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ESTIMATION OF PHYTOCHEMICAL CONSTITUENTS IN THREE PLANTS WIDELY USED FOR TRADITIONAL TREATMENT OF ASTHMA IN MYANMAR

Za Khai Tuang

Department of Botany, University of Kalay, Kalay 02091, Sagaing District, Myanmar.

Keywords:

Datura, *Leucas*, *Piper*, Antimicrobial, Asthma, Phytochemical

Correspondence to Author:
Za Khai Tuang

Lecturer,
Department of Botany, University of Kalay, Kalay 02091, Sagaing District, Myanmar.

E-mail: tuangzakhai@kalayuniversity.edu.mm

ABSTRACT: The crude drug powder extracts from three plants *Datura metel* L., (Solanaceae), *Leucas cephalotes* (Roth.). Spreng, *Piper betel* L., (Piperaceae) were screened for estimating phytochemical constituents. Phytochemical analysis of the extracts revealed that glycoside; tannin, saponin, carbohydrate and phenolic compounds are present in *Piper* and *Leucas* species. Besides glycoside, tannin, saponin, carbohydrate and phenolic compound, alkaloid is also present in *Datura* species. The antimicrobial activity of the plant materials and their usefulness in the treatment of asthma may be due to the presence of active constituents like tannins, alkaloids and phenolic. The present study reported that the phytochemical constituents of three plants being widely used for traditional treatment of asthma in Myanmar, collected from Kalay, Sagaing District.

INTRODUCTION: Plants are good sources of a wide variety of compounds, such as phenolic, terpenoids, nitrogen, vitamins and secondary metabolites, which possess antioxidant, anti-microbial, anti-inflammatory, antitumor, antimutagenic, anti-carcinogenic and diuretic activities [1]. Plants have therefore played a significant role in maintaining human health and improving the quality of human life [2, 3]. Even today, the World Health Organization assumes that as many as 80 percent of people still rely mainly on traditional therapies, such as herbal medicines [4]. *Datura metel* L. has a wide range of traditional applications. Dried powder from the flower of the *D. metel* is used to treat asthma, chronic bronchitis, and pain in Myanmar. The dried leaves powder or fruit powder is smoked to cure asthma [5].

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The whole plant is used externally for earache and smoked to relieve spasmodic asthma. Seeds, leaves, and roots of *Datura* are used for curing insanity, fever with catarrh, diarrhea, skin diseases and cerebral complications [6]. The main constituent of the *Datura* plant is a huge number of tropane alkaloids which increased gradually with an increase in the age of the plant [7, 8, 9]. *Leucas cephalotes* (Roth.) Spreng. is a rainy season weed and commonly found ascending in the hilly regions of Myanmar. The flowers are administered in the form of syrup or with honey for curing cough, colds and asthma. It has also been reported that *L. cephalotes* is used for the treatment of pain, asthma, bronchitis, dyspepsia, inflammation, diarrhea, wounds, jaundice, and fever [10].

The leaf juice of *Piper betel* L. is used for antioxidant, anticancer, anti-allergic, fever, cough, fatigue, asthma, to disinfect wounds externally [11, 12]. The leaf powder of P. betel is traditionally known to be helpful in treating various diseases like boils and abscesses, conjunctivitis, bad breath, constipation, itches, headache, mastitis, otorrhoea,

etc leucorrhoea, rheumatism, cuts and injuries [14]. The present study intends to estimate the phytochemical constituents in extracts of the above three plants collected in Kalay, Sagaing Region, Myanmar, which are widely used for the traditional treatment of asthma in Myanmar. It was detected that the three plants shared very similar phytochemical constituents to each other.

EXPERIMENTAL:

Collection of Plant Samples: Fresh leaves of *D. metel*, *L. cephalotes*, and *P. betel* were collected from Kalay, Sagaing District, Myanmar.

Sample Preparation: The leaves of collected specimens were carefully washed with sterile water, dried in room temperature, then powdered, and kept in an airtight container for further use.

Materials: Electronic balance, water bath, beaker, test tube, glass tube, and airtight container were used in this study.

