonal. The parenchyma cell contain patches of starch were present (Fig. 1.7-1.8).

Vascular bundle were 6 to 8 in numbers. Vascular bundle consisted of phloem toward the outer and xylem toward the inner. Medullary rays were multiseriate radialy elongated or more or less rectangular. Pith occurred at the centre of the root and made up 5 to 6 layers of parenchymatous cell. It is polygonal shaped. Intercellular space, needle shape and cluster of Calcium Oxalate Crystal were present in pith parenchyma.

Antimicrobial activities of various solvent extracts from whole plants *Crepis japonica* (L.) Benth.

In the experiment, the antimicrobial activities of crude extracts were carried out by using various solvent such as petroleum ether (60-80ºC), chloroform, acetone, ethyl acetate, ethanol, methanol and distilled water (Fig 1.9-1.10)

According to this experiment, the whole plant extracts with chloroform, acetone, methanol and ethanol showed effective antimicrobial activity on six different microorganisms. Pet-ether (60-80°C) extracts showed antimicrobial activity on *Pseudomonas aeruginosa* and *E.coli*. The ethyl acetate extracts showed antimicrobial activity on *Pseudomonas aeruginosa*. The watery extracts did not showed antimicrobial activity on six microorganisms (Table 1.1).

Microscopical Characters of Leaves of *Crepis japonica* (L.) Benth.

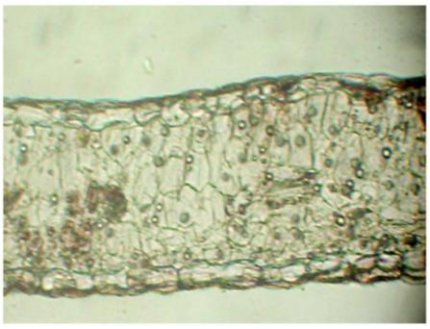


Fig. (1.1) T.S of lamina (x 100)

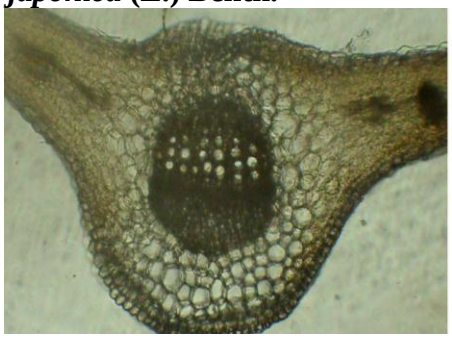


Fig. (1.2) T.S of midrib (x 100) (Apical Region)

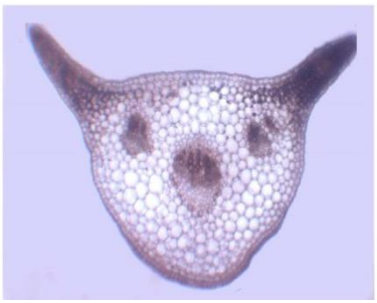


Fig. (1.3) T.S of petiole (basal region) (x 40)

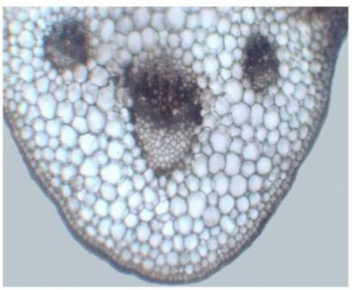


Fig. (1.4) T.S of petiole (top region) (x 40)

Microscopical Characters of Stems and Roots of *Crepis japonica* (L.) Benth.

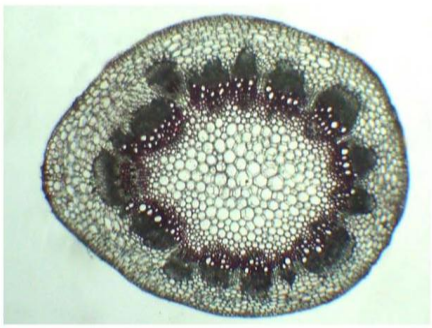


Fig. (1.5) Outline (x 40)

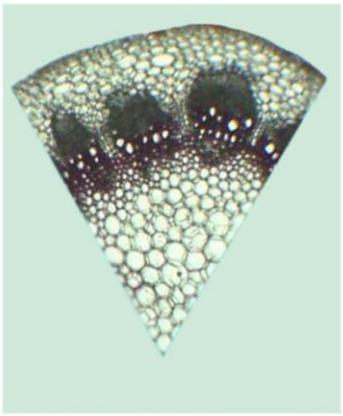


Fig. (1.6) Close up view of stem bundle (x 400)

