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Evaluation of Toxicological and Standardization Parameters and Phytochemical Investigation of *Ficus deltoidea* Leaves

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ABSTRACT

Medicinal herbs were the primary health care agent over the many centuries before development of modern medicine. The fact that herbal medicines have been employed for such a long time does not guarantee their efficacy and safety. On that basis, an attempt was made on efficacy and safety studies of a well known medicinal herb in Malaysia, *Ficus deltoidea* by evaluation of toxicological parameter like heavy metals and standardization parameters like, physical constants, ash content, Microbial Limit Test (MLT) and screening phytoconstituents are present in the leaves of *F. deltoidea*. Heavy metals analysis was done by atomic absorption spectrometry. Physicochemical determinations, including moisture, volatile and total ash content were carried out by Thermogravimetric Analyzer. Microbial Limit Test was done as per the United State Pharmacopoeia method. Thin layer chromatography was carried out for phytochemical screening with normal silica plate using various chemical reagents. The contents of Cd, Pb and As were found to be 0.069, 0.761 and 0.422 ppm, respectively. While Hg was not detected in *F. deltoidea* leaves. MLT test showed 5.0×10^{6} and 3.3×10^{7} cfu g^{-1} for Total aerobic microbial count and total combined mold and yeast respectively. However, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* sp. and *Escherichia coli* were found to absent. Phytochemical studies of petroleum ether, chloroform, methanol and water extracts showed presence of saponin, amino acid, flavonoids and terpenoids. Hence, there is an urgent need for mandatory evaluation of these parameters in every crude drug before further processing to ensure safety and efficacy of medicinal plants. In conclusion, it was shown that the extracts of *F. deltoidea* leaves were enriched with chemically diversified phytoconstituents which could be useful for various pharmacological activities.

Key words: *Ficus deltoidea*, heavy metals analysis, microbial limit test, phytochemical screening

INTRODUCTION

Natural products, mainly the plant-derived constituents, have long been used as sources of drugs. Herbal remedies and herbal products have been use over 4,000 years for partial treatment

ailments and diseases. It is believed that about 80% of world's population use plants as their primary source of medicinal agents (WHO, 2005).

This is not only due to a general tendency toward the natural products, but also to more available evidence regarding their mild features and low side effects. However, the vast majority of the medicinal herbal products is unlicensed and is not required to demonstrate efficacy, safety or quality. Unknown effects of some of medicinal herbs have been observed. Example of allergic reactions, toxic reactions especially due to heavy metal poisoning, adverse effects related to an herb desired for pharmacological action against possible mutagenic effects, drug interactions, drug contamination and mistaken plant identities are provided (Ernst, 1998).

Contamination by heavy metals such as mercury, lead, copper, cadmium and arsenic in herbal remedies can be attributed to many causes, including environmental pollution and can pose clinically relevant dangers for the health of the user and should therefore be limited (WHO, 1998; Lazarowych and Pekos, 1998; AOAC, 2005).

The potential intake of the toxic metal can be estimated on the basis of the level of its presence in the product and the recommended or estimated dosage of the product. This potential exposure can then be put into a toxicological perspective by comparison with the so-called Provisional Tolerable Weekly Intake values (PTWI) for toxic metals, which have been established by the Food and Agriculture Organization of the World Health Organization (WHO, 1979, 1981; De Smet, 1998).

Microbial Limit Test (MLT) provides information for the estimation of the number of viable microorganisms present and for freedom from designated microbial species in the nutraceuticals and herbal products (United State Pharmacopoeia, 2009). It includes tests for total viable aerobic count (bacteria and fungi) and total yeast and mold counts. The most care must be taken in performing the tests, so that microbial contamination from the outside can be avoided. As the demand for the herbal supplements is increasing, therefore, it is very important to have a good quality control for medicinal herbs in order to protect consumers from contamination.

Ficus deltoidea Jack (Moraceae) is one of the native plants which are widely distributed in several countries in Southeast Asia. *Ficus deltoidea* was traditionally used in treating many diseases including high blood pressure, improve blood circulation, gout, pneumonia, heart problems, diarrhea and skin infection (Hakiman and Mazziah, 2009).