Reagents: Dragendroff's reagents and Mayer's reagents, 10% $FeCl_3$, Benedict's solution, distilled water, 2% NaCl + 1% gelatin, 10% Lead acetate, 1 drop of 5% naphthol+ 5 drops of conc: H_2SO_4 were used.

Test for Alkaloids: 2 g of dried powder sample was boiled with 10ml of dilute hydrochloric acid for 30 min, allowed to cool, then filtered. The filtrate was tested with modified Dragendroff's reagent and Mayer's reagent. The presence of alkaloids was confirmed by the formation of an orange and cream precipitate.

Test for Glycosides: 2 g of dried powder sample was boiled with distilled water for 30 minutes, allowed to cool then filtered.

The filtrate was tested with a modified 10% lead acetate reagent. The formation of a pale yellow or yellow precipitate indicates the presence of glycosides.

Test for Reducing Sugar: 2 g of dried powder sample was boiled with distilled water for 30 minutes. A few drops of Benedict's solution were then added to the filtrate. The solution gives to characteristic brick red color indicates the presence of reducing sugar.

Test for Tannins: 2 g of dried powder sample was heated with ethanol in labeled test tubes. Each solution was allowed to cool then filtered. The filtrate was tested with 1% gelatin and 2% sodium chloride. A characteristic gelly block indicates the presence of tannin.

Test for Saponins: 2 g of dried powder sample was vigorously shaken with distilled water in a test tube for 10 min; the formation of persistent froth foam indicates the presence of saponin.

Test for Carbohydrates: 2 g of dried powder sample was boiled with distilled water for 30 minutes and filtered. The filtrate was treated with 1 drop of 5% naphthol and shaken, and the test tube was inclined at an angle of 45°C. Then added 5 drops of concentrated sulphuric acid slowly along the side of the test tube. A white ring formed between layers showed the presence of carbohydrates.

Test for Phenolic Compounds: 2 g of dried powder sample was boiled with ethanol extract and filtered. The filtrate was treated with a few drops of 10% ferric chloride solution. The formation of blue or green color indicates the presence of a phenolic compound.

RESULTS AND DISCUSSION:

Phytochemical Analysis of *Datura metel* L.: The crude extract samples of *D. metel*, commonly called Thorn Apple, and Padaing-phyu in Myanmar name **Fig. 1** were analyzed, and the results were tabulated **Table 1**. The phytochemical analysis of the crude extract indicated glycoside, tannin, saponin, carbohydrate, phenolic compound, and alkaloid were present in *Datura* species **Table 1**, **Fig. 2**.

These compounds are known as biologically active and therefore support antimicrobial activity. *Datura* leaves have also been used for herbal medicine as a bronchodilator, anesthetic, hallucinogenic, anti-spasmodic [15], calm cough, burns, treachitis and to treat laryngitis [16]. In Myanmar, dried or fresh leaves were widely used for the traditional treatment of asthma as oral. However, it was also reported that several *Datura* spp. as potential poisoning due to their hallucinogenic property [17].

TABLE 1: QUALITATIVE ANALYSIS OF PHYTOCHEMICALS IN *D. METEL* LEAVES

S. no.	Compound	Extract	Test Reagent	Observation	Result
1	Alkaloid	1% HCl	(a) Dragendorff's reagent (b) Mayer's reagent	Orange precipitate Cream precipitate	Negative Positive
2	Glycoside	Distilled Water	10% lead acetate	Yellow precipitate	Positive
3	Reducing Sugar	Distilled Water	Benedict solution	Brick Red precipitate	Negative
4	Tannin	Ethanol	1% gelatin and 2% NaCl	Gelly Block precipitate	Positive
5	Saponin		Distilled Water	Frothing precipitate	Positive
6	Carbohydrate	Distilled Water	1 drop of 5% α naphthol and 5 drops of Concentrated sulphuric acid	Violet layer	Positive
7	Phenolic Compound	Ethanol	10% of $FeCl_3$	Blue-green precipitate	Positive

Fig. 1: (A) Habit of *Datura metel* L.; (B) Inflorescence of *Datura metel* L.; (C) Leaves of *Datura metel* L.; (D) Powder of *Datura metel* L.