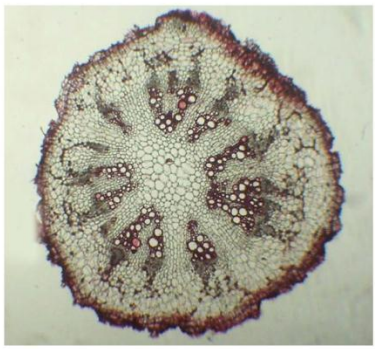


Fig. (1.7) Mature root (x 40)

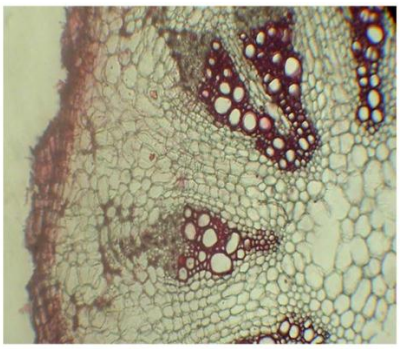
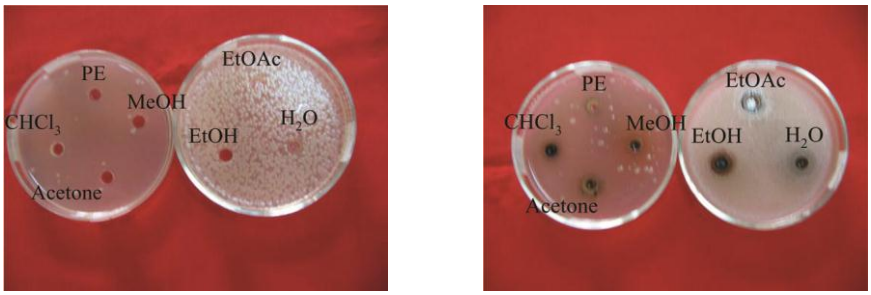


Fig. (1.8) Close up view of vascular bundle (x 400)

Table (1.1) Antimicrobial activity of different solvent extracts of *Crepis japonica* (L.) Benth.

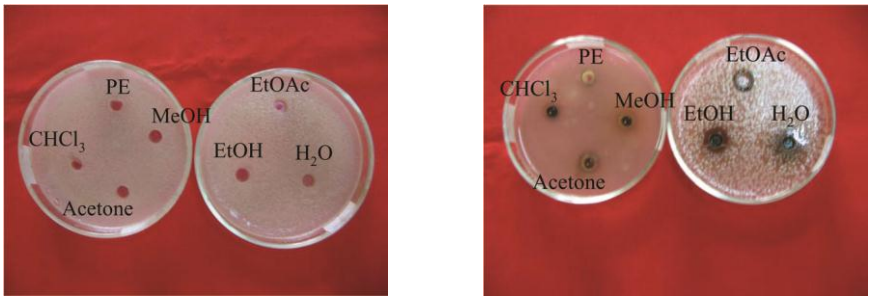
Extracts	Organism					
	Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus pumalis*	*Candida albican*	*Escherichia coli*
Pet-ether (60-80° C)	-	-	12 mm	10 mm	-	14 mm
Chloroform	12 mm	12 mm	10 mm	10 mm	11 mm	10 mm
Acetone	13 mm	14 mm	12 mm	12 mm	14 mm	13mm
Methanol	12 mm	13 mm	12 mm	12 mm	12 mm	13 mm
Ethyl acetate	-	-	12 mm	-	10 mm	10 mm
Ethanol	14 mm	14 mm	14 mm	14 mm	12 mm	14 mm
Water	-	-	-	-	-	-

(Cruickshank, 1975)