Based on this, the present study was conducted on *F. deltoidea* leaves to ensure the quality and efficacy by evaluation of toxicological (heavy metal contents such as lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg)) and standardisation parameters (moisture, volatile, total ash, acid insoluble ash and Microbial Limit Test (MLT) for microbial contamination). Moreover, phytoconstituents present in *F. deltoidea* leaves extracts were investigated.

MATERIALS AND METHODS

Material: The leaves of the plant (*Ficus deltoidea*) were purchased from Herbagus Sdn. Bhd. Penang-Malaysia and identified by Mr. Shunmugam from the school of Biological Sciences, University Sains Malaysia. A voucher sample of the plant, reference number 11204 was deposited at the herbarium of School of Biological Sciences, University Sains Malaysia.

Methods

Standardization parameters

Preparation of the extracts: Cold extraction (maceration) was carried out for the preparation of pet ether (FDP), chloroform (FDC), methanol (FDM) and aqueous extracts (FDA) of *F. deltoidea* leaves under consideration.

Phsicochemical analysis: The moisture, volatile and ash content were determined by using TGA701, Thermogravimetric Analyzer instrument of LECO from USA based on Standard Test Methods (ASTM D5142) (2009). This instrumental test method also cover the calculation of fixed carbon volatile dry and ash dry. Determination of acid insoluble ash was according to the method described by Rao and Xiang (2009), briefly 10 milliliter of dilute hydrochloric acid was added with great care to the ash obtained from the determination of the total ash and the crucible was covered with a watch glass. The crucible was heated on a water bath for 10 minutes. Then the watch glass was rinsed with 5 mL of hot water. The rinsing was added to the crucible and filtered with an ash less filter paper. The residue was transferred to the filter paper with water and the filter paper was washed with water for several times till the filtrate yielded no reactions of chlorides. The filter paper together with the residue was transferred to the original crucible, which was then dried and ignited to constant weight. The acid-insoluble ash was weighed and the percentage of acid-insoluble ash was calculated.

Microbial limit test: Powder dried leaves of *F. deltoidea* were subjected to the Microbial Limit Test (MLT) as per the United State Pharmacopoeia method consists of, total aerobic microbial count, test for *Staphylococcus aureus* and *Pseudomonas aeruginosa*, test for *Salmonella* sp. and *Escherichia coli* and total combined molds and yeasts count (United State Pharmacopoeia, 2009).The procedure used for total aerobic microbial, Total Yeast and Mold Count (via pour plate) were as follows:

For preparation of test solution, 90 mL phosphate buffer (pH 7.2) was added to 10 g of powder dried leaves. One milliliter of test solution was added into the Petri dish (9-10 cm in diameter) aseptically and then 15-20 mL of sterilized molten agar medium (Soybean-Casein Digest agar (SCD) for aerobic organisms and Sabouraud Dextrose Agar (SDA) for Yeast and Mold organisms) were added to the Petri dishes. The plates were incubated at 20-25°C for 5-7 days. After that plates were removed from incubation and the colonies were count.

The procedures for specific microorganisms test were as follows:

- ***Escherichia coli*:** One milliliter of test solution was incubated at 30-35°C for 18-24 hours and then it was added to 100 mL of Mac Conkey broth, incubated at 42-44°C for 24-48 hours, streak onto Mac Conkey agar. After 18-27 hours incubation at 30-35°C the plate was observed for microbial growth
- ***Salmonella* sp.:** Ten milliliter of test solution was incubated at 30-35°C for 18-24 hours. 0.1 mL of test solution was added to 10 mL of RVSEB, incubated at 30-35°C for 18-24 hours, streak onto XLD agar. The plates were incubated at 30-35°C for 18-48 hours and observed for microbial growth
- ***Pseudomonas aeruginosa* and *Staphylococcus aureus*:** One milliliter of test solution was incubated at 30-35°C for 18-24 hours and streak onto agar (CET for *Pseudomonas aeruginosa* and MSA for *Staphylococcus aureus*). After 18-72 hours incubation at 30-35°C it was observed for microbial growth

Toxicological parameter

Determination of heavy metals: The contents of lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) have been detected in *F. deltoidea* by using AAS (Atomic Absorption Spectroscopy)

Table 1: Microwave digestion setting

Stages	Max power (W)	% power	Ramp (min)	Pressure (PSI)	Temperature (°C)	Hold (min)
1	1200	100	15:00	120	200	15:00

Perkin Elmer model AAnalyst 800, auto sampler, as per standard method of British Pharmacopoeia Commission (2008).