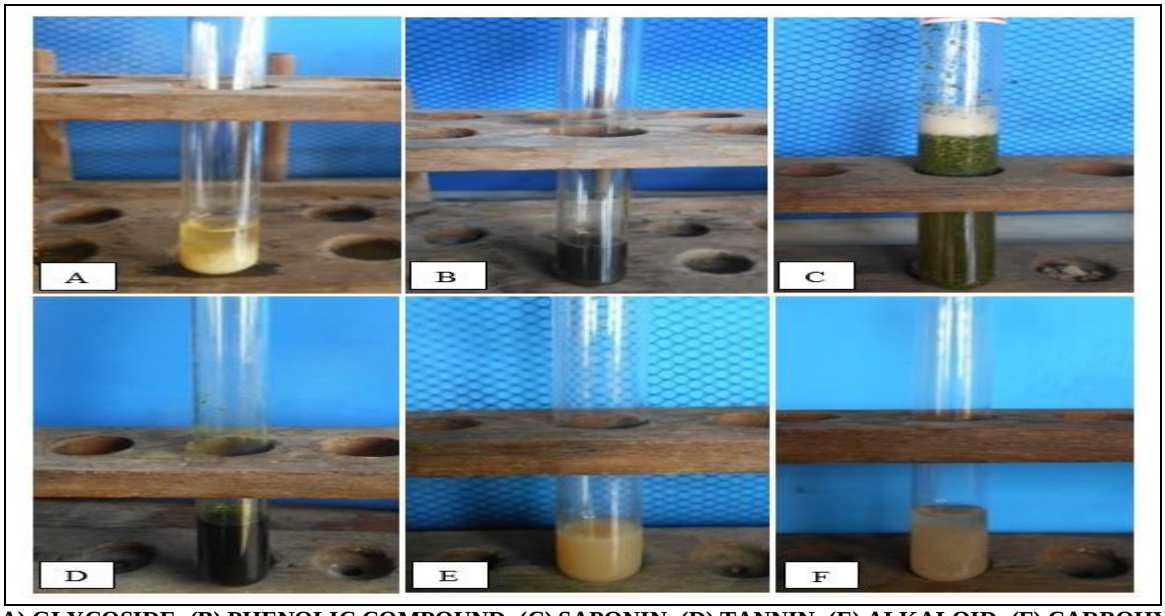

FIG. 2: (A) GLYCOSIDE; (B) PHENOLIC COMPOUND; (C) SAPONIN; (D) TANNIN; (E) ALKALOID; (F) CARBOHYDRATE

Phytochemical Analysis of *Leucas cephalotes* (Roth.) Spreng: The crude extract samples of guma of *L. cephalotes*, locally called as Pingu-htaik-peik in Myanmar **Fig. 3** were also analyzed, and the results were tabulated in **Table 2**. Like *D. metel*, glycoside, tannin, saponin, carbohydrate and phenolic compounds were present in *L. cephalotes*. However, alkaloids could not detect in *L. cephalotes* **Table 2**, **Fig. 4**. Previous reports indicated that tridecanoic acid, lauric acid, adipic acid, labellenic acid, glutaric acid [18], oleanolic acid, triterpenes, flavones and sterols [19] were also found in *Leucas* species. These compounds are suggestive of their possible antimicrobial properties. As long as it is widely used to treat asthma in Myanmar, it is also reported that *Leucas* spp. are traditionally employed for the treatment of urinary complaints, fever, skin diseases, liver disorders, cold and cough [20], snake bite, bronchitis, inflammation, dyspepsia, paralysis and leucoma, urinary discharge [21], chronic, malaria, asthma, bleeding [22], anti-fungal, antioxidant, anti-pyretic, antinociceptive, anti-microbial, analgesic, *etc*. [10, 23, 24]