Control Antimicrobial activity

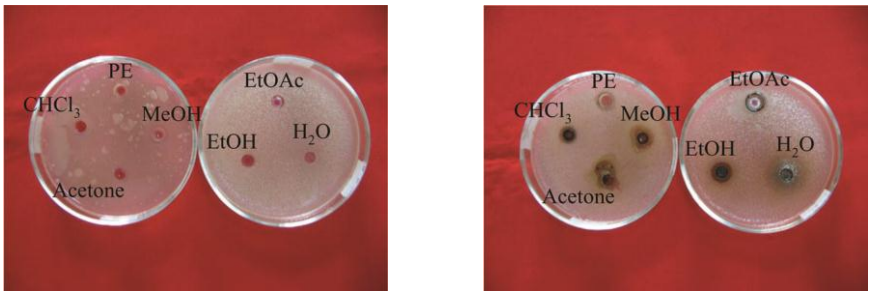
1. ***Bacillus subtilis***



Control Antimicrobial activity

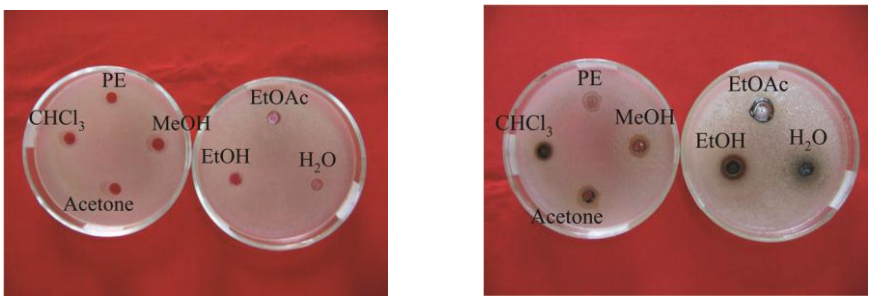
2. ***Staphylococcus aureus***

Fig. (1.9) Antimicrobial activities of various crude extracts against microorganism



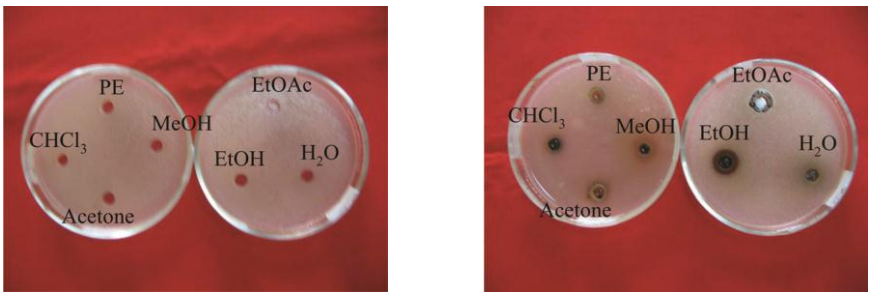
Control Antimicrobial activity

3. ***Pseudomonas aeruginosa***



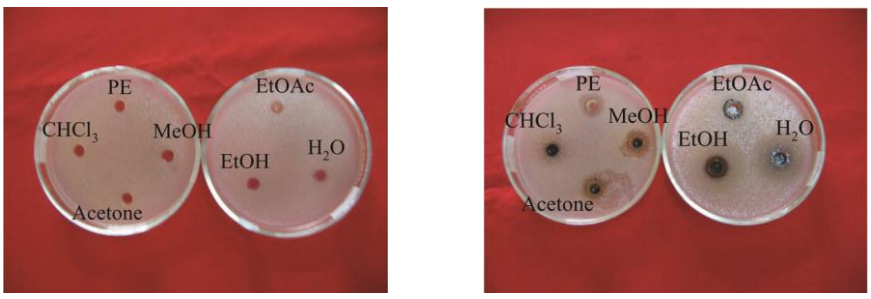
Control Antimicrobial activity

4. ***Bacillus pumalis***



Control Antimicrobial activity

5. ***Candida albican***



Control Antimicrobial activity

6. ***E. coli***

Fig. (1.10) Antimicrobial activities of various crude extracts against microorganism

DISCUSSION AND CONCLUSION

In microscopical study, the anomocytic type of stomata were present on both surfaces and more abundant in lower epidermis. Trichome were absent on both surface. Numerous choloroplasts and oil cells were abundantly found in mesophyll layer. These characters are recorded by Metcalfe and Chalk (1950) and Esau (1965). Sandy calcium oxalate crystals were scattered in parenchymatous cells of leaves and roots. The vascular bundles were collateral and closed type. The bundle sheaths were distinct. These characters are agreement with there given by Pandey (1993), Matcalfe and Chalk (1950).

In antimicrobial activity, ethanolic extracts of the whole plant showed the highest activity on six pathogenic microorganisms. The whole plant extracts of acetone, methanol and ethanol showed effective antimicrobial activity on *Pseudomonas aeruginosa* and *E.coli*. Ethyl acetate extracts showed antimicrobial activity on *Pseudomonas aeruginosa* only. The watery extracts did not show antimicrobial activity on six microorganisms. *E.coli* which was caused diarrhoea and dysentery. Therefore experimental findings from this could be assumed to valuable for human health, especially was in the Myanmar traditional medicine formulation system and valuable for economically product.

ACKNOWLEDGEMENT

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