Approximately 0.5 g of ground sample was accurately weighed and transferred to Teflon vessel. Then 10 mL of HNO_3 was added to the sample vessel. The microwave digestion method is summarized in Table 1. The samples was diluted to 50 mL with distilled water in plastic disposable tubes and filtered with 2 micron Teflon FilterMate. This high dirt trapping FilterMate is especially suitable for trace level analysis and is supplied with lot certification for trace metals. The filtered samples were transferred directly to analyze by AAS using nitric acid as blank solution and standard lead, cadmium, arsenic and mercury solution made with 2% nitric acid as reference.

Phytochemical screening: Four extracts of *F. deltoidea* were screened for the present of various class of compound according to the standard screening method (Trease and Evans, 1983) by using thin-layer chromatography (TLC) using relatively mobile system ethyl acetate – formic acid – acetic acid – water (100 : 11 : 11 : 26). Spraying of the TLC plates using various chemical reagents such as: Anisaldehyde test and Antimony trichloride test for terpenoids, Natural product test for flavonoids, Ninhydrine test for aminoacid, Foam test for saponins, Dragendroff's test for alkaloids, Ethanolic sodium hydroxide test for anthraquinones, Folin-Ciocalteu test for tannins, Aq. sodium hydroxide test for coumarins and Liebermann Burchard test for sterols.

RESULTS

Results obtained from physicochemical analysis of *F. deltoidaea* are giving in Table 2.

Microbial limit test: The results of MLT test are presented in Table 3. All results showed growth after 24 hour incubation for the preparatory testing. Total aerobic microbial count and total combined mold and yeast results showed 5.0×10^6 and 3.3×10^7 cfu g^{-1} respectively while negative results for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* sp. and *Escherichia coli* were found.

Determination of heavy metals: Mercury (Hg), Lead (Pb) and Arsenic (As) were determined in the *F. deltoidea* leaves by using AAS. The content of Cd, Pb and As were found to be 0.069, 0.761 and 0.422 ppm respectively. While Hg was not detected in *F. deltoidea* leaves (Table 4). Level of these four heavy metals in *F. deltoidea* leaves are well within the acceptable limits.

Phytochemical screening: The various extracts weighted and calculated the percentage of yield (Table 5). Percentage of yield for methanol extract was found to be higher rather than the other extracts. It showed methanolic extract contain much more chemical constituents other than other extracts.

The results of phytochemical screening were carried out on the various extracts and recorded as shown in Table 6.

Table 2: Quantitative Physico- chemical analysis of leaves of *F. deltoidea*

Parameters (w/w)	Result
Moisture content	11.209±0.060
Total ash	11.371±0.707
Acid insoluble ash	3.780±0.050
Volatile	67.961±0.202
Fixed carbon	9.460±0.540
Volatile dry	76.540±0.221
Ash dry	12.810±0.799

Table 3: Contamination of microbial in the leaves of *F. deltoidea*

Parameter	Result (cfu g^{-1})
Total viable aerobic count	5.0×10^{6}
Total yeast and mould count	3.3×10^{7}
Escherichia coli	Absent
Staphylococcus aureus	Absent
Salmonella spp.	Absent
Pseudomonas aeruginosa	Absent

Table 4: Heavy metal content of leaves of *F. deltoidea*

Element	Result (ppm)	Specification
Lead	0.761	Not more than 10 ppm
Cadmium	0.069	Not more than 0.3 ppm
Arsenic	0.422	Not more than 5.0 ppm
Mercury	Below detection limit	Not more than 0.5 ppm

Table 5: Percentage yield of extracts (Successive extraction) from *F. deltoidea* leaves