TABLE 2: QUALITATIVE ANALYSIS OF PHYTOCHEMICALS IN *L. CEPHALOTES* LEAVES

S. no.	Compound	Extract	Test Reagent	Observation	Result
1	Alkaloid	1% HCl	(a) Dragendorff's reagent	Orange precipitate	Negative
			(b) Mayer's reagent	Cream precipitate	Negative
2	Glycoside	Distilled Water	10% lead acetate	Yellow precipitate	Positive
3	Reducing Sugar	Distilled Water	Benedict solution	Brick Red precipitate	Negative
4	Tannin	Ethanol	1% gelatin and 2% NaCl	Gelly Block precipitate	Positive
5	Saponin		Distilled Water	Frothing precipitate	Positive
6	Carbohydrate	Distilled Water	1 drop of 5% α naphthol and 5 drops Concentrated sulphuric acid	Violet layer	Positive
7	Phenolic Compound	Ethanol	10% of $FeCl_3$	Blue-green precipitate	Positive

FIG. 3: (A) HABIT OF *LEUCAS CEPHALOTES* (ROTH). SPRENG; (B) INFLORESCENCE OF *LEUCAS CEPHALOTES* (ROTH) SPRENG; (C) LEAVES OF *LEUCAS CEPHALOTES* (ROTH). SPRENG; (D) POWDER OF *LEUCAS CEPHALOTES* (ROTH) SPRENG

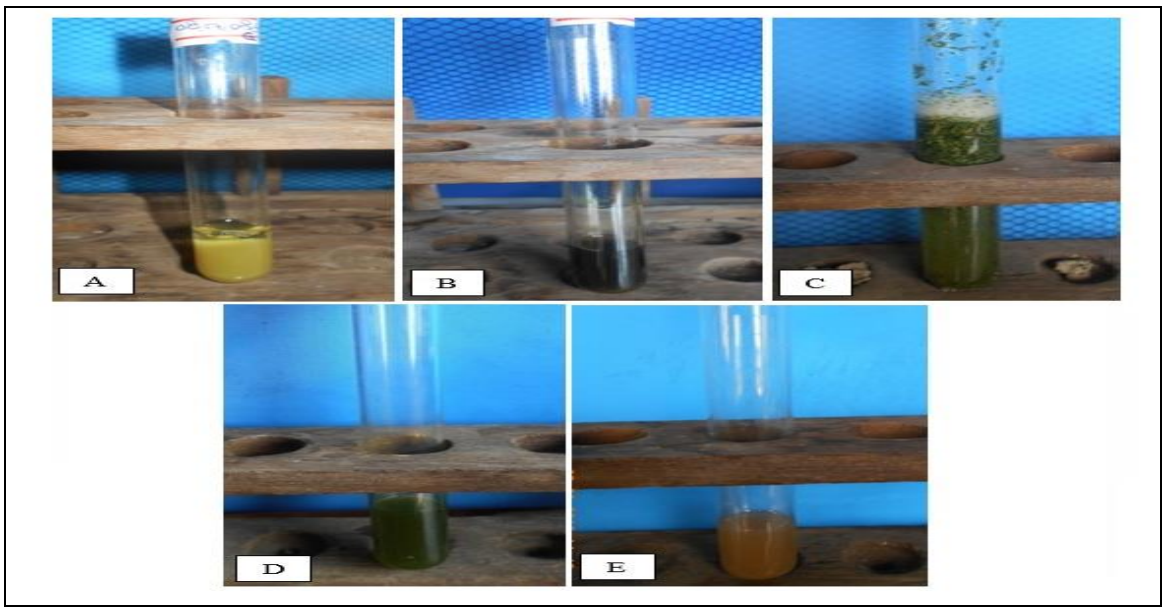

FIG. 4: (A) GLYCOSIDE; (B) TANNIN; (C) SAPONIN; (D) PHENOLIC COMPOUND; (E) CARBOHYDRATE

Phytochemical Analysis of *Piper betle* L.: A common name betel of locally called Kun in Myanmar of *P. betle* **Fig. 5** is widely used for the treatment of various ailments since century [25, 11]. The crude extract of *P. betle* was also studied in the current work, and the phytochemical analysis results were tabulated in **Table 3**, **Fig. 6**. As shown in **Table 3**, the present study detected that glycosides, tannins, and saponins compounds were present in betel leaves. Additionally, it was reported that the betel leaf contains minerals, protein, carbohydrates, fats, fibers, water, essential oils [26], alkaloids, different vitamins like vitamin C, nicotinic acid, vitamin A, thiamine, riboflavin, calcium, iron, and iodine [13]. It has also been suggested that the presence of phenolic natural compound flavonoids in the plant extracts showing antioxidant activity [27-29]. Hence, the phenolic compounds identified in the betel leaves extract might contribute to the antioxidant activity [27]. Betel is also believed that a blessed as an evergreen and perennial plant that God has given the shape of his own heart [14]. Besides used as an asthma treatment, betel leaves are also used for curing eye injury, baby lotion for the newborn, coughs [30], constipation [31], digestive and pancreatic lipase stimulant activities [30, 32], bad breath [30], conjunctivitis, headache, itches, mastoiditis, mastitis, otorrhoea, leucorrhoea, rheumatism [33], bronchitis and dyspnea [34], respiratory catarrhs and antiseptic [35], antibacterial [36], anti-acetylcholinesterase [26], inhibit male reproductive competence [37], suggested to against Covid-19 due to the presence of aurantiamide [38], and also used to improve meat cholesterol levels of Bali duck with fermented rice husk [39].

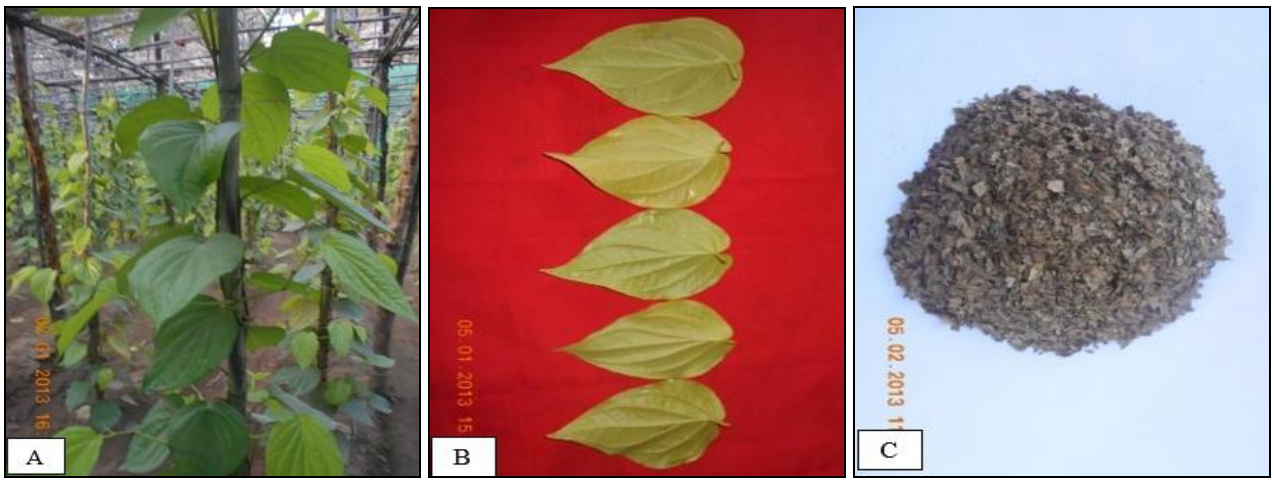

FIG. 5: (A) HABIT OF *PIPER BETLE* L.; (B) LEAVES OF *PIPER BETLE* L.; (C) POWDER OF *PIPER BETLE* L.