	Percentage of yield of extracts			
Part of plant	Petroleum ether	Chloroform	Methanol	Water
Leaves	2.8537	3.3452	5.984	7.985

Table 6: Summery of phytochemical screening

Phytochemical category	Reagents	Pet. Et extract	Chloroform extract	Water extract	Methanol extract
Terpenoids	Anisaldehyde test	+	+	+	+
	Antimony trichloride test	+	+	+	+
Flavonoids	Natural product test	+	+	+	+
Amino Acid	Ninhydrine test	+	+	+	+
Saponin	Foam test	+	+	+	+
Alkaloids	Dragendroff's reagent	-	-	-	-
Anthraquinones	Ethanolic sodium hydroxide	-	-	-	-
Tannins/ Phenols	Folin-Ciocalteu reagent	-	+	+	+
Coumarins	Dilute sodium hydroxide	-	-	-	-
Sterols	Liebermann Burchard reagent	-	-	-	-

+ : Present - : Absent

DISCUSSION

Medicinal plants have been contributing principally to global health. The quality of a plant product is determined by the prevailing conditions during growth which includes seed selection,

growth conditions, use of fertilizers, harvesting, drying and storage hence, they are capable of variation. Apart from these criteria, factors such as the method of extraction, contamination with microorganisms, heavy metals and pesticides can alter the quality, safety and efficacy of herbal drugs (De Smet, 1998).

In the present work, physical constants such as: moisture, volatile, total ash, acid insoluble ash, fixed carbon, volatile dry and ash dry were determined in *F. deltoidea* leaves as standardisation parameter. Total ash and acid insoluble ash content are important indices to illustrate the quality as well as purity of herbal medicine. Total ash includes "physiological ash", which is derived from the plant tissue itself and "non-physiological ash", which is often from environmental contaminations such as sand and soil. Total ash content alone is not sufficient to reflect the quality of herbal medicines, since the plant materials often contain considerable levels of physiological ash, calcium oxalate in particular. Thus, the acid-insoluble ash content is another index to illustrate the quality of herbal medicine (Rao and Xiang, 2009).

Microbial Limit Test (MLT) were done in *F. deltoidea* leaves for estimation of the number of viable microorganisms present and for freedom from designated microbial specie, including; total aerobic microbial count, total combined molds and yeasts count, test for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* sp. and *Escherichia coli*. The study revealed the microbial safety aspect of the herbal supplements. Overall, no serious microbial contamination was observed in the sample tested and the contamination was still within the acceptable limit (United State Pharmacopoeia, 2009).

Atomic absorption spectroscopy was used to measure heavy metals content in *F. deltoidea* leaves. Microwave digestion method was used due to its speed and lower reagent consumption. The amount of Pb, Cd, As and Hg were found within the acceptable limits. Thus, in order to ensure the safety and quality of medicinal herbs, the analysis of heavy metals must be carried out on routine basis using simple and robust atomic absorption spectrometry (AAS) technique.

Preliminary phytochemical revealed the presence of phenolic, saponin, amino acid, flavonoids and terpenoids in all extracts of *F. deltoidea* leaves. Results reveal that the all extract has large number of phytoconstituents, which may be responsible for many pharmacological activities such as antioxidant, anti-inflammatory, anti cancer and etc. Plant polyphenols are known to have antioxidant properties (Noro *et al*., 1983). The presence of saponins protects plant from microbial pathogens. Flavonoids act as an anti-inflammatory response in the same way as the non-steroidal anti-inflammatory drugs, i.e. by inhibiting the enzymes that cause the synthesis of prostaglandins (Kumar and Kumud, 2010).

CONCLUSION

The results confirm that *F. deltoidea* leaves does not contain toxic element. However, medicinal herbs may be contaminated easily during growing and processing (Basgel and Erdemoglu, 2006). It is important to have a good quality control for herbal medicines in order to protect consumers from contamination. In addition, present study showed that *F. deltoidea* large number of phytoconstituents therefore research on pharmacological properties of *Ficus deltoidea* becomes a challenge due to the lack of knowledge in this species.

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