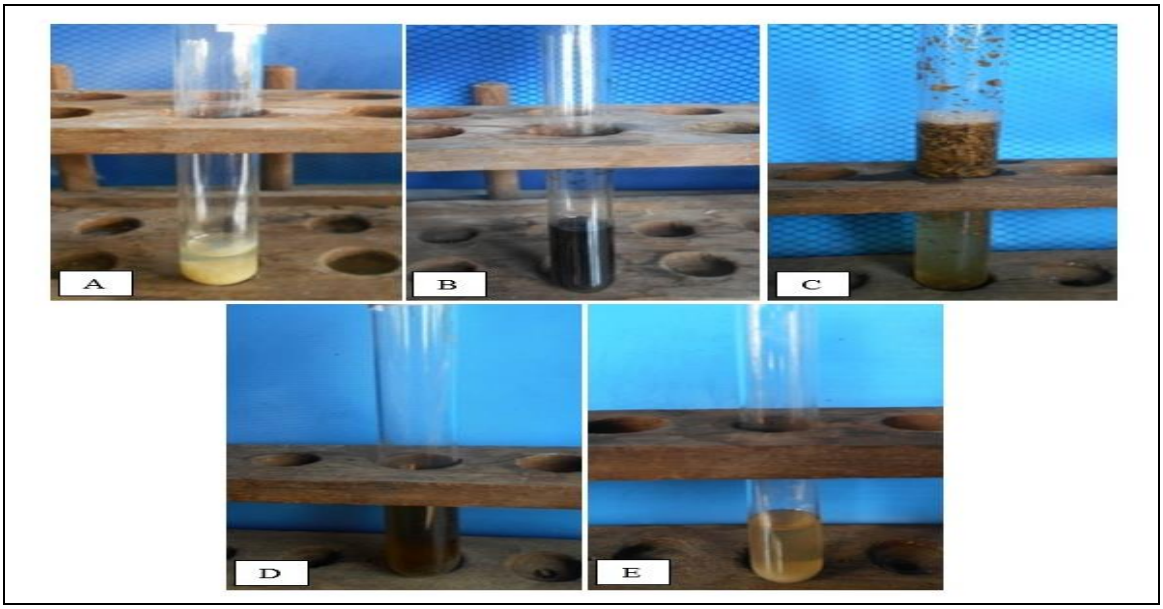

FIG. 6: (A) GLYCOSIDE; (B) PHENOLIC COMPOUND; (C) SAPONIN; (D) TANNIN; (E) CARBOHYDRATE

TABLE 3: QUALITATIVE ANALYSIS OF PHYTOCHEMICALS IN *PIPER BETLE* L. LEAVES

S. no.	Compound	Extract	Test Reagent	Observation	Result
1	Alkaloid	1% HCl	(a) Dragendorff's reagent	Orange precipitate	Negative
			(b) Mayer's reagent	Cream precipitate	Negative
2	Glycoside	Distilled Water	10% lead acetate	Yellow precipitate	Positive
3	Reducing Sugar	Distilled Water	Benedict solution	Brick Red precipitate	Negative
4	Tannin	Ethanol	1% gelatin and 2% NaCl	Gelly Block precipitate	Positive
5	Saponin		Distilled Water	Frothing precipitate	Positive
6	Carbohydrate	Distilled Water	1 drop of 5% α naphthol and 5 drops of Concentrated sulphuric acid	Violet layer	Positive
7	Phenolic Compound	Ethanol	10% of $FeCl_3$	Blue-green precipitate	Positive

CONCLUSION: From the above studies, it was found that the three plants widely used to treat asthma as a traditional medicine in Myanmar shared very close phytochemical constituents **Table 4**. It is concluded that traditional plants represent sources of biologically active components and antimicrobial with stable that can establish a scientific base for the use of plants in modern medicine [10, 40-42]. Hence, it is hoped that scientifically evaluated ethnomedical preparation can be extended for future exploration into the field of pharmacology and other biological actions for drug discovery.

TABLE 4: COMPARISON OF PHYTOCHEMICAL CONSTITUENTS BETWEEN THE THREE PLANTS

S. no.	Compound	Observation	Results		
			D. metel	***L. cephalotes***	***P. betle***
1	Alkaloid	Orange precipitate	-	-	-
		Cream precipitate	-	+	-
2	Glycoside	Yellow precipitate	+	+	+
3	Reducing Sugar	Brick Red precipitate	+	-	-
4	Tannin	Gelly Block precipitate	+	+	+
5	Saponin	Frothing precipitate	+	+	+
6	Carbohydrate	Violet layer	+	+	+
7	Phenolic Compound	Blue-green precipitate	+	+	+

+ = positive; - = negative

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